The influence of exotic salmonids on native host-parasite dynamics

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Summary

Native parasite acquisition provides introduced species with the potential to modify native host-parasite dynamics by acting as parasite reservoirs (with the ‘spillback’ of infection increasing the parasite burdens of native hosts) or sinks (with the ‘dilution’ of infection decreasing the parasite burdens of native hosts) of infection. Exotic salmonids are frequently shown to acquire native parasites; however, as research into the threats posed by exotic salmonids has largely focused on predation and competition, threats posed by shared native parasites are poorly understood.

I used a multiple-pronged approach combining field observations, experimental infections and dynamic population modelling to investigate whether native parasite acquisition by exotic salmonids alters host-parasite dynamics in native fish populations from streams and lakes in New Zealand and Argentina. I also used a meta-analysis approach to investigate which trait(s) influence native parasite acquisition by exotic freshwater fish.

My research demonstrated that two key factors strongly influence whether the dynamics of native parasites will be affected by exotic fish. On one hand, the competency of exotic fish for native parasites is an important determinant of whether native parasite populations are likely to increase or decrease. On the other hand, the relative abundance of the exotic species determines whether its competency for a native parasite will actually translate into altered native host-parasite dynamics, with highly abundant exotic species more likely to induce changes in native parasite dynamics. I also demonstrated how exotic species may be able to override the influence of low host abundance, or competency by altering native host behaviour.

The meta-analysis suggested that traits known to influence parasite richness in native fish or invasion success of exotic species are not reliable predictors of native parasite acquisition by exotic fish. Instead, it is more likely that complex interactions between a variety of biological, geographical and historical factors govern parasite acquisition by exotic species, making it difficult to predict whether native parasites will be acquired.

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Table of Contents

List of Tables

List of Figures

CHAPTER 1

General Introduction

Native *Galaxias* sp. avoiding exotic brown trout *Salmo trutta* (Photo: K. Miller)

1.1 Exotic species introductions

Native biotas face increasing pressures from anthropogenic changes to their environment, suffering the consequences of habitat loss and a rapidly changing climate, in addition to experiencing increasing impacts mediated by the intentional introduction or accidental release of exotic species (Sala *et al.* 2000). Trends in global transport and trade suggest that exotic species will continue to invade new regions (Levine & D'Antonio 2003) and, given that anthropogenic changes to habitat and climate can facilitate the establishment of exotic species (Didham *et al.* 2007, Rahel & Olden 2008), native populations are likely to face ever-increasing threats to their survival. Traditionally, these threats have been thought to come from predation and resource competition exerted by exotic species (e.g. Witte *et al.* 2000, Wiles *et al.* 2003). However, disease–mediated impacts are increasingly recognised as a considerable threat to native biodiversity (eg. Woodroffe 1999, Daszak, Cunningham & Hyatt 2000, Cunningham, Daszak & Rodriguez 2003).

1.2 Disease associated with exotic species

Disease threats associated with exotic species are often assumed to originate from introduced or novel parasites that have spilled-over into native populations (Sutherland *et al.* 2008). This view is understandable, given that numerous introduced diseases have had devastating impacts on native populations (eg. canine distemper virus in Serengeti lions (Roelke-Parker *et al.* 1996), avian malaria in Hawaiian birds (Van Riper, Van Riper & Hansen 2002), squirrel pox virus in British red squirrels (Tompkins, White & Boots 2003)). In modern societies, however, mechanisms are in place to screen exotic species for pathogens prior to intentional introduction, which limits the concomitant introduction of new parasites primarily to those occurring through accidental host introductions. Establishment of newly arrived parasites may also be hampered by a lack of suitable native species necessary to complete the parasite's lifecycle, resulting in exotic species being released from the regulatory influences of their former parasite burdens (Torchin *et al.* 2003).

However, the disease-free status of introduced species is often short-lived as exotic species acquire native parasites (Torchin *et al.* 2003, Kelly *et al.* 2009b). If exotic species are competent hosts for these native parasites, exotic species may act as infection reservoirs that amplify or "spillback" native infection to native hosts (Kelly *et al.* 2009b). Alternatively, exotic species with poor competency for native parasites may act as infection sinks that dilute native infection, because parasite propagules infecting exotic hosts are lost from the system

(e.g. Telfer *et al.* 2005). Thus, exotic species have the potential to affect native populations through mechanisms beyond the more commonly invoked influences of predation, competition and the introduction of novel parasites. Understanding how exotic species influence native populations, through shared native parasites, is a missing element in our understanding of the threats posed to native biodiversity by exotic species.

1.3 Disease associated with exotic freshwater fish

The consequences of novel parasites spilling over from exotic to native fish hosts are well documented. For example, the invasive Asian topmouth gudgeon, *Pseudorasbora parva* (Chordata: Actinopterygii: Cypriniformes), is threatening European fish diversity by exposing native fish to a deadly, non-specific fish parasite (Rosette-like Agent, closely related to *Sphaerothecum destruens*, Gozlan *et al.* 2005, Pinder, Gozlan & Britton 2005). Similarly, the introduced eel-specific nematode *Anguillicola crassus*, originally from Asia, but accidentally imported to Europe inside live eels from Taiwan, has caused marked declines in the native European eel *Anguilla anguilla* (Taraschewski 2006). Like other introduced species, exotic fish have been translocated to new regions as parasite-free eggs or fry, which reduces the chance of co-introducing their original parasites. Despite the initial release from parasites, many exotic fish species now support diverse parasite assemblages gained following introduction to a new region (e.g. Dix 1968, Ortubay *et al.* 1994, Poulin & Mouillot 2003, Salgado-Maldonado 2006), though it is unknown how the acquisition of native parasites by exotic fish may affect native host-parasite dynamics.

1.4 Salmonid introductions

Salmonids are native to cold water environments of the Northern Hemisphere; however, through extensive translocation, they are now present in all continents except Antarctica (MacCrimmon & Marshall 1968, MacCrimmon 1971). The extensive translocation of salmonids can be largely attributed to their high value as recreational angling and aquaculture resources. Nevertheless, salmonids are less valued for their detrimental impacts on freshwater ecosystems following their introduction. These impacts range from altering the behaviour and distribution of macroinvertebrates and fish through predation, competing with native fish for food and space, to changes in ecosystem functioning (Simon & Townsend 2003, Townsend 2003, Vigliano, Fabian & Aquaculture 2007).

In New Zealand, seven salmonid species have been successfully introduced for recreational angling (McDowall 1990). The brown trout, *Salmo trutta*, was introduced as parasite-free eggs in 1867 from Tasmania, Australia, from stock originating in England. It is considered the most widely distributed salmonid species in the country, forming self-sustaining populations in upland lakes and streams to lowland rivers and coastal waters (MacCrimmon & Marshall 1968, McDowall 1990). The rainbow trout, *Oncorhynchus mykiss*, introduced in 1883 from California, USA (MacCrimmon 1971), is the second most abundant salmonid in New Zealand, though its distribution is more localised, with some populations supported by artificial stocking (McDowall 1990). All other introduced salmonid species have limited distributions, despite efforts to establish new populations throughout the country, and consequently receive less attention from anglers and researchers. The detrimental impacts of salmonids, especially brown trout, on native fish communities in New Zealand have been extensively studied over recent decades (e.g. Crowl, Townsend & McIntosh 1992, Simon & Townsend 2003, Townsend 2003, McIntosh *et al.* 2010). However, prior to my study, no links had been made between exotic salmonid presence and disease-mediated impacts on native fish communities.

1.5 Thesis outline

In this thesis, I investigate the influence of exotic freshwater salmonids on native host-parasite dynamics, through a combination of field observations, experimental infections and dynamic population modelling. I also investigate the factors that influence the likelihood of exotic freshwater fish acquiring native parasites using a meta-analysis approach.

CHAPTER 2 – Brown trout are known to have caused population declines and localised extinction of native fish populations in New Zealand (Townsend & Crowl 1991). In this chapter, I investigate whether exotic brown trout also influence native disease patterns in native fish populations of Otago streams. Data from this chapter have been published (see Kelly *et al.* 2009a), for which first authorship was determined by mutual agreement to reflect the collaborative nature of this project.

CHAPTER 3 – This chapter builds on the results of the previous one, investigating the mechanisms through which exotic brown trout alter the dynamics of the native acanthocephalan *Acanthocephalus galaxii* in Otago streams. A revised submission of this chapter, for which I am first author, has been submitted to the Journal of Animal Ecology.

CHAPTER 4 –Field-based observations by Rauque *et al*.(2003) suggest that the exotic rainbow trout is a major contributing host to populations of the native acanthocephalan *Acanthocephalus tumescens* in an Argentine lake invaded by multiple salmonid species. In this chapter, I experimentally assess the assumptions of Rauque *et al*.(2003), and model the influence of multiple salmonid species on native host-parasite dynamics in Lake Moreno, Argentina. This chapter represents an ecosystem-scale 'replicate' to the study of *A. galaxii* in New Zealand, and serves to assess whether the impact of introduced salmonids is similar across different geographical areas.

CHAPTER 5 – This chapter builds on the results of Chapters 3 and 4 that suggest rainbow trout may have greater fitness impacts for native parasites than brown trout. Here I simultaneously evaluate the influence of brown trout and rainbow trout on two native trematode parasites, *Telogaster opisthorchis* and *Stegodexamene anguillae*, in Lake Pearson, New Zealand. This chapter also provides an opportunity to determine whether the influences of exotic salmonids on native host-parasite dynamics are specific to native acanthocephalans only, or can be applied to other native parasites with more complex lifecycles.

CHAPTER 6 – While previous chapters have examined scenarios where native parasite species are either known or assumed to be acquired by exotic hosts, here I use a meta-analysis approach to investigate which trait(s) influence native parasite acquisition by exotic freshwater fish. Unlike previous chapters, the scope of this chapter extends beyond New Zealand and Argentina, using a compilation of published data from around the globe on exotic fish species that have acquired native parasites. Chapters 4-6 are in preparation for journal submission. I will be first author on all publications resulting from these chapters, with co-authorship from my supervisors.

CHAPTER 7 – Here, the results of the previous chapters are synthesised, and I discuss what determines whether a native parasite will be acquired by an exotic fish, and whether native host-parasite dynamics will be altered. I also discuss further areas of research focus stemming from this thesis, such as surveying salmonid-free locations and degraded lowland environments.

APPENDIX 1 and 2 – Here, I include two co-authored publications that I have contributed to during my thesis; first, discussing the concept of parasite spillback in invasion ecology (APPENDIX 1, Kelly *et al.* 2009b), and second, the dynamics of diseases and biological invasions in freshwater ecosystems (APPENDIX 2, Poulin *et al.* 2011).

CHAPTER 2

Has the introduction of brown trout altered disease patterns in native New Zealand fish?

Roundhead galaxias (*Galaxias anomalus*) heavily infected with trematode metacercarial cysts

2.1 Introduction

Exotic species are a major driver of biodiversity loss (Sala *et al.* 2000), with predation and competition commonly regarded as the main mechanisms through which exotic species impact native populations (e.g. Wiles *et al.* 2003, Bohn, Amundsen & Sparrow 2008). Increasingly however, exotic species are shown to alter native species survival through altered disease dynamics (Daszak, Cunningham & Hyatt 2000, Prenter *et al.* 2004). The introduction and transmission of novel parasites by exotic species has resulted in novel infections in native species. These are either maintained by the exotic species acting as an infection reservoir (parasite spillover), or self-sustaining novel infections forming in native populations subsequent to transmission from the exotic host (pathogen pollution; Daszak, Cunningham & Hyatt 2000, Cunningham, Daszak & Rodriguez 2003). While the introduction of novel parasites can have detrimental consequences to native populations, the frequency that this occurs may be reduced through (i) small founder population sizes, (ii) selection of disease free individuals for translocation, (iii) loss of novel parasites during translocation, and (iv) failure of novel parasites in new environments to find suitable hosts to complete their lifecycles (Torchin *et al.* 2003, MacLeod *et al.* 2010). Therefore, many exotic species arrive without novel parasites to transmit to native populations.

Exotic species, however, do not remain free from parasites in their new ranges as native diseases are readily acquired, providing exotic hosts with various opportunities to impact native populations through altered native disease dynamics (Torchin *et al.* 2003, Kelly *et al.* 2009b). They may spillback native parasites to native hosts, by acting as competent reservoir hosts for native parasites (Daszak, Cunningham & Hyatt 2000, Tompkins & Poulin 2006, Kelly *et al.* 2009b). Alternatively, exotic species may cause parasite dilution, by acting as poor hosts for native parasites that decrease disease burdens in native hosts (Telfer *et al.* 2005, Kopp & Jokela 2007). It is also possible that the acquisition of native parasites by an exotic species may not alter native parasite dynamics, or only cause subtle changes that are undetectable.

Freshwater fish communities in New Zealand provide spatially discrete models to test the influence of exotic species on native disease dynamics. Although it is widely recognised that exotic fish in New Zealand, predominately salmonids, have caused the decline and localised extinction of many native fish populations due to predation and competition (Townsend 2003); the role of exotic fish in altering disease dynamics has received little attention (Tompkins & Poulin 2006). This is likely due to the introduction of salmonids as parasite-free eggs (MacCrimmon & Marshall 1968), which reduced the likelihood of parasite

spillover to occur, and thus the potential for novel diseases to be implicated in native species declines. However, studies have documented the acquisition of native parasites by salmonids in New Zealand (e.g. 10 native parasites in brown trout *Salmo trutta* Dix 1968, Hine, Jones & Diggles 2000). Thus, salmonids may be impacting native fish through alterations to native host-parasite dynamics.

This study assesses whether the introduction of brown trout has altered native disease patterns in New Zealand stream fish by surveying multiple streams that vary in native and exotic fish composition. First, the extent of native parasite acquisition by introduced trout in steams of varying native fish community composition was assessed. Second, the potential for parasite spillback or dilution to occur is determined by evaluating native parasite infections in native fish hosts in relation to trout abundance. Finally, mechanisms that may be responsible for altered parasite dynamics are identified, and their implications for native host populations are discussed.

2.2 Materials and Methods

2.2.1 Fish sampling

The influence of brown trout presence on native parasite patterns was determined by sampling native and exotic fish populations from 14 stream sites in the Manuherikia and Taieri River catchments, Otago, New Zealand (Table 1; Figure 2.1). 'Trout-invaded' and 'trout-free' sampling locations were selected from previous studies by Townsend and Crowl (1991), Leprieur *et al*. (2006) and the New Zealand freshwater fish database (NZFFD). All sites were sampled during austral winter (June - August 2007); a time at which the infection prevalence of many fish parasites increases in invertebrate intermediate hosts (see Lagrue & Poulin 2009). The fish community at Swin Burn (Q1), which was considered 'trout-free' in June 2007, was re-sampled in October 2007 as trout were observed in a related study from this site.

Fish densities at each site were assessed by single-pass electric fishing a stream reach (70-100 m) that included both riffle and pool sections. A random sub-set of trout and native fish from each site were euthanised, measured for fork length (FL) and weight (g), prior to being preserved in 10% formalin. In the laboratory, the alimentary canal (oesophagus to anus) of each fish was removed and split longitudinally to examine for parasites. Metacercarial cysts from all internal organs and body tissues of small fish (galaxias and bullies) were identified by compressing thin pieces of tissue between glass plates. Body tissues of large fish

(predominantly trout) were screened for metacercarial cysts by digesting body tissues in a pepsin solution (6 g pepsin in 7 ml HCl in 1 L of distilled water at 40 °C for 4 hours, see McFarland, Mouritsen & Poulin 2003).

2.2.2 Intermediate host sampling

The presence and relative abundance of invertebrate intermediate hosts were assessed at each site from a 3–5 min kick-sample (using a D-frame pole net) spread diagonally across the stream to sample all microhabitats. Invertebrates were preserved in 10% formalin. The abundance of the gastropod *Potamopyrgus antipodarum* and the amphipod *Paracalliope fluviatilis* were assessed from each sample, representing the main intermediate hosts for known parasites of freshwater fish in New Zealand.

2.2.3 Statistical Analyses

The relationship between fish length and weight were analysed using a linear regression ($\log_{10}$ transformed) in STATISTICA 6.0 (Sat Soft, Tulsa, OK, U.S.A.). The variation in infection intensity amongst sites was analysed using a Generalised Linear Mixed Model (GLMM) computed with the program *R* (R Development Core Team 2008; version 2.7.1). Separate GLMMs were constructed for each native parasite to assess whether trout altered disease patterns in native hosts. The response variable for all analyses was the intensity of infection [$\log_{10}$ or $\log_{10}(x + 1)$ transformed]. As infection intensity may increase with fish size (and age; Poulin 2000), and the presence of trout may alter the size structure of native fish (McIntosh, Crowl & Townsend 1994); infection intensity was investigated with respect to host size (length, weight), pooled across sites. Sites of zero parasite prevalence were excluded from all analyses, as it was unknown whether the absence of parasites was due to historical absence from the site or environmental factors.

Three predictor variables were used for each GLMM (index of trout abundance, total fish abundance and intermediate host abundance). The index of trout abundance was calculated from the ratio of trout to native fish captured per site. It was assumed that if trout were competent hosts for a native parasite, an increase in the ratio would amplify infection in native fish; whereas if trout were incompetent hosts, an increase in the ratio would dilute infection in native fish. Total fish abundance was calculated from the total number of trout and native fish. This predictor variable was included as the number of available hosts may

also influence per capita infection intensity (Fauchald *et al.* 2007). Relative abundance of intermediate hosts was also included in analyses as it is assumed that differences in infection amongst sites may be explained by the abundance of intermediate hosts, and therefore the supply of infective stages to hosts (e.g. Thieltges & Reise 2007).

Two random factors, 'fish within site' and 'fish body mass' (used as a measure of fish body size), were included in each GLMM to control for non-independence, and avoid pseudoreplication (Crawley 2002). A global model was fitted including all predictor variables and random factors, followed by successive removal of unimportant predictor variables (Crawley 2002). Log-likelihood ratio tests that compared models before and after removal of a given variable were used to assess the importance of each variable. Non-significant variables were removed. The final model was based on hierarchical ordering using the Akaike Information Criterion (AIC), with the best model having the lowest AIC score. However, if the best model (lowest AIC score) contained a term that was not significant at the $P = 0.05$ level; the model with the next lowest AIC and significant term was chosen (Crawley 2002).

2.3 Results

Native fish communities of the Manuherikia River catchment were comprised mostly of upland bully *Gobiomorphus breviceps*, which co-occurred with brown trout at seven sites (Table 1). The Hound Burn, Taieri River catchment was the only site where upland bully occurred in the absence of brown trout, with roundhead galaxias *Galaxias anomalus* also present there. Roundhead galaxias was the most abundant native fish species of the Taieri catchment, occurring in sympatric populations with brown trout at four sites. Brown trout were less abundant than upland bully at all sympatric sites (1 trout : 3.5-40 bully), while the relative abundance of trout to galaxias was variable between sites (1 trout : 0.8-41 galaxias).

Seven native parasite species (six trematodes and one acanthocephalan) were present in fish communities of the Manuherikia and Taieri catchments (Table 2.2). Upland bully acted as a definitive host to the trematode *Cotiocaecum parvum* (0.22 – 14 parasites per fish) and as a second intermediate host for three other trematode species (*Telogaster opisthorchis, Stegodexamene anguillae* and *Apatemon* spp.) that occurred as metacercarial cysts embedded in body tissues. Abundance of *S. anguillae* and *T. opisthorchis* metacercariae, which utilise predatory fish as definitive hosts, ranged in abundance from low to moderate, and low to high respectively. The abundance of *Apatemon* spp., which utilises birds as definitive hosts, was

highly variable and of the three fish species surveyed, *Apatemon* spp. occurred in highest abundances in upland bully.

Parasite species richness was greatest in roundhead galaxias, with six trematodes and one acanthocephalan present (Table 2.2). Trematode metacercariae infections were dominated by *S. anguillae* and *T. opisthorchis* occurring at low to high abundances, with minor *Apatemon* spp. infections present at three sites. Of the three adult trematodes observed from roundhead galaxias, the intestinal trematode *Deretrema minutum* was most common in both prevalence and abundance, followed by the gall bladder trematode *Deretrema philippae.* The only population of roundhead galaxias infected with *C. parvum* occurred at the Hound Burn, with light infections noted. The acanthocephalan *Acanthocephalus galaxii* was recovered from fish of Old Hut Creek and the Swin Burn region, with infections occurring at low to high prevalence and low abundance.

Two native parasites, *T. opisthorchis* and *A. galaxii,* were recovered from brown trout. Both metacercarial and adult stages of *T. opisthorchis* infection occurring at low to moderate prevalence and low abundance. Infections of *A. galaxii* occurred in trout from the Swin Burn catchment where roundhead galaxias were co-infected, with greater prevalence and abundance of acanthocephalans observed in the exotic host.

2.3.1 Infection of native fish in relation to trout presence

Both roundhead galaxias and upland bully body mass regressions versus fish length (FL) were significant (r = 0.9 and 0.94, respectively, both P = 0.0001). Non-significant Durbin-Watson statistics indicated that linear models were the appropriate fit to the data (Fry 1993), therefore fish body mass was used as a random effect in the GLMMs examining relationships with parasite intensity.

Infection intensities of *Apatemon* spp. and *S. anguillae* in upland bully were positively related to the density of the intermediate snail host (Table 2.3), while infection intensities of *S. anguillae* and *T. opisthorchis* metacercarial cysts were negatively related to the trout index (Table 2.3; Figure 2.2). While a model could not be fitted to the intensity of infection of *C. parvum,* a simple linear regression demonstrated that *C. parvum* infection intensity was also negatively related to the trout index (r = -0.37, $P < 0.05$; Table 2.3).

The intensity of *T. opisthorchis* in roundhead galaxias was negatively correlated to the abundance of the intermediate snail host, while the intensity of *D. philippae* infections were

negatively related to trout abundance (Table 2.3; Figure 2.3). Models were not fitted for infection intensities of *A. galaxii*, *S. anguillae* or *D. minutum*.

2.4 Discussion

Both the parasite's origin and the exotic host's competency for the parasite determine how exotic hosts may influence native populations through disease (Daszak, Cunningham & Hyatt 2000). Due to the absence of introduced parasites in native and exotic fish populations in Otago streams, both pathogen pollution and parasite spillover driven by the introduction of novel parasites are unlikely. In contrast, the presence of two native fish parasites, *T. opisthorchis* and *A. galaxii*, in brown trout provides this exotic host with the opportunity to alter native host-parasite dynamics. However, no evidence was found to support the process of parasite spillback, as the acquisition of native parasites by brown trout did not correspond to increased parasite infections in native fish populations. Negative relationships between trout abundance and infection intensities of four native parasites (*C. parvum*, *D. philippae, S. anguillae* metacercariae, *T. opisthorchis* metacercariae) suggest that the dilution effects of trout on native infection are not necessarily restricted to the parasite species observed in trout.

Although field surveys indicate that brown trout may dilute infection in native fish, a number of potential mechanisms could be responsible for the observed patterns of dilution. For example, the presence of an additional species may decrease the likelihood that an infective stage will encounter a suitable host to complete its lifecycle (encounter reduction, see Keesing, Holt & Ostfeld 2006). Field infection patterns of the bacterium *Bartonella* in native wood mice *Apodemus sylvaticus* in the presence of an incompetent parasite host, the introduced bank vole *Myodes glareolus*, demonstrates how this mechanism can reduce infections in native hosts (Telfer *et al.* 2005). Infection dilution mediated by an non-host has the potential to cause major changes to the transmission rate of native parasites to their hosts, with Johnson *et al*. (2009) also demonstrating experimentally that *Schistosoma mansoni* infections in the native snail *Biomphalaria glabrata* may be reduced by 30% in the presence of sympatric non-host snail populations. In this study, infection dilution mediated by the presence of a non-host also provides a possible explanation for the negative relationship between trout abundance and parasite infection in native hosts, since brown trout are likely to encounter infectious stages sustained by sympatric native populations.

For native parasites that are able to establish in exotic hosts, the hosts' competency for acquired native parasites continues to have a major influence on whether native host-parasite

dynamics will be altered. Native parasites that experience reduced establishment, survival and/or fecundity in their exotic host, will have reduced overall fitness at the population level. Although adult and metacercarial stages of *T. opisthorchis* were noted in brown trout in the current study, low infection prevalence and intensity in this host compared with relatively high infection burdens in sympatric native hosts, suggest that brown trout have poor host competency for *T. opisthorchis*, thus leading to infection dilution.

Predatory and competitive behaviour of trout may also contribute to decreased infection in native fish by reducing the abundance of native hosts for infectious stages to encounter. This could also cause localised extinctions of the parasites themselves, if insufficient hosts are available in which native parasites can complete their lifecycle. Predation of heavily infected native fish could further dilute native infection if brown trout are incompetent hosts for native parasites and occur at relatively high abundances. As a response to the presence of trout in streams, galaxiids may also demonstrate reductions in activity levels and feeding durations (Edge, Townsend & Crowl 1993), in addition to being outcompeted for preferred microhabitats (McIntosh, Townsend & Crowl 1992). Behavioural changes by native fish are likely to alter the rate at which native hosts ingest infected intermediate invertebrate hosts and may influence the probability of encountering trematode cercariae.

Although *A. galaxii* infections in roundhead galaxias showed no relationship with trout abundance, the presence of similar or higher prevalence and infection intensity in trout to the native host indicates that *A. galaxii* has the strongest likelihood of being influenced by the spillback of infection from an additional host species. While field surveys did not indicate that spillback was occurring, any amplification of infection by introduced trout may be masked by trout predating on heavily infected individuals. Experimental infections are required to determine the host competency of brown trout for *A. galaxii*, and may reveal why a pattern between trout abundance and parasite infections in roundhead galaxias were not observed from the field (see Chapter 3). Experimental infections would also be beneficial in determining which mechanism(s) are responsible for dilution patterns observed for trematode species.

Table 2.1. Site characteristics and number of fish captured in streams of the Manuherikia and Taieri River catchments in winter 2007.

Site	Fish community	Date	Reach width m	Reach length m	NZ grid E	NZ grid N	Fish abundance fish captured per reach		
							S. trutta	*Go. breviceps*	*Ga. anomalus*
Manuherikia River catchment									
Station Creek	Sympatric (Bully)	June	2	80	22°61'100	55°83'800	6	25	-
Mata Creek	Sympatric (Bully)	Aug	3	80	22°60'800	55°83'640	4	14	-
McCleans Creek	Sympatric (Bully)	Aug	1.5	80	22°40'415	55°64'317	3	20	-
Becks Creek	Sympatric (Bully)	June	1.5	70	22°52'635	55°73'016	5	20	-
Millers Creek	Sympatric (Bully)	June	1.2	70	22°50'000	55°74'500	10	35	-
Lauder Creek	Sympatric (Bully)	June	4.5	100	22°48'800	55°74'500	2	35	-
Chatto Creek	Sympatric (Bully)	June	1.5	80	22°35'730	55°65'892	1	40	-
Ida Burn	Sympatric (Galaxias)	June	2.5	80	22°54'900	55°66'600	15	-	18
Taieri River catchment									
Hound Burn	Sympatric (Galaxias + Bully)	Aug	1.5	70	22°98'735	55°53'055	-	7	27
Old Hut Creek	Sympatric (Galaxias)	June	2.5	70	22°94'000	55°68'734	27	-	21
Camp Creek	Sympatric (Galaxias)	Aug	1.75	80	22°97'704	55°67'874	1	-	41
Swin Burn Q1	Trout-free (Galaxias only)	June	3	80	22°96'900	55°58'700	-	-	28
Swin Burn Q1	Sympatric (Galaxias)	Oct					6	-	65
Swin Burn Q2	Sympatric (Galaxias)	Oct	2.5	80	22°98'300	55°59'400	22	-	40

Table 2.2. Prevalence (%) and intensity of infection (worms per fish) of parasites of bully, galaxias and trout from stream sites of the Manuherikia and Taieri catchments.

		Metacercariae			Adult trematodes				Acanthocephala
Site	N	*S. anguillae*	*T. opisthorchis*	*Apatemon spp.*	*T. opisthorchis*	*C. parvum*	*D. minutum*	*D. philippae*	*A. galaxii*
Trout									
Station Creek	6	0	0	0	0	0	0	0	0
Mata Creek	7	0	0	0	14.3% 2.4	0	0	0	0
McCleans Creek	3	0	33.3% 0.3	0	0	0	0	0	0
Becks Creek	5	0	0	0	0	0	0	0	0
Millers Creek	4	0	0	0	0	0	0	0	0
Lauder Creek	1	0	0	0	0	0	0	0	0
Chatto Creek	1	0	0	0	0	0	0	0	0
Ida Burn	4	0	25% 0.75	0	0	0	0	0	0
Old Hut Creek	10	0	0	0	0	0	0	0	0
Swin Burn Q1 Oct.	6	0	0	0	0	0	0	0	83.3% 10.4
Swin Burn Q2 Oct.	14	0	0	0	0	0	0	0	85.7% 9
Across site mean		0	5.3% 0.1	0	1.3% 0.2	0	0	0	15.35% 1.75
Bully									
Station Creek	9	0	22% 0.33	8% 17.9	0	11.1% 0.22	0	0	0
Mata Creek	5	100% 1.2	40% 1.4	60% 16.2	0	80% 3.4	0	0	0
McCleans Creek	8	100% 35.6	100% 37.5	100% 225	0	100% 14	0	0	0
Becks Creek	10	60% 8.1	90% 14.8	100% 125	0	0	0	0	0
Millers Creek	9	66.6% 2.8	88.8% 11.5	100% 237	0	11.1% 0.22	0	0	0

Lauder Creek	8	62.5% 1.6	0	25% 0.5	0	0	0	0	0
Chatto Creek	13	84.6% 30.1	84.6% 23	84.6% 3.6	0	83.3% 3.9	0	0	0
Hound Burn	3	0	100% 176	100% 29.3	0	0	0	0	0
Across site mean		59.2% 9.9	65.6% 33.3	69.7% 81.8	0	35.7% 2.7	0	0	0
Galaxias									
Ida Burn	6	100% 8.2	33.3% 7.3	0	0	0	33.3% 0.8	16.6% 0.33	0
Hound Burn	4	100% 27.7	100% 138	0	0	25% 0.5	50% 1	0	0
Old Hut Creek	10	100% 39.3	100% 21.5	10% 0.1	0	0	33.3% 7.4	22.2% 0.22	11.1% 0.11
Camp Creek	6	100% 24.5	100% 105.2	16.63% 1.6	0	0	50% 25.3	16.6% 0.17	0
Swin Burn Q1 June	10	90% 7.3	40% 3.1	0	0	0	90% 3.7	40% 1	40% 1.1
Swin Burn Q1 Oct.	27	100% 11.2	92.6% 4.4	0	0	0	96.3% 6	66.6% 1.9	40.7% 2.7
Swin Burn Q2 Oct.	20	100% 38.8	75% 2.6	0	0	0	85% 2.3	40% 0.75	80% 9
Across site mean		98.5% 22.4	77.3% 40.3	3.8% 0.24	0	3.6% 0.07	62.5% 6.6	28.8% 0.62	24.4% 1.8

Table 2.3. Generalised linear mixed models examining the relationships between environmental factors and infection intensity in upland bully and roundhead galaxias.

Native host	Parasite species	Candidate models	AIC	d.f.	Variable(s)	Estimate	SE	*P*-value
Upland bully	*Apatemon* spp	T.N + *P. anti* +fish	85.1	7				
		P. anti + fish	83.3	6				
		P. anti	82.5	5	*P. anti*	0.48	0.1	<0.005
	S. anguillae	T.N + *P. anti* +fish	89.7	7	T.N	-4.88	0.99	<0.05
					P. anti	0.28	0.06	<0.05
					Fish	-2.2	0.64	NS
		T.N + *P. anti*	95.4	6				
		T.N + fish	97.3	6				
		Fish + *P. anti*	98.2	6				
	T. opisthorchis	T.N + *P. anti* +fish	93.3	7				
		T.N + *P. anti*	93.3	6				
		T.N	94.9	5	T.N.	-4.2	1.6	<0.05
	C. parvum	Not fitted						
Roundhead galaxias	*A. galaxii*	Not fitted						
	S. anguillae	Not fitted						
	T. opisthorchis	T.N + *P. anti* + fish	36.7	7				
		P. anti + fish	34.7	6				

		P. anti	35.7	5	*P. anti*	-0.39	0.18	<0.05
	D. minutum	Not fitted						
	D. philippae	T.N + fish	17.7	6				
		T.N	16.1	5	T.N	-0.27	0.07	<0.05

To control for non-independence of data, ‘site’ and ‘ fish body weight’ were included as random effect. For each response variable, the parameter estimates for the best model are indicated.

AIC, Akaike information criterion; T.N, Trout index; *P. anti* , *Potamopyrgus antipodarum*; fish, total fish abundance.

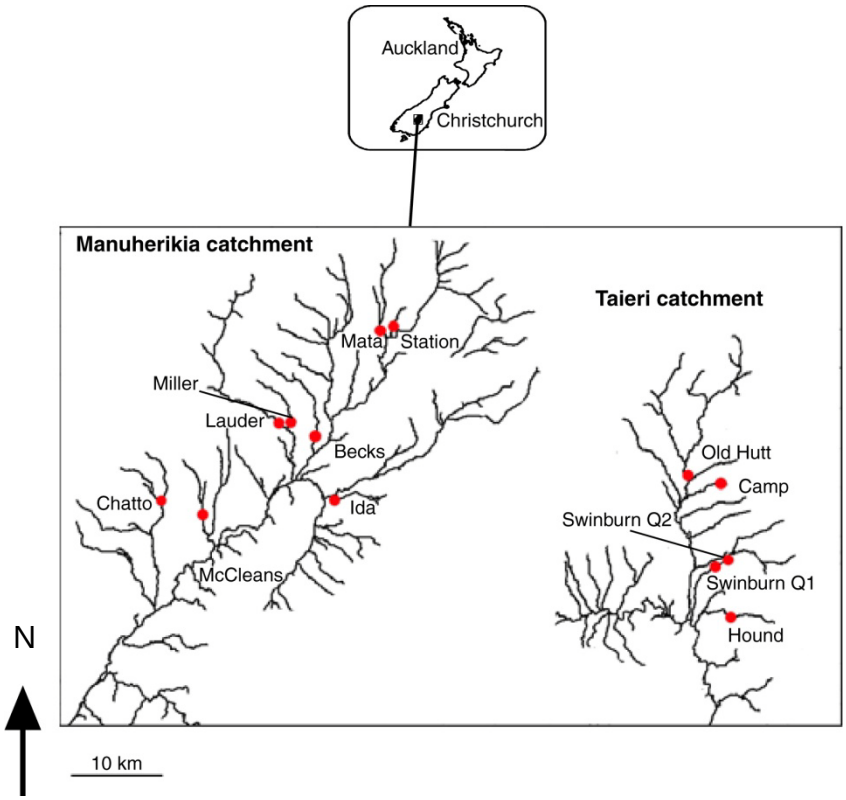

Figure 2.1. Map showing locations of stream sites in the study area (see Table 2.1).

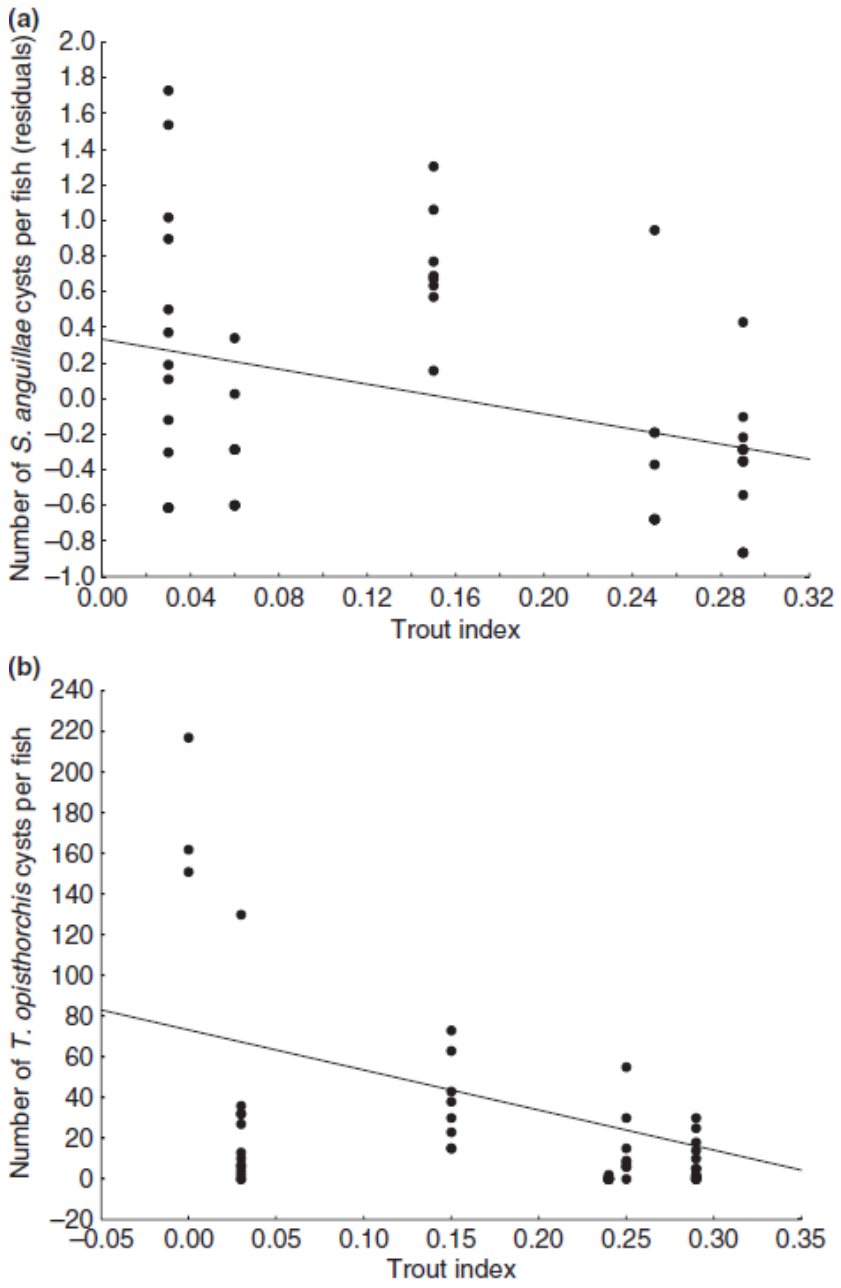

Figure 2.2. Relationship between infection intensity of (a) *Stegodexamene anguillae* and (b) *Telogaster opisthorchis* cysts in upland bully and the index of trout abundance. Note that in (a) the *y*-axis represents partial residuals and this accounts for the independent effects of *Potamopyrgus antipodarum* (see Table 2.3).

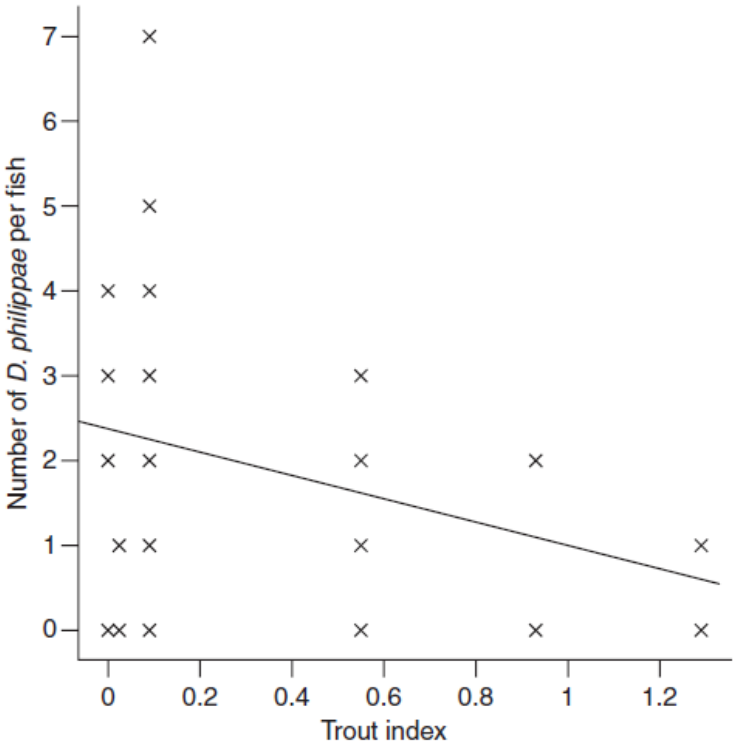

Figure 2.3. Relationship between infection intensity of *Deretrema philippae* in roundhead galaxias, and the index of trout abundance. The line represents the linear relationship predicted by regression.

CHAPTER 3

Introduced brown trout alter native acanthocephalan infections in native fish

Mature female acanthocephalan *Acanthocephaus galaxii*

3.1 Introduction

Introduced species have the potential to modify native host-parasite dynamics through the acquisition of native parasites. Recent reviews have demonstrated that introduced animals may acquire about five native parasites on average, with some gaining more than 16 (Torchin *et al.* 2003, Kelly *et al.* 2009b). These reviews highlight that not only do a great variety of introduced species acquire native parasites, but also that the acquired parasites are highly diverse.

The frequency with which introduced species acquire native parasites has led to increasing attention to the ways they can modify native parasite dynamics. Parasite 'spillback' to a native host may result from the acquisition of native parasites by an introduced species, provided that it becomes a competent host that can act as a reservoir of infection (Daszak, Cunningham & Hyatt 2000, Tompkins & Poulin 2006, Kelly *et al.* 2009b). The spillback of parasites may act as an additional threat to native biodiversity via increased disease impacts on species already facing mounting pressures from other sources such as competition, predation, habitat loss, pollution and climate change. Recent studies have also documented how the acquisition of native parasites by introduced species may lead to parasite 'dilution', whereby native hosts experience lower parasite burdens in the presence of introduced hosts (Telfer *et al.* 2005, Kelly *et al.* 2009a, Thieltges *et al.* 2009). While reduced parasite burdens in native hosts may appear of less concern, alterations to processes that regulate population densities may have flow-on effects on native species dynamics, such as the modification of competitive advantage between conspecifics (Kopp & Jokela 2007), which could result in subsequent alterations to community dynamics and ecosystem functioning. The frequency with which introduced species acquire native parasites and attain high population densities means there is much potential for either parasite spillback or dilution to occur.

Our current understanding of the potential impacts of introduced species on native host-parasite interactions relies largely on field-based observations of introduced populations, using a combination of parasite fitness parameters at individual (e.g. size, fecundity) and population scales (e.g. prevalence, infection intensity, abundance; Bush *et al.* 1997) to assess the competency of introduced species as native parasite hosts (Rauque, Viozzi & Semenas 2003). Such assessments have been made with or without sympatric native host populations (Galli *et al.* 2005), and occasionally native parasite dynamics have been surveyed within native-only host populations to provide baselines from which the effects of an introduced host are judged (Dubois,

Marcogliese & Magnan 1996, Kelly *et al.* 2009a). However, native populations free from the effects of introduced species are not always available for such comparisons to be made.

While field-based observations are essential to ascertain which native parasites have been acquired by introduced species and to identify patterns, differences in parasite fitness parameters between native and introduced hosts may not provide a true reflection of a parasite's overall performance. Field observations can obscure differences in establishment success, survival/mortality, maturation, growth and relative fecundity. For example, poor host competency of an introduced host in terms of parasite establishment could be masked if this species was more likely to encounter infectious stages, resulting in similar levels of prevalence and/or infection intensity in native and introduced populations. Similarly, comparable numbers of mature parasites in native and introduced hosts might suggest equal compatibility for a native parasite in both hosts, but field observations of parasite maturity cannot take into account differing maturity rates between native and introduced hosts.

Recognising the potential limitations of field observations, some researchers have used experimental infection trials to allow differences in parasite fitness between hosts to be verified under standard conditions (King, van Oosterhout & Cable 2009, Thieltges *et al.* 2009). However, even this approach fails to account for the dynamics of the whole host-parasite system in terms of relative abundances of host populations, infection encounter rates by intermediate hosts, and potential host behavioural changes caused by the presence of an introduced species. For example, hosts that occur at high densities are more likely to influence parasite dynamics than less common hosts.

Here, an in-depth assessment of parasite fitness in native and introduced hosts in a system previously identified as one in which the acquisition of native parasites by an introduced species results in the dilution of parasites in native hosts is provided (Kelly *et al.* 2009a). A multiple-scale approach of field observations, experimental trials and dynamic population modelling was used to examine this complex system and identify mechanisms responsible for observed field patterns.

3.2 Materials and Methods

3.2.1 Study system

In New Zealand, the introduced brown trout (*Salmo trutta* L.) is a dominant freshwater fish considered responsible for the decline and local extinction of native fish species (Townsend 2003). Since its introduction as parasite-free eggs in 1867 (MacCrimmon & Marshall 1968), more than 10 native parasite species have been acquired by brown trout, including the generalist freshwater acanthocephalan *Acanthocephalus galaxii* Hine (Hine, Jones & Diggles 2000). In the Otago province of the South Island of New Zealand, this acanthocephalan has been reported from sympatric stream populations of native roundhead galaxias (*Galaxias anomalus* Stokell) and brown trout (Kelly *et al.* 2009a). The parasite matures in the intestine of the definitive fish host and releases eggs into the water column via fish faeces. Cystacanth stages develop in the intermediate amphipod host *Paracalliope fluviatilis* Thomson after egg ingestion, with the life cycle of *A. galaxii* completed following predation of infected amphipods by the definitive host (Hine 1977). Brown trout are a major predator of galaxiids (Crowl, Townsend & McIntosh 1992, Townsend 1996), and thus may acquire additional *A. galaxii* infections via post-cyclic transmission when roundhead galaxias are eaten. This additional transmission route has been reported in many acanthocephalan species, including native acanthocephalans transmitted from native galaxias to introduced trout in Argentina (Rauque, Semenas & Viozzi 2002), but has not been experimentally demonstrated for *A. galaxii*. Preliminary field observations suggest that *A. galaxii* attains equal or higher prevalence and infection intensity in brown trout (Kelly *et al.* 2009a), indicating the potential for modification of parasite-host dynamics by this introduced host.

3.2.2 Field surveys

Field surveys were conducted to determine the prevalence (the percentage of infected hosts) and infection intensity (the number of worms per host; Bush *et al.* 1997) of *A. galaxii* in its definitive and intermediate host species, and the relative densities of these hosts at three sites previously identified by Kelly *et al.* (2009a) as ‘sympatric’ for roundhead galaxias and brown trout infected with *A. galaxii*: Swin Burn Q1 (22°96’90’’E, 55°58’70’’N), Swin Burn Q2 (22°98’30’’E, 55°59’40’’N) and Old Hut Creek (22°94’00’’E, 55°68’73’’N), all in the upper

Taieri River catchment in Otago. Each site was visited on 4-5 occasions between June 2007 and August 2008 to monitor temporal changes in host and parasite populations. Fish densities at each site were assessed by single-pass electric fishing the entire width (2.5-3.0m) of the same reach (area 230-416 m^2) on each occasion. Random sub-samples of roundhead galaxias (n = 5-27) and brown trout (n = 1-16) selected from pooled fish collected at each site were euthanized, measured for fork length (FL) to the nearest 1 mm and preserved in 10% buffered formalin. In the laboratory, the alimentary canal from oesophagus to anus of each fish was removed and split longitudinally. The abundance of acanthocephalans was noted, and the length (μm) and sex of each worm were recorded. In addition, for female worms, developmental status (F1- immature with ovarian balls only, F2 – maturing eggs, F3 – fully mature eggs) and proportion of mature eggs in a sub-sample of 50 eggs were also recorded. The numbers of *P. fluviatilis* and prey fish present in fish stomachs were also enumerated, to assess the relative importance of cystacanth and post-cyclic infection pathways.

The prevalence of infection in the intermediate amphipod host *P. fluviatilis* was measured on each site visit from a 3-5 minute kick sample spread diagonally across the stream in an attempt to sample all microhabitats present (ie. edge/centre, pool/riffle and gravel/macrophyte habitats). Amphipods were preserved in 10% formalin. In the laboratory, all amphipods were removed from kick net samples, and up to 1000 individual amphipods from each sample were examined for the presence of cystacanths.

3.2.3 Infection experiment

An infection experiment was conducted to determine the fitness of *A. galaxii* worms in both definitive hosts, from which relative rates of parasite establishment, maturation and mortality could be estimated. Brown trout (131-191 mm) and roundhead galaxias (60-75 mm) were collected by electric fishing from the Cap Burn (22°95’56’’E, 55°46’22’’N) and a tributary of the Kye Burn (22°93’23’’E, 55°63’67’’N), and acclimatised in the University of Otago’s controlled climate facilities (13 hour day/11 hour night period, 10°C, 15% daily water change, fed *ad libitum* with commercial pellets) for six months prior to experimentation. All experimental fish were treated with the anthelmintic Tetramisole HCl (10mg L^{-1} 48 hours x 2 repeat) at the beginning of the acclimatisation period to remove any naturally acquired parasites prior to artificial infection. Infection status prior to the experiment was investigated by autopsy of five

treated and five untreated fish per species, during which no intestinal parasites were found in treated or un-treated fish. Six experimental fish of each species were randomly assigned to two infection time-periods (2 or 6 weeks). During the 48 hours prior to experimental infection, each fish was measured (FL) and placed without food into an individual tank (tank size: 15 L – roundhead galaxias, 30 L – brown trout).

Intermediate infective stages of *A. galaxii* were obtained by collecting naturally infected *P. fluviatilis* amphipods by handnet from the Swin Burn (22°98'30"E, 55°59'40"N). Infection status was determined under a dissection microscope. A sub-sample of 15 amphipods identified as infected were autopsied to assess observer error in correctly identifying the presence of *A. galaxii*, with single infections of *A. galaxii* correctly identified in all amphipods. Ten live infected *A. galaxii* amphipods were placed into each tank. The number of amphipods consumed by each fish was monitored by checking for uneaten individuals 24 and 48 hours post-exposure. All unconsumed amphipods were removed at 48 hours post-exposure, and fish resumed their diet of *ad libitum* commercial fish pellets. At the completion of each infection period (2 or 6 weeks), fish were euthanized and immediately examined for parasites prior to fixing all acanthocephalans in 10% buffered formalin. Worm measurements were made as in the field survey.

3.2.4 Statistical analyses

Statistical analyses of field survey and experimental infection data were conducted using SPSS Statistic 15.0 (SPSS 2006). Seperate General Linear Models (GLMs) were used to test for the influence of species, sex and/or season on prevalence, infection intensity and worm length from the field survey. Infection intensity data was natural log transformed to meet the normality assumptions of analysis of variance (ANOVA). Seperate one-way ANOVA were used to test for differences in establishment and length of male and female worms in galaxias and trout at 2 weeks post-infection.

3.2.5 Population model

A population model was constructed using Model Maker 4.0 (Cherwell 2000) to dynamically simulate the influence of brown trout presence on *A. galaxii* in its definitive hosts.

3.2.5.1 Model formulation

The life cycle of *A. galaxii* was modelled using a series of linked differential equations that simulate (on a daily basis, scaled to 1 m^2) the populations of uninfected (A_{NP}) and infected (A_P) amphipods, the populations of immature and mature *A. galaxii* worms in roundhead galaxias (I_G, W_G) and trout (I_T, W_T), and the number of *A. galaxii* eggs in the environment (E), in discrete time-steps:

$$\frac{dA_{NP}}{dt} = \left(\lambda_A S + \sigma e^{-x(A_{NP}+A_P)} - 1 - C_T T - C_G G - vE\right)A_{NP} \quad \text{Equation 1a}$$

$$\frac{dA_P}{dt} = vEA_{NP} + \left(\left(\sigma e^{-x(A_{NP}+A_P)} - 1\right)X + (C_T T + C_G G)Y\right)A_P \quad \text{Equation 1b}$$

$$\frac{dI_G}{dt} = C_G G Y \gamma A_P - (m_G + \alpha_G + b_G + DT)I_G \quad \text{Equation 2a}$$

$$\frac{dI_T}{dt} = \gamma T(C_T Y A_P + D I_G) - (m_T + \alpha_T + b_T)I_T \quad \text{Equation 2b}$$

$$\frac{dW_G}{dt} = m_G I_G - (\alpha_G + b_G + DT)W_G \quad \text{Equation 3a}$$

$$\frac{dW_T}{dt} = m_T I_T + \gamma DT W_G - (\alpha_T + b_T)W_T \quad \text{Equation 3b}$$

$$\frac{dE}{dt} = (W_G + W_T)\lambda_W - (\varepsilon + vA_{NP})E \quad \text{Equation 4}$$

The amphipod population was dynamically modelled to reflect changing amphipod densities throughout the year while fish densities were held constant (T and G for trout and galaxias respectively), because field surveys indicate the latter remain relatively constant throughout the year and there is no evidence of parasite impact on fitness (Kelly *et al.* 2009a).

The **population of uninfected amphipods A_{NP}** (**Equation 1a**) increases as a function of amphipod fecundity (λ_A) (toggled by S, a logic switch denoting the breeding season), and decreases as a function of natural mortality (σ-1, where σ is survival) influenced by density dependence (x), predation by trout (C_T) and galaxias (C_G), and infection (ν).

The **population of infected amphipods A_P** (**Equation 1b**) increases as a function of infection (v), and is modified by a combination of natural mortality (σ-1) modified by density dependence (x) and infection (X), and the predation of infected amphipods by trout (C_T) and galaxias (C_G), also modified by infection (Y). The parameter Y acknowledges that the presence of

the parasite can be expected to increase the likelihood that an amphipod will be eaten. If the rate at which uninfected amphipods ingest acanthocephalan eggs exceeds the number of uninfected amphipods, all amphipods are considered to be infected.

The **population of immature worms in galaxias** I_G (**Equation 2a**) increases with the predation of infected amphipods ($C_G Y$), modified by establishment success (γ). The number of immature worms decreases with worm maturation (m_G), parasite mortality (α_G), natural host mortality (b_G) and post-cyclic transmission of worms from galaxias to trout (D). The **population of immature worms in trout** I_T (**Equation 2b**) increases with the predation of infected amphipods ($C_T Y$) and post-cyclic transmission (D), both modified by establishment success (γ), and decreases with worm maturation (m_T), worm mortality (α_T) and natural host mortality (b_T).

The **population of mature worms in galaxias** W_G (**Equation 3a**) increases as a function of worm maturation (m_G) and decreases with parasite mortality (α_G), natural host mortality (b_G) and post-cyclic transmission (D). The **population of mature worms in trout** W_T (**Equation 3b)** increases as a function of worm maturation (m_T) and the establishment (γ) of post-cyclically transmitted worms from galaxias to trout (D), and decreases due to the effects of parasite mortality (α_T) and natural host mortality (b_T). A density dependence parameter influencing worm survival was not incorporated into immature or mature worm equations, as very low field infection intensities of *A. galaxii* were observed in the species modelled in contrast to burdens recorded from other galaxias hosts in New Zealand (cf. *Galaxias maculatus* mean infection intensity 127 per fish (Hine 1977).

The **number of** *A. galaxii* **eggs in the environment** E (**Equation 4**) increases as a function of the number of eggs produced (λ_W) from mature worms in both galaxias (W_G) and trout (W_T), and decreases with natural mortality (ε) and the ingestion of eggs by amphipods (v).

3.2.5.2 Model parameterisation

Parameter estimates were obtained, where possible, from the field surveys and experimental infections reported here, and from the published literature and unpublished data (see Tables 3.1 & 3.2). Host densities (G, T) were calculated as the average number of fish m^{-2} at Swin Burn Q1 and Q2 combined (Old Hut Creek was not included in the parameterization of the model as only a single *A. galaxii* infection was recorded there). The daily mortality rate of brown

trout was based on a maximum lifespan of 5-6 years for non-migratory brown trout (Huryn 1996), whereas the mortality rate of roundhead galaxias was estimated from a closely related species, *Galaxias paucispondylus* (Bonnett 1990).

Mean amphipod density was estimated as 250 amphipods m^{-2} in North Otago streams (Weller 2003), with natural survival estimated from the maximum lifespan of 1 year (F. Wilhelm, unpublished data). The daily fecundity rate of 0.058 new individuals per amphipod during the breeding season (September to March, austral spring to end of austral summer) was estimated from a maximum of 3.5 broods of 5 eggs per female and a 0.5 sex ratio (F. Wilhelm, unpublished data). However, since a proportion of females are known to breed year round (Towns 1981), breeding from April to August (austral autumn and winter) was set at 20% of the maximum.

The probability of amphipods being consumed by each definitive host was calculated as the average number of amphipods in the host gut (with an assumed gut clearance time of 48 hours; (Tekinay, Guner & Davies 2003) relative to amphipod density (field survey). The potential for post-cyclic transmission of parasites from galaxias to trout was calculated from the average number of roundhead galaxias present in the stomachs of brown trout in the field surveys.

Although acanthocephalans have been shown to markedly alter intermediate host behaviour (Baldauf *et al.* 2007), often increasing the likelihood that infected individuals will be eaten (Moore 1984, Lagrue *et al.* 2007), no information is available about how *A. galaxii* alters the risk of being eaten (Y) or mortality (X) of its amphipod host. Likewise, no information is available about amphipod density dependence (x) or parasite egg ingestion rate by amphipods (v). These parameters were estimated by model optimisation to the values that result in predicted levels of prevalence and infection intensity, in both intermediate and definitive hosts, equivalent to those observed in the field (a mean population size of 250 m^{-2} for uninfected amphipods, a mean prevalence of infected amphipods of 0.1%, and a mean worm population size of 0.1 m^{-2} at equilibrium dynamics; Table 3.2). Once an amphipod becomes infected with a single *A. galaxii*, it is assumed that additional eggs are not consumed. This assumption does not affect model dynamics as eggs are highly abundant in the environment, and prevalence of amphipod infection is low.

The establishment, maturation and mortality rates of *A. galaxii* in each definitive host were estimated from the infection experiment. Parasite establishment at day one and the daily mortality rate of *A. galaxii* in galaxias were estimated from the slope of the line calculated from

the percentage of worms present at 2 and 6 weeks post-infection. No worms were present in trout at 6 weeks post-infection, so parasite establishment rate in trout was assumed to be the same as in galaxias, while parasite mortality rate was estimated from the decline to zero parasites at six weeks. No mature worms were present in trout from either the experimental infection or field survey; thus the maturity rate in trout was set at zero.

The daily rate of parasite egg production was estimated from the acanthocephalan, *Polymorphus minutus* (1700 eggs per female per day; (Crompton & Whitfield 1968), divided by the overall parasite male:female ratio observed in the current study. Egg mortality was based on aquatic acanthocephalan eggs being known to survive a maximum of 6-9 months (Crompton 1970).

3.2.5.2 Model simulation

Although brown trout are known to have altered freshwater fish communities in New Zealand, resulting in the declining abundance and localised extinction of some *Galaxias* species (Townsend 2003), their influence on native host-parasite dynamics is largely unknown. Here, I use model simulations to examine the influence of brown trout on native parasite dynamics in roundhead galaxias by investigating varying ratios of fish community composition that represent (i) fish communities prior to trout introduction (galaxias scenario), (ii) trout presence in native communities (galaxias + trout scenario), and (iii) localised extinction of native fish (trout scenario). To understand how the presence of the exotic host might be influencing observed levels of infection in the native host, I use sensitivity analyses to test the validity of potential contrasting hypotheses in the galaxias + trout scenario: (i) brown trout outcompete roundhead galaxias for infected amphipods (varying C_T), (ii) brown trout prey on roundhead galaxias (varying D), and (iii) brown trout modify the behaviour of roundhead galaxias, resulting in altered consumption of infected amphipods (varying C_G).

Simulations were run varying the above parameters ± 100% around their estimated value in 10% increments, to investigate the influence of each parameter on the infection intensity of *A. galaxii* in roundhead galaxias. For each scenario, I monitored the peak density of *A. galaxii* in the roundhead galaxias population (m^{-2}) after 3000 daily iterations.

3.3 Results

3.3.1 Field survey

Roundhead galaxias was the most abundant definitive host in surveyed streams, with average densities of 0.05-0.20 fish m^{-2} for roundhead galaxias and 0.005-0.07 fish m^{-2} for brown trout. A total of 219 of 537 roundhead galaxias (39-91 mm), and 84 of 126 brown trout (63-189 mm) were examined for *A. galaxii*. At Old Hut Creek, 72 galaxias and 34 trout were examined but only a single *A. galaxii* infection was recovered from one roundhead galaxias on a single occasion (in June 2007). This site was removed from further analysis of field survey observations.

Infection prevalence did not differ significantly between sites or host species (GLM; $P > 0.05$), with the percentage of fish infected ranging from 12.5-85% for roundhead galaxias and 0-100% for brown trout (Table 3.3). Infection intensities were highest in brown trout (natural log transformed: General Linear Model (GLM) species; $F_{1,93} = 4.977$, $P = 0.028$) but did not differ between sites or seasons. Acanthocephalans were larger in roundhead galaxias than brown trout (GLM species; $F_{1,220} = 24.803$, $P < 0.001$), with female worms larger than male worms (GLM sex; $F_{1,220} = 5.965$, $P = 0.015$). All female worms recovered from brown trout were immature with ovarian balls present only, while on average 18.6% of female worms in roundhead galaxias contained developing eggs (F2) or fully mature shelled eggs (F3) (Figure 3.1).

Examination of stomach contents showed that 71% of brown trout stomachs contained amphipods, with an average of 14.9 amphipods per fish, while 17% of roundhead galaxias stomachs contained amphipods, with an average of two amphipods per fish. Fourteen percent of brown trout stomachs also contained roundhead galaxias, with between one and three galaxias present. Prevalence of infection in amphipods collected from kick samples was very low at all sites, with a maximum of 0.1% of amphipods infected.

3.3.2 Infection experiment

Similar percentages of worms had established in each host species at two weeks post-infection, with an average of 38.6% of worms establishing in roundhead galaxias and 28.0% in brown trout (one-way ANOVA species; $P = 0.465$; Figure 3.2). The intensity of infection

declined in both species between two and six weeks post-infection, with 19.2% of worms remaining in galaxias and no worms in trout (Figure 3.2).

Male *A. galaxii* were larger in brown trout than in roundhead galaxias at two weeks post-infection (one-way ANOVA male; $F_{1,14} = 5.948$, $P = 0.030$), while female worms were of similar length ($P = 0.136$, Table 3.4). Worm size increased between two and six weeks post-infection, with average male and female lengths in galaxias at six weeks post-infection greater than in trout at two weeks post-infection. No gravid female worms were present in either host species at two weeks post-infection, while one of four female worms present in galaxias at six weeks post-infection was gravid.

3.3.3 Population model

Model simulations of varying fish community composition indicated that a minimum proportion of 70% galaxias in the total fish community is the threshold for the survival of *A. galaxii*, below which the native parasite rapidly goes extinct (Figure 3.3). At the current field densities of the total fish community (0.088 individuals m^{-2}), densities of *A. galaxii* infection in galaxias are predicted to be only slightly lower than would be expected if trout were absent from the system, as the ability of trout to act as an infection sink is weakened by the low relative density of this introduced host.

Changing the rate of predation by brown trout on either amphipods or roundhead galaxias had little influence on the density of *A. galaxii* in the modelled roundhead galaxias population (Figures 3.4a and 3.4c). In contrast, *A. galaxii* densities in roundhead galaxias were highly sensitive to changes in the rate of predation on amphipods by this fish (Figure 3.4b), with predation rates below 0.004 amphipod^{-1} host^{-1} day^{-1} (-20%) resulting in extinction of the parasite.

3.4 Discussion

We used a combination of field observations, experimental infections and dynamic population modelling to examine the influence of an introduced species on a native host-parasite system. The field observations indicated that although the prevalence and infection intensity of *A. galaxii* were similar in introduced trout and native galaxias, the native parasite attained smaller sizes and failed to mature in the introduced host. Experimental infection results showed trout to

be similar to galaxias in its competence as a host in terms of parasite establishment and initial growth, but trout was found to be unsuitable for maintaining *A. galaxii* populations, as worms did not reach maturity. Hence, the field and experimental results suggested that trout were likely to act as an infection sink due to poor host competency. However, the population model indicated that the influence of this mechanism on the dynamics of *A. galaxii* in its native host would be limited due to only a small proportion of the total fish community being comprised of trout. In contrast, the model suggested that trout presence may have more substantial consequences for the native parasite population by altering amphipod consumption by roundhead galaxias, with such consequences being greater at higher trout densities.

3.4.1 Field observations

Field observations are often the first source of information used to determine the influence of introduced species on host/parasite dynamics. For example, Telfer *et al.* (2005) used field surveys to determine the influence of the introduced bank vole (*Myodes glareolus*) on *Bartonella* sp. infections in native wood mice (*Apodemus sylvaticus*), concluding that infection prevalence in wood mice decreased with increasing bank vole density. Moreover, Rauque *et al.* (2003) used the results of field surveys to demonstrate that parasites in the introduced rainbow trout (*Oncorhynchus mykiss*) contributed 20% of total egg output by the native acanthocephalan parasite *Acanthocephalus tumescens*. Field observations may provide useful insights into the potential impacts of introduced species on native parasite-host dynamics. However, as demonstrated by this study, assessment of parasite maturation is needed, in addition to observations of infection prevalence and intensity, to determine the impact of an introduced species.

3.4.2 Infection experiment

Experimental infections generally supported the field based conclusions that trout are inferior hosts of *A. galaxii*, yet interestingly *A. galaxii* initially experienced similar establishment success, and equivalent or greater growth in this host. Although worms in galaxias were relatively slower growing, in the long term they attained larger sizes than in trout. Most

importantly, *A. galaxii* worms failed to reach maturity in brown trout; thus, the introduced host was unsuccessful at supporting this stage of the parasite's life cycle.

3.4.3 Population model

The population model revealed that the density of *A. galaxii* in roundhead galaxias may only be slightly reduced as a result of parasite dilution by trout. In sensitivity analyses, modifying either the rate at which trout feed on roundhead galaxias or on amphipods caused little change to the native parasite's density in its native roundhead galaxias host. However, decreasing the rate of amphipod predation by roundhead galaxias markedly decreased the parasite's adult population. The model also revealed that parasite burdens were highly sensitive to relative host abundances. Hence, a correlation between increasing trout presence and decreasing native parasitism of roundhead galaxias, as observed for the parasite *Deretrema philippae* by Kelly *et al.* (2009a), may just as likely be caused by trout reducing galaxias density or altering their foraging behaviour, rather than trout serving as an infection sink. This conclusion is supported both by demonstrations that trout predate galaxias (Crowl, Townsend & McIntosh 1992, Townsend 2003) and experimental studies of habitat use by native galaxiids in the presence of brown trout (McIntosh, Townsend & Crowl 1992), which showed that trout outcompete *Galaxias* spp. in streams, forcing them to forage in areas with lower invertebrate drift.

Native parasite loss through dilution or via other mechanisms, such as altered predation on intermediate hosts by native species, is a largely unrecognised consequence of the invasion of introduced species. Most commonly, invasion biology research has focused on the impacts of introduced species on native conspecifics via predation and/or competition, and through the introduction of novel parasites. However, since native parasites play major roles in the structuring of native communities, neglecting the potential for native parasite loss subsequent to the arrival of exotic species overlooks a potentially important consequence of invasions.

3.4.4 Limitations and strengths of dynamic population models

We acknowledge that the strength of population simulation models in depicting 'real world' dynamics is only as good as the data used to parameterise them. In the present study, I

utilised field information from multiple sites and seasons, experimental infections and published literature to generate the majority of parameter estimates. However, I was unable to draw on these sources to estimate the amphipod's mortality and predation risk, although the dynamic model suggested that *A. galaxii* infections are strongly influenced by both parameters. Manipulation of intermediate host behaviour to enhance transmission success via predation is a common strategy of parasites that utilise hosts at different trophic levels, and is frequently adopted by acanthocephalans (Moore 1984, Poulin 1995). Acanthocephalan infections in amphipods have also been reported to alter host immune responses to bacterial infections, potentially increasing intermediate host mortality (Cornet *et al.* 2009). Experimental studies are required to fully quantify the mechanism and degree to which *A. galaxii* infection alters the mortality and predation risk of amphipods, and to identify whether behavioural responses of infected amphipods to predator presence differ between native and exotic fish.

Table 3.1. Definitive host parameter definitions and estimates used in the population model.

Parameter	Galaxias		Trout		Units	Source
	Symbol	Value	Symbol	Value		
Density	G	0.0763	T	0.0117	m^{-2}	This study
Predation of amphipods	C_G	0.0004	C_T	2e-05	$amphipod^{-1}\ host^{-1}\ day^{-1}$	This study
Parasite establishment	γ	0.5078	γ	0.5078	$worm^{-1}$	This study
Parasite mortality	a_G	0.0186	a_T	0.0416	$worm^{-1}\ day^{-1}$	This study
Parasite maturation	m_G	0.006	m_T	0.000	$worm^{-1}\ day^{-1}$	This study
Natural host mortality	b_G	0.0007	b_T	0.0004	$host^{-1}\ day^{-1}$	Bonnett (1990), Huryn (1996)
Predation of galaxias	-	-	D	0.01	$galaxias^{-1}\ trout^{-1}\ day^{-1}$	This study

Table 3.2. Intermediate host and parasite parameter definitions and estimates used in the population model.

Parameter	Symbol	Value	Units	Source
Amphipod breeding rate	λ_A	0.058	amphipod^{-1} day^{-1}	F. Wilhelm, unpublished data
Amphipod natural mortality	σ	0.0027	amphipod^{-1} day^{-1}	F. Wilhelm, unpublished data
Parasite fecundity	λ_W	807.5	worm^{-1} day^{-1}	Crompton & Whitfield (1968)
Ingestion rate of eggs by amphipods	N	6e-06	egg^{-1} amphipod^{-1} day^{-1}	This study
Egg mortality	E	0.005	egg^{-1} day^{-1}	Crompton (1970)
Amphipod density dependence	x	0.0001	amphipod^{-1} day^{-1}	Model optimization
Parasite influence on amphipod mortality	X	26.68	amphipod^{-1} day^{-1}	Model optimization
Parasite influence on amphipod predation	Y	40.36	amphipod^{-1} day^{-1}	Model optimization

Table 3.3. Seasonal prevalence, infection intensity and length *A. galaxii* in naturally infected roundhead galaxias and brown trout from sites Q1 and Q2 on the Swin Burn.

Site	Host Species	Season	No. of hosts	Prevalence (%)	Infection intensity (mean ± SD)	Male length μm (mean ± SD)	Female length μm (mean ± SD)	No.parasites
Q1	Roundhead galaxias	Jun 07	10	40	2.8 ± 1.5	1725 ± 527	4800 ± 566	11
		Oct 07	27	40.7	6.6 ± 6.3	2376 ± 1023	3002 ± 1844	72
		Mar 08	16	12.5	5	2600 ± 650	4350 ± 1222	10
		May 08	20	45	16.6 ± 19.7	1699 ± 563	2447 ± 963	149
		Aug 08	19	52.6	11.2 ± 16	2057 ± 393	3403 ± 1221	112
	Brown trout	Jun 07	0	-	-	-	-	-
		Oct 07	6	83.3	12.6 ± 14.6	1210 ± 323	1141 ± 266	63
		Mar 08	3	100	13.7 ± 16.8	1039 ± 239	1162 ± 232	41
		May 08	1	0	-	-	-	-
		Aug 08	0	-	-	-	-	-
Q2	Roundhead galaxias	Oct 07	20	80	11.2 ± 22.4	1851 ± 713	2520 ± 1374	179
		Mar 08	10	80	1.5 ± 0.5	2346 ± 940	2720 ± 952	12
		May 08	20	85	4.1 ± 3.8	2108 ± 716	3349 ± 1665	65
		Aug 08	5	60	5.7 ± 4.7	1642 ± 391	3255 ± 900	17
	Brown trout	Oct 07	14	85.7	10.8 ± 12.1	943 ± 193	967 ± 180	129
		Mar 08	4	75	6.3 ± 3.8	1378 ± 370	1295 ± 341	19
		May 08	2	50	27	1254 ± 192	1405 ± 208	27
		Aug 08	2	100	10	1478 ± 311	1475 ± 293	20

Table 3.4. Prevalence, infection intensity, length and reproductive status *A. galaxii* in experimentally infected roundhead galaxias and brown trout. Gravid females represent females with maturing eggs (F2) and fully mature shelled eggs (F3).

Host	Week	No. of hosts	Prevalence %	Infection intensity (mean ± *SD*)	Parasite sex	Length µm (mean ± *SD*)	No. of parasites	Gravid females
Roundhead galaxias	2	6	100	3.33 ± 2.5	M	1806.25 ± 84.1	8	0
					F1	2097.92 ± 108.8	12	
	6	6	50	2.33 ± 1.5	M	2191 ± 141.7	3	25
					F1	2783.33 ± 65.1	3	
					F2	2425	1	
Brown trout	2	6	100	2.67 ± 1.9	M	2128 ± 103.7	7	0
					F1	2338.89 ± 103.8	9	
	6	6	0	0	-	-	-	-

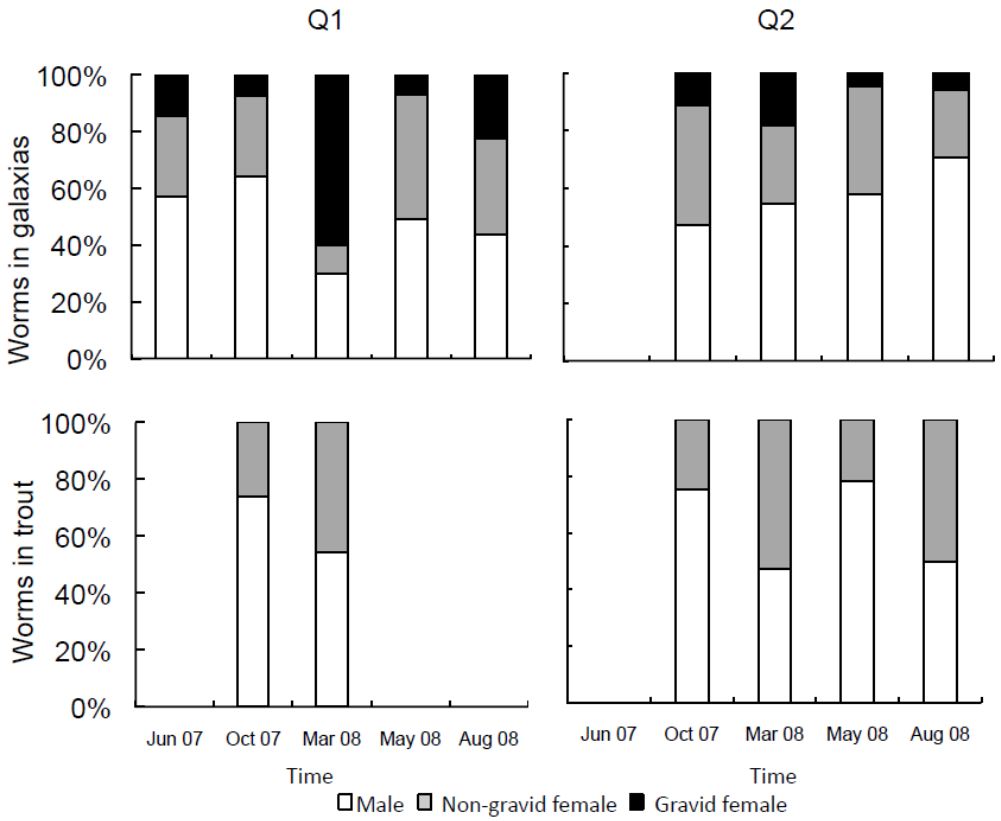

Figure 3.1. Seasonal composition of *A. galaxii* sex and reproductive status in roundhead galaxias and brown trout at sites Q1 and Q2 on the Swin Burn. Gravid female worms with maturing eggs (F2) or fully mature shelled eggs (F3) were absent from brown trout throughout the study period.

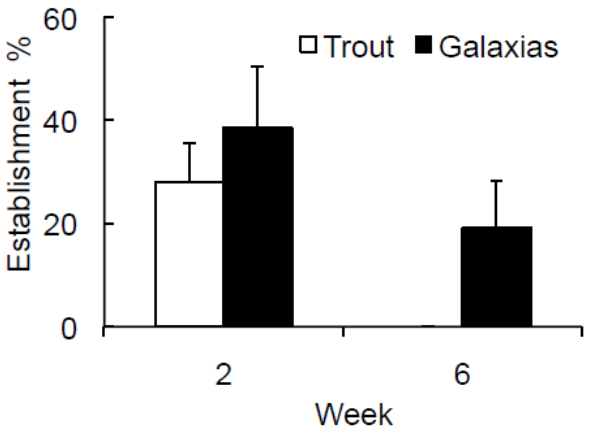

Figure 3.2. Establishment (2 weeks) and survival (6 weeks) of *A. galaxii* in experimentally infected roundhead galaxias and brown trout. Establishment success did not differ between hosts ($P > 0.05$).

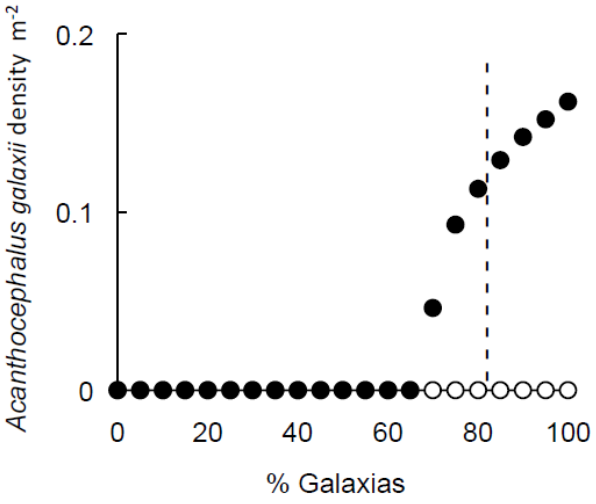

Figure 3.3. Density of *A. galaxii* infections in roundhead galaxias (filled circle) and brown trout (open circle) in relation to percentage of galaxias in the combined total fish population (0.088 fish m^{-2}) predicted from model simulations. The dashed line represents the current percentage of galaxias in the total fish population as recorded in the field survey.

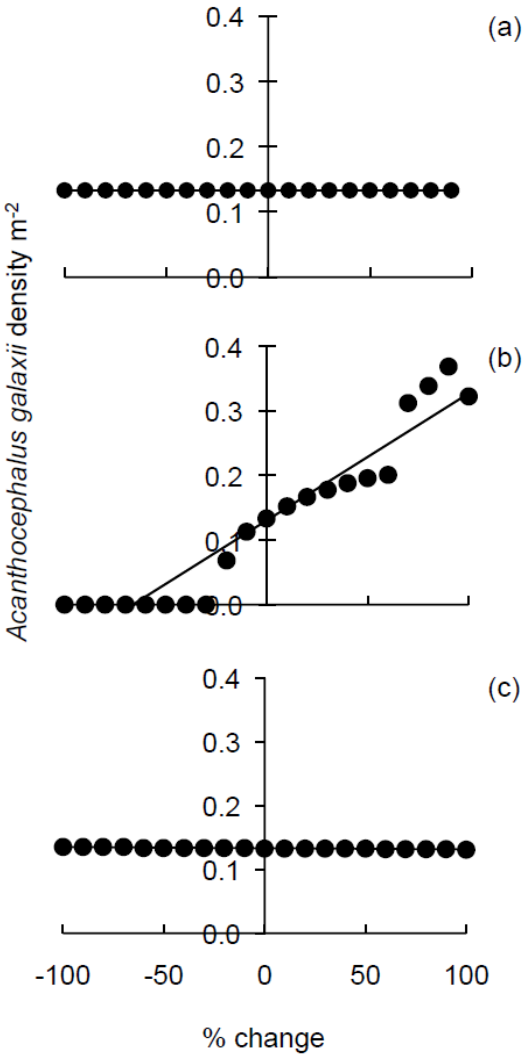

Figure 3.4. Simulating the effect on the density of *A. galaxii* infections in roundhead galaxias of altering (a) trout predation on amphipods C_T, (b) galaxias predation on amphipods C_G and (c) trout predation on galaxias (D). Each estimate was modified ± 100% of current estimates in 10% increments.

CHAPTER 4

High exotic host competency for native infection does not guarantee parasite spillback: a case study from salmonids introduced to Argentina

Lake Moreno, Bariloche, Argentina

4.1 Introduction

Whether through intentional release or accidental introduction, the deleterious effects of exotic species on their native counterparts are evident (Sato *et al.* 2010), with many reviews predicting such impacts will be a major driver of future biodiversity loss (e.g. Sala *et al.* 2000, Kolar & Lodge 2001, Jenkins 2003). The mechanisms underlying the effects of exotic species have been a popular research focus for decades, with predation, competition and introduced diseases frequently cited (e.g. Miller 1989, Tompkins, White & Boots 2003, Wiles *et al.* 2003). In the freshwater fish literature, for example, exotic salmonids are widely acknowledged to cause local declines and extinctions of native fish populations via predation and resource competition (Townsend 2003, Macchi, Pascual & Vigliano 2007, Arismendi *et al.* 2009), although impacts mediated by disease have largely been discounted. This is because most salmonid introductions have occurred as parasite-free eggs (Clements 1988, Kennedy & Bush 1994, Poulin & Mouillot 2003), limiting the likelihood of introducing novel diseases that could spill over into native populations. However, the parasite-free status of introduced salmonids does not preclude them from influencing native fish populations via disease. Exotic salmonids have been shown to acquire native parasites subsequent to their introduction (Dix 1968, Ortubay *et al.* 1994, Poulin & Mouillot 2003). If exotic salmonids are competent hosts for such parasites, they may increase disease burdens in native hosts by acting as infection reservoirs from which parasites "spill back" (Daszak, Cunningham & Hyatt 2000, Tompkins & Poulin 2006, Kelly *et al.* 2009b).

There is good evidence that such impacts are occurring in Argentinean freshwater lakes into which rainbow trout, *Oncorhynchus mykiss,* have been introduced. Thus, a population study of the native acanthocephalan *Acanthocephalus tumescens* in Lake Moreno indicated that adults of this parasite in the exotic trout may contribute approximately 20% of acanthocephalan egg outputs, altering native-host parasite dynamics (Rauque, Viozzi & Semenas 2003). In addition, Revenga *et al*. (2005) have shown that with increasing proximity to an *O. mykiss* fish farm, native *Galaxias maculatus* in the lake experience increased *A. tumescens* infection prevalence and intensity.

Acanthocephalus tumescens is a freshwater acanthocephalan that matures in the intestine of a definitive fish host and releases eggs into the water with fish faeces. Larval cystacanth stages develop in the amphipod intermediate host, *Hyalella patagonica*, following egg ingestion, with the lifecycle of *A. tumescens* completed following amphipod predation by the definitive host fish.

Fish may also acquire additional *A. tumescens* infections via post-cyclic transmission, as the small native host fish *G. maculatus* is consumed by the majority of *A. tumescens* definitive hosts, in addition to being a major diet component of some exotic salmonids including *O. mykiss* (Rauque, Semenas & Viozzi 2002, Rauque, Viozzi & Semenas 2003, Macchi, Pascual & Vigliano 2007). This transmission pathway, whereby adult worms in definitive hosts are transmitted to other hosts via predation (Nickol 1985, Kennedy 1999), has been previously documented for *A. tumescens* in *O. mykiss* (Rauque, Semenas & Viozzi 2002), though the relative success of the transmission pathway has not been compared between native and exotic fish hosts.

Here I investigate whether parasite spillback is indeed occurring in the Lake Moreno system, through a combination of experimental infections and dynamic population modelling. First, I assessed *A. tumescens* fitness in native and exotic fish through both transmission pathways – larval cystacanth infections from amphipods, and post-cyclic infections from *G. maculatus*. Second, I examined the extent to which *A. tumescens* acquisition by exotic salmonids is predicted to alter native host-parasite dynamics. Finally, I identified the mechanisms likely to be responsible for observed patterns and discuss their implications for native host populations.

4.2 Materials and Methods

4.2.1 Study site

Lake Moreno (41°05'S, 71°32'W) is a small lake (10.6 km^2) situated in Nahuel Huapi National Park, Rio Negro Province, Argentina, whose fish community is comprised of five native (*Diplomystes viedmensis, G. maculatus, Galaxias platei, Odontesthes hatcheri, Percichthys trucha*) and three exotic (*O. mykiss, Salmo trutta, Salvelinus fontinalis*) hosts of *A. tumescens* (Table 4.1). *Galaxias maculatus* is the most abundant native host, while *O. mykiss* is the most abundant exotic host, comprising approximately 99.99 and 0.005% of the total fish community, respectively (Vigliano *et al.* 2008). Although Rauque *et al*. (2003) detected no *A. tumescens* infection in the native *O. hatcheri* (n = 19) or in the exotic *S. trutta* (n = 1) in Lake Moreno, field observations from other localities show that both species are hosts to *A. tumescens* (Semenas & Trejo 1997). An *O. mykiss* cage farm in Lake Moreno produces approximately 20-30,000 fish/year (M. Beveraggi, unpublished data), with the possibility of some farmed fish escaping into the wild population.

4.2.2 Infection Experiments

4.2.2.1 Study organisms

Two native hosts, *G. platei* and *P. trucha*, in addition to exotic *O. mykiss*, were selected for experimental infection. These species were assumed to be significant contributors to the *A. tumescens* population based on their relative densities, infection flow rates and parasite egg outputs quantified from field surveys (Rauque, Viozzi & Semenas 2003, Vigliano *et al.* 2009).

Infection experiments were carried out at the Centro de Salmonicultura, Nacional Universidad del Comahue, (Bariloche, Rio Negro Province), which provided *O. mykiss* (mean fork length 189 mm) from commercially raised stock. *Galaxias maculatus* (mean fork length 59 mm) were collected from Bahía Verde, Lake Mascardi, Río Negro Province (41°17'S, 71°38'W) by seine-netting. Gill nets were used to collect *P. trucha* (mean fork length 358 mm) from Laguna Blanca, Neuquen Province (39°03'S, 70°22'W) and *G. platei* (mean fork length 153 mm) from Laguna Coyte, Chubut Province (42°25'S, 71°22'W). *Percichthys trucha* and *G. platei* were treated for fungal growth associated with capture (20g NaCl/L H_20 for 45 minutes x twice weekly x 3 weeks) and acclimatized in outdoor aquaria for seven weeks prior to the experiment. Fish were maintained *ad libitum* on different diets because a common food consumed by all fish species was not available: *O. mykiss* and *P. trucha* - commercial fish pellets, *G. maculatus* and *G. platei* - *Tubifex* worms.

During acclimatisation, experimental fish were treated with an anthelmintic (Praziquantel 10mg/L x 3hrs in a water bath). Anthelmintic efficacy was assessed by comparing parasite infections in sub-sets of five treated and five un-treated fish per species. Ten *G. maculatus* collected from Lake Mascardi were substituted for *G. platei* to test anthelmintic efficacy, as few *G. platei* were available for experimentation. Previous studies recorded moderate prevalence (59-75%) and infection intensities (mean 7-14 worms/fish) in *G. maculatus* at this site (Rauque, Semenas & Viozzi 2002), so they provided a suitable substitute to test anthelmintic efficacy. Treated and untreated fish were autopsied 48 hours post-anthelmintic exposure, with 80% of untreated *G. maculatus* containing *A. tumescens* (infection intensity 4.25 worms/fish), while no intestinal parasites were recovered from treated fish or untreated *O. mykiss* and *P. trucha*.

Hyalella patagonica amphipods, naturally infected with *A. tumescens*, were collected by sweep net from Bahía Verde, Lake Mascardi. Acanthocephalan infection in amphipods was ascertained under a dissecting microscope.

4.2.2.2 Cystacanth Infection

A larval infection experiment was conducted to determine the fitness of *A. tumescens* transmitted from cystacanths in infected amphipods to native (*P. trucha*, *G. platei*) and exotic (*O. mykiss*) hosts, from which relative rates of larval establishment, growth, fecundity and survival could be estimated. Six fish per species were randomly assigned to individual tanks and maintained without food for 48 hours prior to experimental infection. Ten cystacanths were removed from infected amphipods under a dissecting microscope, and placed into a gelatine capsule (length 19.4 mm, diameter 6.9 mm). Using a pipette, each capsule was orally inserted into the stomach of an experimental fish previously anesthetised in a Benzocaine solution (100 ppm). Fish were placed in a tank of freshwater and observed for five minutes post-recovery to ensure regurgitation did not occur. Fish were then returned to their individual tanks and maintained for a further four weeks on their respective diets.

At the completion of the infection period, fish were euthanised with a sharp blow to the head and immediately examined for parasites by removing the alimentary canal from oesophagus to anus and splitting longitudinally. The abundance of acanthocephalans was noted prior to fixing worms in 2% formalin. Length (μm) and sex of each worm were recorded. In addition, developmental status (F1 - immature with ovarian balls only, F2 - maturing eggs, F3 - fully mature eggs) and the proportion of mature eggs in a sub-sample of 50 eggs were also recorded for female worms.

4.2.2.3 Post-cyclic Infection

A post-cyclic infection experiment was conducted to determine the fitness of post-cyclically transmitted *A. tumescens* from infected *G. maculatus* to native (*P. trucha, G. platei*) and exotic (*O. mykiss*) hosts, from which relative rates of post-cyclic establishment, growth and fecundity could be estimated. These experiments also examined whether post-cyclically

transmitted worms in predatory hosts achieved greater fitness than in their original definitive host, *G. maculatus*. Six *P. trucha* and six *O. mykiss* were randomly selected for post-cyclic experiments, while only five *G. platei* were used due to the limited availability of specimens. All fish were maintained as for cystacanth infections prior to experimental infection.

Naturally infected *G. maculatus* were captured one day prior to experimental infections and transported live to the laboratory. Forty live adult *A. tumescens* were carefully removed from the intestine of freshly euthanised *G. maculatus*, and placed into gelatine capsules. Capsules were administered to anesthetised fish, as for larval infections, with infected fish maintained in individual tanks for four weeks post-infection on their respective diets.

At Day 0 of the post-cyclic infection experiment, 60 randomly selected *G. maculatus* (source group) were autopsied to assess parasite fitness (abundance, length, female maturity). An additional 60 *G. maculatus* (control group) were maintained for 4 weeks on a diet of *Tubifex* worms, to allow *A. tumescens* growth, maturity and survival in *G. maculatus* to be compared with post-cyclically transmitted individuals. At the completion of the infection period, all fish were euthanised and immediately examined for parasites, with acanthocephalans preserved and measured as for cystacanth infections.

4.2.3 Statistical analyses

Statistical analyses of experimental infection data were conducted using SPSS Statistic 15.0 (SPSS 2006). With the exception of cystacanth infection establishment and male worm length data, analysis of variance (ANOVA) tests were used for all analyses, for which data were transformed when necessary to meet the normality assumptions of parametric tests. Cystacanth infection establishment and male worm length data could not be transformed to meet assumptions of normality; therefore, non-parametric tests were used to assess differences in establishment (Kruskal-Wallis test) and male length (Mann-Whitney U test) between hosts. Male and female worm lengths were only compared between *O. mykiss* and *P. trucha* from the cystacanth infection experiment, as too few worms were present in *G. platei* for analysis. Post-cyclically transmitted male and female worms in *P. trucha* and male worms in *G. platei* were also excluded from length analysis due to insufficient worms.

4.2.4 Population model

A population model was constructed using Model Maker 4.0 (Cherwell 2000) to dynamically simulate the influence of exotic salmonids on *A. tumescens* in its native definitive hosts.

4.2.4.1 Model formulation

The lifecycle of *A. tumescens* was modelled using a series of linked differential equations, which simulated (on a daily basis, scaled to $1m^{-2}$) the number of immature (I_{GM}) and mature (W_{GM}) worms in *G. maculatus*, the number of immature (I_i) and mature (W_i) *A. tumescens* worms in the i^{th} predatory definitive host, and the number of eggs in the environment (E), in discrete time-steps:

$$\frac{dI_{GM}}{dt} = C_{GM}AEN_{GM}\gamma_i{}^{-\sigma(I_{GM})} - (m_{GM} + \alpha_{GM} + b_{GM} + D_i)I_{GM} \quad \text{Equation 1a}$$

$$\frac{dW_{GM}}{dt} = m_{GM} - (\alpha_{GM} + b_{GM} + D_i)W_{GM} \quad \text{Equation 1b}$$

$$\frac{dI_i}{dt} = (C_iAE + D_i)N_i\gamma_i{}^{-\sigma(I_{GM})} - (m_i + \alpha_i + b_i)I_i \quad \text{Equation 2a}$$

$$\frac{dW_i}{dt} = \gamma D_i W_{GM} - (\alpha_T + b_T - m_T)W_i \quad \text{Equation 2b}$$

$$\frac{dE}{dt} = W_i\lambda_W - (\varepsilon + A)E \quad \text{Equation 3}$$

The **number of immature worms in *G. maculatus*** I_{GM} **(Equation 1a)** increases with the number of eggs in the environment (E) at a rate directly proportional to the consumption rate of amphipods (C_{GM}) by *G. maculatus* (N_{GM}), modified by a constant *(A)* that incorporates the egg ingestion rate by amphipods, the rate at which ingested eggs successfully develop to cystacanths in amphipods and the loss of cystacanths due to amphipod mortality. This is additionally modified by the rate at which cystacanths, acquired by the consumption of infected amphipods, successfully establish to become immature worms (γ_{GM}), and density dependence (σ) acting on worm survival. The number of immature worms is influenced by worm maturation (m_{GM}), post-cyclic transmission of worms from *G. maculatus* to predatory fish (D_i), and the natural mortality of both the parasite (α_{GM}) and its host (b_{GM}). The **number of mature worms in *G. maculatus*** W_{GM} **(Equation 1b)** increases as a function of worm maturation (m_{GM}) and is modified by post-

cyclic transmission of worms from *G. maculatus* to predatory fish (D_i), and the natural mortality of both the parasite (α_{GM}) and its host (b_{GM}).

The **number of immature worms in the *i*th predatory fish host** I_i **(Equation 2a)** increases with the number of eggs in the environment (E), as for immature worms in *G. maculatus*, in addition to post-cyclically transmitted worms from *G. maculatus* to predatory fish (D_i). The number of immature worms is influenced by worm maturation (m_i) and the natural mortalities of both the parasite (α_i) and its host (b_i). The **number of mature worms in the *i*th predatory fish host** W_i **(Equation 2b)** increases as a function of worm maturation (m_i) and is modified by the establishment (γ_i) of post-cyclically transmitted worms from *G. maculatus* to predatory fish (D_i), and the effects of parasite (α_i) and natural host mortality (b_i). Only the post-cyclic transmission of immature and mature *A. tumescens* from *G. maculatus* to predatory fish species was included in the model. Although other fish species are occasionally consumed by native and exotic host species, *G. maculatus* is the main prey species in Lake Moreno (Macchi *et al.* 1999).

The **number of** *A. tumescens* **eggs in the environment** E **(Equation 3)** increases as a function of the number of eggs produced (λ_W) from mature worms in fish hosts (W_{Gm} and W_i), and decreases with natural mortality (ε) and a constant (A).

4.2.4.2 Model parameterisation

Parameter estimates (daily values averaged over seasons) were obtained, where possible, from experimental infection trials (this study), the published literature and unpublished data (Tables 4.1 & 4.2). *Galaxias maculatus* density was estimated from galaxias production and survival averaged over seasons (Vigliano *et al.* 2009). Densities (N_i) for all other species were estimated from gill-netting and hydroacoustic surveys (Vigliano *et al.* 2009). Daily mortality rates for each fish species were estimated from maximum known life spans in Lake Moreno (*O. mykiss* 7 years, *P. trucha* 8 years, *G. maculatus* 6 years, *O. hatcheri* 5 years; Bello 1995, Milano 1996, Vigliano *et al.* 2009) or the surrounding catchment (S. trutta 7 years, G. platei 9 years; Milano 2003, Valiente *et al.* 2010), with the lifespan of *S. fontinalis* assumed to be equivalent to other exotic salmonids. The maximum lifespan of *D. viedmensis* was estimated from the mean age for North American Siluriformes (7.6 years; Winemiller & Rose 1992).

Daily predation rates on amphipods and *G. maculatus* by each fish species were calculated as the average number of each prey item in the stomach of each host species (P.J. Macchi, unpublished data; D. Milano, unpublished data), corrected for 48 hour gut clearance duration (Tekinay, Guner & Davies 2003).

Larval *A. tumescens* establishment rates in salmonids and galaxiids were estimated from experimental infections of *O. mykiss* and *G. platei*, respectively. Larval establishment in *D. viedmensis* and *O. hatcheri* was estimated from the mean establishment rate in experimentally infected native fish (*P. trucha* and *G. platei*). Parasite maturation in salmonids was estimated from *O. mykiss*, while maturation rates for native hosts were estimated from another acanthocephalan native to southern hemisphere galaxiids (*Acanthocephalus galaxii*; Paterson *et al*. in prep), as no *A. tumescens* had reached maturity in native fish by four weeks post-infection. Parasite mortality in all hosts of *A. tumescens* were also estimated from mortality rates in *A. galaxii* native hosts, as *A. tumescens* mortality rates could not be calculated since the presence of parasites was only assessed on one occasion post-infection. Post-cyclic establishment rates were estimated from experimental infections as per larval establishment rates, while the mortality of post-cyclically transmitted parasites was assumed to be equal to that of larval transmitted worms. Post-cyclic maturation rates were calculated from the difference between the proportion of mature female worms in *G. maculatus* on Day 0 and in each host on Day 28.

The daily rate of egg production of *A. tumescens* was unknown and was estimated from the acanthocephalan, *Polymorphus minutus* (1700 eggs per female per day; Crompton & Whitfield 1968), divided by the overall parasite male:female ratio of 0.41:0.59 (Rauque, Semenas & Viozzi 2006). Egg mortality was estimated from a maximum survival period of six to nine months for aquatic acanthocephalan eggs (Crompton 1970).

Amphipod populations were not dynamically modelled because the density of *H. patagonia* in Lake Moreno, or other Patagonian lakes, was unavailable from the literature. The rate of egg ingestion, the establishment and development of eggs to cystacanths in amphipods and the loss of cystacanths due to amphipod mortality were also unknown. A constant (A) was used to represent the infection maintained by the amphipod population in Lake Moreno. Additionally, the influence of density dependence on worm survival (σ) was unknown for *A. tumescens*, but experimental studies on other acanthocephalan species suggest a negative relationship between worm abundance and survival (e.g. Brown 1986). Both A and dW were thus estimated by model

optimisation to the values that resulted in predicted levels of prevalence and infection intensity in definitive hosts, equivalent to those observed in the field (a mean prevalence of 1.8%, and a mean worm population size of 1.55 m^{-2} in infected *G. maculatus* at equilibrium dynamics).

4.2.4.3 Model simulation

Model simulations were conducted to examine the influence of exotic salmonids on the dynamics of the native parasite *A. tumescens* in its native definitive hosts by investigating different fish community compositions representing (i) the fish community prior to exotic salmonid introduction (native only scenario), and (ii) the current fish community with exotic salmonids present (native + exotic scenario). To determine the relative influence of each exotic salmonid on the modelled native host-parasite dynamics, fish communities comprised of all native fish species together with just one of the exotic salmonids (*O. mykiss* only, *S. fontinalis* only, or *S. trutta* only) were separately simulated. Additionally, because a field survey has suggested that the presence of an *O. mykiss* fish farm in Lake Moreno may increase the prevalence of *A. tumescens* infections in *G. maculatus* (Revenga, Torres & Baiz 2005), though the *A. tumescens* infection status in farmed *O. mykiss* themselves is unknown. Fish communities representing the current fish community with a fish farm present (native + exotic + fish farm scenario), and the fish community comprised of all native fish species with wild and farmed *O. mykiss* (*O. mykiss* + fish farm) were separately simulated.

Highly abundant host species often have the greatest influence on host-parasite dynamics (e.g. Mastitsky & Veres 2010). In Lake Moreno, *G. maculatus* is highly abundant in relation to all other fish species; therefore *G. maculatus* is likely to strongly influence the dynamics of *A. tumescens* infections. *Galaxias maculatus* is also a major prey item for exotic salmonids, comprising up to 37% of prey consumed by *O. mykiss* in Lake Moreno (Vigliano *et al.* 2009). Thus, high abundances of *A. tumescens* infections in *O. mykiss* could be attributed to post-cyclic transmission from native *G. maculatus*, and the high abundance of *G. maculatus* in Lake Moreno relative to other fish species. In light of this, sensitivity analyses were used to investigate whether *A. tumescens* infection in Lake Moreno is (1) maintained by *G. maculatus* (varying *G. maculatus* abundance) and (2) strongly influenced by post-cyclic infection from *G. maculatus* (varying post-cyclic transmission rates).

4.3 Results

4.3.1 Cystacanth infection

Acanthocephalus tumescens established equally well in *P. trucha* and *O. mykiss*, with fewer worms establishing in *G. platei* (Kruskal-Wallis $\chi = 5.839$, $P = 0.054$; Table 4.3). Worms of both sexes achieved greater length in *O. mykiss* than *P. trucha* (Mann-Whitney U test male: $U = 13.00$, $P = 0.01$; one-way ANOVA female: $F_{1,20} = 21.670$, $P < 0.001$). Seventy-five percent of female worms in *O. mykiss* were gravid at four weeks post-infection, containing either maturing eggs (F2 status) or fully mature shelled-eggs (F3 status). All female worms in *G. platei* and *P. trucha* were immature, containing ovarian balls only (F1 status).

4.3.2 Post-cyclic infection

Post-cyclically transmitted *A. tumescens* experience greater establishment success in *O. mykiss* than in native *G. platei* or *P. trucha* (log x+1 transformed; one-way ANOVA: $F_{2,14} = 7.619$, $P = 0.006$; Tukey *post-hoc test*; Table 4.4). Neither male nor female worms in post-cyclic hosts or control *G. maculatus* (Day 28) differed in length from the source *G. maculatus* (Day 0; one-way ANOVA male: $P = 0.157$, female: $P = 0.360$). Female worms were consistently more abundant than males in all infection groups. The proportion of gravid female worms in *G. maculatus* declined during the 28 day infection period, with a similar proportion of gravid worms in the *O. mykiss* and *G. maculatus* control groups. All female worms recovered from *P. trucha* (n = 1) and *G. platei* (n = 4) were gravid.

4.3.3 Population model

The population model successfully replicated the population of *A. tumescens* in the most abundant host, *G. maculatus*, but it slightly underestimated the infection in other less abundant host species. Model simulations of fish communities, with and without exotic salmonids, indicated that salmonid presence is predicted to have caused only very minor increases in *A. tumescens* infection in all native fish (Figure 4.1). *Salvelinus fontinalis* had a greater influence on *A. tumescens* infections than *O. mykiss*, and *S. trutta* maintained only a very small proportion of the acanthocephalan population. The inclusion of the *O. mykiss* fish farm in model simulations

increased the influence of this salmonid on infection levels, although overall increases in infection attributed to *O. mykiss* presence were still minor (Figure 4.1).

Model exploration indicated that *G. maculatus* maintains the greatest proportion of the *A. tumescens* population (1.54 worms m^{-2}), with minor contributions made by all other fish species (<0.31 worms m^{-2}; Table 4.5). Differences in the mean *A. tumescens* infection in all native hosts with and without salmonids present, and at varying *G. maculatus* densities, indicated that, on average, native fish would experience only slight increases in infection with decreasing *G. maculatus* abundance (Figure 4.2). Post-cyclic transmission was shown to have little effect on overall infection dynamics. Altering post-cyclic transmission rates had little influence on the density of worms in native and exotic fish, with the exception of *S. trutta,* which requires post-cyclic transmission to become infected (and then only at low intensity; Table 4.5).

4.4 Discussion

A combination of experimental infections and dynamic population modelling were used to determine whether exotic salmonids influence *A. tumescens* infections in native freshwater fish of Lake Moreno in Argentina. Experimental cystacanth infections demonstrated that *A. tumescens* in exotic *O. mykiss* had equal establishment success, and superior growth and maturity, compared to values for parasites infecting native hosts. Post-cyclic infections demonstrated that *O. mykiss* was also a competent host of *A. tumescens*, showing greater parasite establishment success than in native hosts. However, this transmission pathway was not linked to greater parasite fitness, in terms of growth or maturity, in either native or exotic post-cyclic hosts. Dynamic population modelling, incorporating data from experimental infections and previous field studies, revealed that of the three salmonids introduced to Lake Moreno, *S. fontinalis* may make the greatest contribution to the dynamics of *A. tumescens*. However, the overall influence of exotic salmonids (including the *O. mykiss* fish farm) on *A. tumescens* infection in native hosts is predicted to be very limited. The model also indicated that *G. maculatus* maintains the majority of *A. tumescens* individuals in the system, with sensitivity analysis suggesting that changes in the rate of post-cyclic transmission of *A. tumescens* from infected *G. maculatus* has minimal influence on infection in other native and exotic hosts.

Although exotic host competency for native parasites is important to the determination of whether or not parasite spillback may occur (Kelly *et al.* 2009b), this study demonstrates that low

relative density of exotic hosts diminishes the potential influence of exotic competency. The high competency of *O. mykiss* for *A. tumescens* observed in infection experiments did not result in infection "spill back" to native fish in the model, because the low relative host density of salmonids weakened the potential for infection amplification. Minimal changes to native disease dynamics by exotic hosts occurring at low relative densities have also been reported for native *Ichthyophonus* sp. fungal infections acquired by American shad, *Alosa sapidissima*, a fish introduced to the Pacific coast of North America. Although American shad are highly susceptible to the disease, population densities of this exotic species have remained low until recent years (Hershberger *et al.* 2010). American shad densities now exceed those of native salmonids and the exotic species is considered responsible for the amplification of *Ichthyophonus* sp. infections, although this has yet to be linked to infection spillback in native fish (Hershberger *et al.* 2010). Where spillback of infection has been suggested to occur, exotic species tend to occur in high abundances relative to native hosts. Such is the case in Lake Naroch, Belarus, where the exotic mollusc, *Dreissena polymorpha*, is highly abundant in relation to native molluscs and is thought to have increased the abundance of metacercarial stages of the trematode *Echinoparyphium recurvatum* that infect waterfowl as a final host (Mastitsky & Veres 2010).

As demonstrated in this study, when multiple transmission pathways are available, and these differ in fitness benefits to a parasite, taking into account the relative contribution of each pathway to the flow of infection to a host is important when assessing that host's influence on the overall dynamics of the parasite. While *A. tumescens* achieved greater fitness in salmonids when transmitted at the cystacanth stage, this transmission pathway was not the major source of parasites for salmonids. Instead, *A. tumescens* infections in salmonids were maintained by post-cyclic infection, a transmission pathway shown to generate minimal fitness benefits to the parasite. Differences in parasite fitness between transmission pathways also provide an explanation for the model prediction that *S. fontinalis* may have a greater influence on *A. tumescens* infections than *O. mykiss*, as the former species acquires a greater proportion of parasites through cystacanth infections, while the latter obtains more parasites through post-cyclic transmission. The relative importance of each transmission pathway may also explain the minimal change in *A. tumescens* infections attributed to the presence of *S. trutta*, as this species not only occurred at low densities, but also relied on post-cyclic transmission to acquire infections. The relative contributions of multiple transmission pathways may be obscured when relying on field observations alone to provide indications about a parasite's fitness. Thus,

utilising both experimental infections and population modelling is necessary to first, experimentally assess the relative parasite fitness associated with different transmission pathways and hosts, and second, to dynamically model their influence on the parasite population.

Prior to salmonid introduction, little information was available regarding the composition and functioning of the Lake Moreno fish community (Macchi, Pascual & Vigliano 2007, Pascual *et al.* 2009). Population modelling provides an opportunity to reconstruct native host-parasite dynamics prior to the introduction of exotic species, and enables the evaluation of the relative importance of each host species to a parasite's population. Model simulations indicate that it is unlikely that exotic salmonids have negatively affected native fish communities in Lake Moreno by acting as competent reservoirs of native infection that spillback into native hosts. Declining populations of native species such as *D. viedmensis* and *O. hatcheri* are more likely explained by competition and predation by salmonids, rather than slight increases in *A. tumescens* infections driven by the exotic fish. This conclusion is also supported by diet surveys of native and exotic fish that show *D. viedmensis* were only present in the guts of salmonids and thus *D. viedmensis* may not have experienced predation pressure prior to the introduction of salmonids (Macchi *et al.* 1999).

Although previous observations indicated that *A. tumescens* infections in *G. maculatus* increased in prevalence and infection intensity with decreasing distance to the *O. mykiss* fish farm (Revenga, Torres & Baiz 2005), modelling suggests that the presence of the fish farm in Lake Moreno causes little change to infection of native hosts overall. The increases in parasite infection observed by Revenga *et al.* (2005) may be localised around the fish farm due to high densities of *O. mykiss* in a relatively confined space, and indicate that if salmonid densities increased significantly, native fish might experience greater parasite burdens. As neither the parasite burdens in farmed *O. mykiss*, nor the density or infection status of amphipods in proximity to the fish farm, were reported by Revenga *et al.* (2005), it is difficult to speculate whether the relationship between parasite burdens and proximity to the farm is due to spillback of infection from exotic hosts, or increasing intermediate host density and enhanced transmission success.

4.4.1 Model limitations

The ability to realistically model the dynamics of *A. tumescens* relies on assumptions about amphipod dynamics and fish densities, which influence the conclusions derived from the population model. The lack of amphipod density information from the literature prevented the infection of amphipods being dynamically modelled. While the amphipod population was substituted for a constant to represent the infection in that population, which assumed that the proportion of infected amphipods is relatively constant throughout the year, the natural fluctuations of infection observed by Rauque and Semenas (2007) could not be incorporated.

Recent advances in acoustic fisheries assessment have provided more accurate measurements of densities of larger fish species in Lake Moreno than in previous studies, while larval galaxiid abundance, production and survival studies have enabled densities of *G. maculatus* to be quantified (Vigliano *et al.* 2009). These density estimates, which were used in modelling, suggest that *G. maculatus* has a significantly greater abundance than other fish species in Lake Moreno. Sensitivity analysis demonstrates that even if the density of this key species was over-estimated or under-estimated in the model, in relation to that for other fish, salmonids would still only cause negligible increases of infection in native fish.

In conclusion, this study suggests that although exotic salmonids are competent hosts of *A. tumescens*, their introduction to Lake Moreno is unlikely to have impacted native fish populations through the amplification of infection. A low density of salmonids, relative to native fish, and their reliance on post-cyclic transmission to obtain infection, weakens the potential spillback effects of enhanced parasite fitness in the exotic host. It is likely that salmonids have had greater impacts on native fish through predation and competition, than via modified native host-parasite dynamics. Futhermore, the results of this study indicate that researchers must be cautious when assessing the influence of exotic species on native host-parasite dynamics based on field observations alone.

Table 4.1. Definitive host parameter definitions and estimates used in the population model.

Parameter	Symbol	*GM*	*GP*	*PT*	*DV*	*OH*	*OM*	*SF*	*ST*	Units	Source
Density	N_i	38.68	0.00054	0.00206	8.96e-06	6.14e-05	0.00180	0.00058	6.14e-05	m^{-2}	This study
Natural host mortality	b_i	0.00046	0.00030	0.00034	0.00036	0.00055	0.00039	0.00039	0.00039	$fish^{-1}\ day^{-1}$	Milano (1996); Vigliano(2009); Bello(1995); Valiente(2010); Winemiller (1992); Milano (2003)
Amphipod predation	β_i	0.115	8.12	6.1	5	3.867	0.07	1.37	0	day^{-1}	P.J. Macchi, unpublished data; D. Milano, unpublished data; *Grupo de Evaluación y Manejo de Recursos Icticos* (GEMARI), unpublished data
G. maculatus predation	D_i	-	2.80e-07	4.78e-06	0	5.82e-08	1.41e-04	1.53e-05	1.48e-06	$G.maculatus^{-1}\ host^{-1}\ day^{-1}$	P.J. Macchi, unpublished data; Daniela, unpublished data; GEMARI, unpublished data
Larval establishment	γ_i	0.166	0.166	0.350	0.258	0.258	0.335	0.335	0.335	$worm^{-1}$	This study

Post-cyclic establishment	T_I	-	0.025	0.009	0.017	0.017	0.256	0.256	0.256	worm^{-1}	This study
Parasite maturation	φ_i	0.022	0.022	0.022	0.022	0.022	0.027	0.027	0.027	worm^{-1} day^{-1}	This study
Parasite mortality	α_i	0.0187	0.01869	0.01869	0.01869	0.01869	0.01869	0.01869	0.01869	worm^{-1} day^{-1}	Paterson *et al*. in press

Notes: *GM – Galaxias maculatus, GP – Galaxias platei, PT - Percichthys trucha, DV - Diplomystes viedmensis, OH - Odontesthes hatcheri, OM – Oncorhynchus mykiss, SF – Salvelinus fontinalis, ST – Salmo trutta.*

Table 4.2. Intermediate host and parasite parameter definitions and estimates used in the population model.

Parameter	Symbol	Value	Units	Source
Parasite fecundity	λ_W	807.5	$worm^{-1}\ day^{-1}$	Crompton & Whitfield (1968)
Egg mortality	E	0.005	$egg^{-1}\ day^{-1}$	Crompton (1970)
Worm density dependence survival	d	4.96606	$worm^{-1}\ day^{-1}$	Optimised
Amphipod constant	A	9.10408e-06	-	Optimised

Table 4.3. Prevalence, establishment, length and reproductive status of *A. tumescens* in fishes experimentally infected with cystacanths. Gravid females represent females with maturing eggs (F2) and fully mature shelled eggs (F3).

Host	n	Prevalence %	Establishment % (mean ± SE)	Parasite sex	Length μm (mean ± SE)	n	Gravid females % (F2 + F3)
Oncorhynchus mykiss	6	83.3	33.5 ± 9.5	M	2694.5 ± 225.5	8	75.0
				F	3370.1 ± 150.9	12	
Percichthys trucha	6	100	35 ± 5	M	1913.1 ± 17.9	11	0
				F	2414.0 ± 19.7	9	
Galaxias platei	6	16.6	8.3 ± 8.3	M	2385.6	1	0
				F	2300.4	1	

Table 4.4. Prevalence, establishment, length and reproductive status of *A. tumescens* in fishes experimentally infected with post-cyclically transmitted worms. Gravid females represent worms with maturing eggs (F2) or fully mature shelled eggs (F3). * Prevalence calculated from first 30 *G. maculatus* autopsied.

Host	Infection level	No. of fish	Prevalence %	Establishment % (mean ± SE)	Parasite sex	Length µm (mean ± SE)	No. of worms	Gravid females % (F2 + F3)
Oncorhynchus mykiss	40	6	83.3	21.8 ± 5.7	M F M:F	2968.8 ± 132.4 4289.2 ± 181.6 1:2.5	15 38	73.7
Percichthys trucha	40	6	16.6	0.9 ± 0.5	M F M:F	3777.2 5339.2 1:1	1 1	100
Galaxias platei	40	5	40	2.5 ± 0.7	M F M:F	3294.4 4700.2 ± 490.1 1:4	1 4	100
Galaxias maculatus	Source group	60	55	-	M F M:F	3056.2 ± 110.2 4430.4 ± 91.2 1:2.1	33 73	81.3
	Control group	54	41.7*	-	M F M:F	3398.5 ± 104.4 4574.4 ± 82.6 1:2	55 109	73.5

Table 4.5. Simulating the effects of altering post-cyclic transmission rates of exotic salmonids on *A. tumescens* infection densities (worms m^{-2}) in native and exotic hosts.

Parameter changed	% change	*GP*	*PT*	*DV*	*OH*	*OM*	*SF*	*ST*
Post-cyclic consumption	100	0.0491255	0.316902	0.001454	0.0074762	0.0062210	0.0368457	3.76E-09
	0	0.0491260	0.316904	0.001454	0.0074763	0.0062158	0.0368459	1.88E-09
	-100	0.0491265	0.316906	0.001454	0.0074764	0.0062106	0.0368461	0

Notes: *GP – Galaxias platei, PT - Percichthys trucha, DV - Diplomystes viedmensis, OH - Odontesthes hatcheri, OM – Oncorhynchus mykiss, SF – Salvelinus fontinalis, ST – Salmo trutta.*

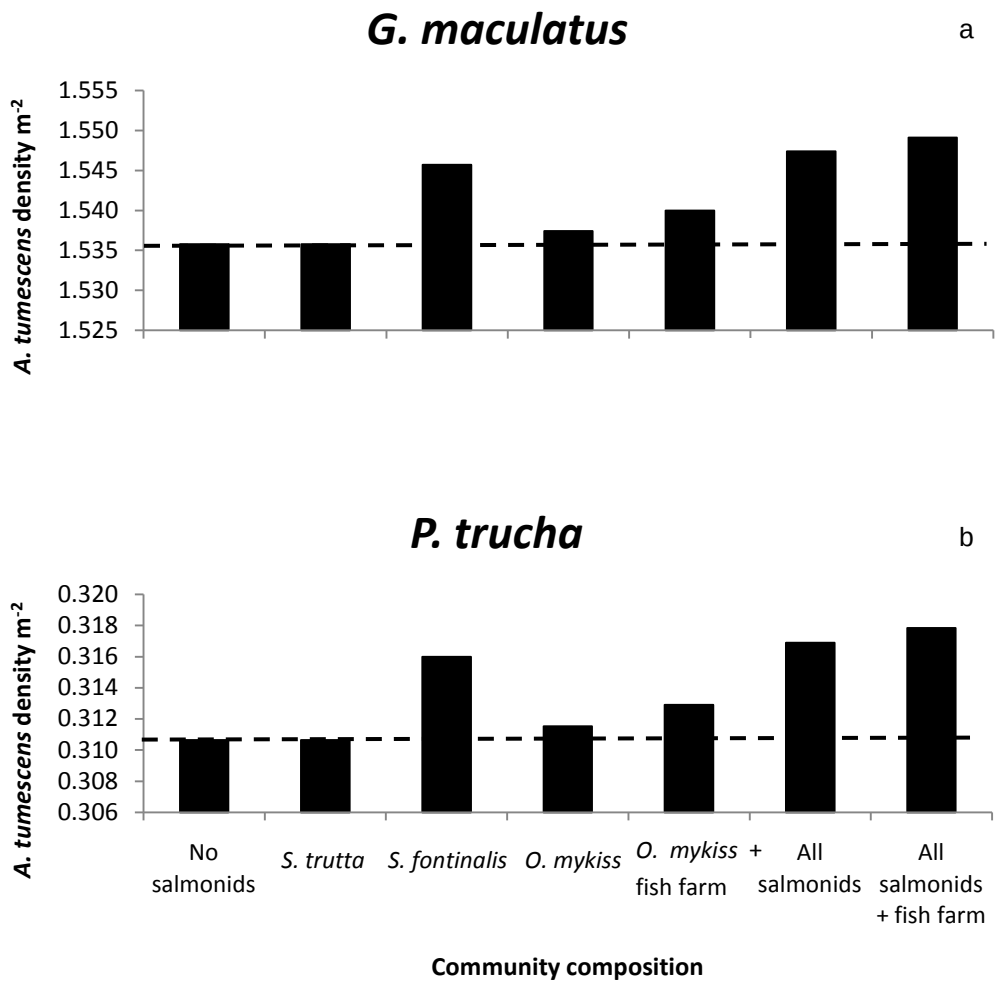

Figure 4.1. Influence of salmonid presence on *A. tumescens* infections in native (a) *G. maculatus* and (b) *P. trucha*. Note Y-axis does not begin at 0 and scale differs for each species. Dashed line represents infection density in native host populations in absence of salmonids.

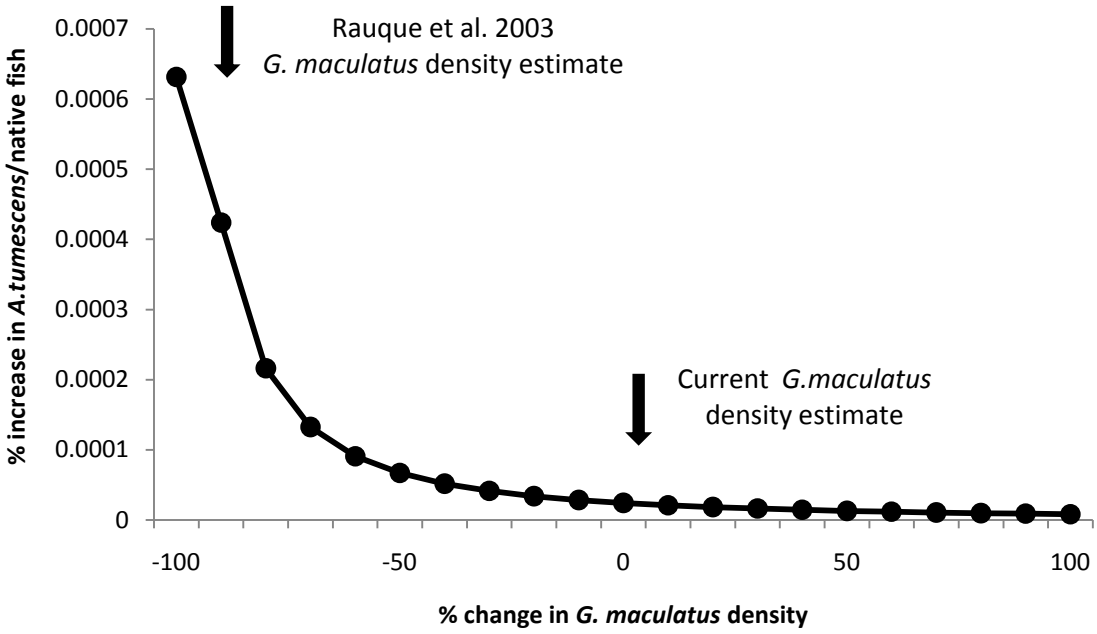

Figure 4.2. Simulating the effect of altering *G. maculatus* abundance on *A. tumescens* infections in native fish. Values shown are outcomes of changes of ± 100% in current estimates, in 10% increments.

CHAPTER 5

Exotic species competency for native parasites: do all salmonids pose equal threats to native host-parasite dynamics?

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5.1 Introduction

Whether through accidental introductions or intentional releases, many species attain distributions far beyond their natural ranges, often forming novel interactions with native counterparts. Interactions mediated by predation and competition are frequently cited as detrimental outcomes resulting from the presence of exotic species in novel habitats (e.g. Ricciardi, Neves & Rasmussen 1998, Wiles *et al.* 2003). However, interactions between exotic and native species, mediated by disease, are also increasingly recognised (e.g. Van Riper, Van Riper & Hansen 2002, Tompkins, White & Boots 2003, LaDeau, Kilpatrick & Marra 2007). Exotic salmonid fishes, for example, have long been known to influence native communities through novel predatory and competitive interactions (Crowl, Townsend & McIntosh 1992, Townsend 2003), with the possibility of additional interactions mediated by disease highlighted only recently (Tompkins & Poulin 2006, Kelly *et al.* 2009a).

Although salmonids are native to cold water environments of the Northern Hemisphere, they have been widely translocated around the globe for angling and aquaculture (Crawford & Muir 2008). This global translocation has resulted in both the introduction of fish to regions where salmonids were traditionally absent (e.g. Australasia, South America), and also the exchange of salmonids between regions where different salmonid species naturally occur (e.g. rainbow trout *Oncorhynchus mykiss* native to the Pacific Coast of North America introduced to the United Kingdom, and brown trout *Salmo trutta* native to Eurasia introduced to North America; MacCrimmon & Marshall 1968, MacCrimmon 1971). While the process of translocation is often an opportunity for exotic species to escape natural enemies such as predators and disease (Torchin, Lafferty & Kuris 2001, Torchin *et al.* 2003), the presence of native salmonids in the introduced ranges of exotic fish means that translocated salmonids are unlikely to escape natural enemies completely.

In contrast, salmonids introduced to the Southern Hemisphere have largely escaped salmonid-adapted diseases. The introduction of parasite-free ova or fry (Kennedy & Bush 1994), and the use of multiple-stage translocations (e.g. New Zealand brown trout originated from trout introduced to Australia, MacCrimmon & Marshall 1968), have also severely limited the likelihood of co-introducing diseases during translocations. However, salmonids introduced to the Southern Hemisphere have not escaped disease burdens entirely, as they often acquire generalist parasites from native fish communities (Dix 1968, Ortubay *et al.* 1994). In some cases, exotic salmonids have achieved equal or greater parasite species richness than in their area of origin, irrespective of the presence of native salmonid species (Poulin & Mouillot 2003).

The ease with which they acquire native parasites facilitates the potential for exotic salmonids to alter native host-parasite dynamics, by acting as competent parasite reservoirs that "spillback" infection into native hosts (see Daszak, Cunningham & Hyatt 2000, Kelly *et al.* 2009b), or as incompetent native parasite hosts that dilute infection burdens in native hosts (e.g. Telfer *et al.* 2005, Kopp & Jokela 2007). While few studies have investigated the influence of exotic salmonids on native host-parasite dynamics, assessments of host competency for two closely related acanthocephalans, *Acanthocephalus tumescens* in rainbow trout and *Acanthocephalus galaxii* in brown trout, suggest that host competency for native disease differs between salmonid species (Chapters 3 & 4). Thus the potential for exotic salmonids to act as drivers of parasite spillback or dilution, and their influence on native fish populations beyond predation and competition, may differ between species.

In Lake Pearson, New Zealand, the co-existence of introduced populations of rainbow and brown trout presents an opportunity to simultaneously compare the competency of two exotic salmonids for native fish parasites. Although native host-parasite dynamics were previously unknown in Lake Pearson, native fish populations are comprised of hosts for the native trematodes *Stegodexamene anguillae* MacFarlane 1951 and *Telogaster opisthorchis* MacFarlane 1945. Both trematodes co-infect their definitive native eel hosts, *Anguillae* sp. (*S. anguillae* - upper intestine, *T. opisthorchis* - lower intestine, MacFarlane 1945, MacFarlane 1951), and adult worms are also reported from exotic salmonids (Dix 1968, Hine, Jones & Diggles 2000, Kelly *et al.* 2009a). These trematodes have similar lifecycles, with eggs released from adult worms reaching the water column with eel faeces, and the first intermediate host, the snail *Potamopyrgus antipodarum*, becoming infected by either ingesting eggs (*T. opisthorchis*) or encountering free-living larvae that emerge from eggs (*S. anguillae*). Second intermediate hosts, the native fish *Galaxias* spp. and *Gobiomorphus* spp., become infected by contact with cercarial infective stages released from snails, with the parasites then encysting as metacercariae within fish tissues. Eels acquire the worms following the consumption of second intermediate hosts, and salmonids frequently encounter trematode metacercarial cysts as they are known to prey on both *Galaxias* and *Gobiomorphus* in New Zealand lakes (e.g. McCarter 1986, Rowe, Konui & Christie 2002). Therefore, exotic salmonids may not only alter the abundance of second intermediate hosts through predation, but also could alter trematode infection burdens in both intermediate and definitive hosts through the spillback or dilution of parasites.

Here, a multiple-pronged approach is applied to (i) quantify the influence of exotic salmonids on native trematode infections in native fish populations, and (ii) determine

whether salmonid species differ in their influence on native host-parasite dynamics. First, I use field data to evaluate the relative acquisition of native trematodes by native and exotic hosts. Second, I use experimental infections to determine whether host competency for native parasites differs between exotic salmonids, and between native and exotic hosts. Third, I discuss how the acquisition of native parasites by exotic salmonids may influence native host-parasite dynamics.

5.2 Methods

5.2.1 Study Site

Lake Pearson (24°10’520E, 57°87’570N) is a small, shallow lake (1.79 km^2, maximum depth 17 m) situated in the Waimakariri River Catchment, Canterbury, South Island, New Zealand. The fish community consists of eight species, including four exotic salmonids, of which rainbow and brown trout are the most numerous. Lake trout, *Salvelinus namaycush*, were successfully introduced in 1907; however, declining fish size and condition has been noted since 1950, and this species is now considered rare (Hutchison 1981, Rowe & Graynoth 2002). Sporadic occurrence of chinook salmon, *Oncorhynchus tshawytscha,* has also been reported in this lake (Hutchison 1981).

5.2.2 Field surveys

Field surveys were conducted to determine the prevalence (proportion of infected individuals) and infection intensity (number of parasites per host , Bush *et al.* 1997) of the trematodes *T. opisthorchis* and *S. anguillae* in second intermediate and definitive fish hosts, and the relative densities of hosts. Fish and invertebrate communities were sampled during April - May (Austral Autumn) and September 2008 (Austral Spring) from four sites (approximately 200 m shore length per site). A variety of fishing techniques were used to assess relative fish densities as no single fish capture method was suitable to catch all species. Exotic salmonids were sampled using sinking 25 m multi-panel gill nets (mesh sizes: 13, 25, 38, 56, 70 mm) set perpendicular to the shoreline for 1-2 hours during daylight hours. Longfin eels were sampled using unbaited fyke nets (wing length 450 cm, stretched mesh size 20 mm) set overnight at the lake shore. *Gobiomorphus* and *Galaxias* species were sampled using unbaited minnow traps (height 25 cm, length 45 cm, stretched mesh size 5 mm), also set overnight. Additionally, seine nets were used to capture *Gobiomorphus* and *Galaxias* when

fewer than 30 individuals per species were obtained from minnow traps. The number of fish captured by each sampling technique was used to determine the relative abundance ratios of fish hosts, comparable only between the same sampling techniques.

A random sub-sample of up to 30 fish per species per sampling period were euthanized (47 brown trout, 41 rainbow trout, 40 common bully *Gobiomorphus cotidianus*, three upland bully *Gobiomorphus breviceps*, two koaro *Galaxias brevipinnis*). Fork length (mm) and total weight (g) were recorded for each fish prior to preserving the alimentary canal (oesophagus to anus) in 10% buffered formalin. Remaining body tissues of bullies and koaro were frozen for later examination for metacercarial cysts. Body tissues of salmonids were not examined for metacercarial cysts as field surveys by Kelly *et al*. (2009a) demonstrated very low prevalence and infection intensity of metacercarial cysts in *S. trutta*, although sympatric native fish had moderate infections. Longfin eels in Lake Pearson could not be examined for intestinal parasites because eels are of high conservation status in this lake. To provide a reference of trematode prevalence, infection intensity and fitness in native definitive hosts, nine longfin eels were collected from nearby Lake Sumner (24°42’440E, 58°33’810N) on the same sampling occasions as Lake Pearson.

In the laboratory, the numbers of trematodes in the alimentary canals of trout and eels were recorded. The development status (non-gravid or gravid, based on the presence of eggs in utero) and worm size (calculated from the surface area of an ellipse = π x (L/2) x (W/2), L = length in μm and W = width at widest point in μm) were determined for each worm. The number of eggs in the uterus, and egg volume ($V=\pi \times L \times W^2/6$, where L = length and W = width) of a subsample of five eggs were measured for each gravid worm. Stomach contents of each definitive host were examined to enumerate *Galaxias* and *Gobiomorphus* consumed by each fish.

Field surveys also determined density of the first intermediate snail host *P. antipodarum* in Lake Pearson. Density was estimated from three surber samples per site collected from random locations within 5 m of the shore during April-May 2008 (n = 12), with samples preserved in 10% formalin prior to enumeration of snails.

5.2.3 Experimental infection

An infection experiment was conducted to determine the fitness of *T. opisthorchis* and *S. anguillae* in native and exotic definitive hosts, from which relative rates of parasite

establishment, fecundity and mortality could be estimated. Brown trout (107 – 148 mm) were collected by electric fishing from the Cap Burn (22°95'560E, 55°46'220N), Taieri River catchment, Otago. Rainbow trout (182 – 244 mm) were obtained from the Otago Fish and Game Macraes hatchery, Otago; while wild caught longfin eels (460 – 550 mm) were sourced from NZ Eels Limited, Te Kauwhata, Waikato. All fish were acclimatised in a 200 L tank in the University of Otago's controlled climate facilities (13 hour day/11 hour night period, 10°C, 15% daily water change) for two weeks prior to experimentation. A common food consumed by all fish species was not available; therefore fish were maintained on different diets fed *ad libitum* (longfin eel and brown trout – blood worms; rainbow trout – commercial fish pellets).

During acclimatisation, experimental fish were treated with an anthelmintic, Praziquantel, in tablet form (25 mg - longfin eel; 12.5 mg - rainbow and brown trout), to rid them of potential pre-existing worm infections. The anthelmintic was administered to fish that had been anaesthetised in a Benzocaine solution (100 ppm), using a pipette to orally insert each tablet into the fish's stomach. Fish were placed in a tank of freshwater and observed for five minutes post-recovery to ensure regurgitation did not occur. Experimental fish were maintained for a further seven days prior to experimental infection, to allow for the elimination of residual anthelmintic from body tissues and its excretion into tank water (Björklund & Bylund 1987). Reabsorption of active ingredients excreted from fish was minimised through daily water changes. Anthelmintic efficacy was tested by assessing parasite infections in a sub-set of five treated and un-treated fish per species. Treated and untreated fish were autopsied 48 hours post-anthelmintic exposure, with no intestinal parasites recovered in brown and rainbow trout in either treatment. Intestinal parasites were also absent from anthelmintic treated eels, while all untreated eels contained trematodes (mean infection intensity of both species combined = 64 adult trematodes per individual).

For both trematode species (*T. opisthorchis* and *S. anguillae*), five experimental fish of each species were randomly assigned to three infection time-periods (5, 15 or 25 days), and placed into individual 30 L tanks without food for 48 hours prior to experimentation. Common bully naturally infected with *T. opisthorchis* and *S. anguillae* metacercariae were collected from Lake Waihola (22°84'950E, 54°61'500N), Otago, and transported live to the laboratory. Live metacercariae were carefully removed from the body tissues of freshly euthanized fish and placed into gelatine capsules at densities reflecting the average infection intensity of trematodes in bullies of Lake Pearson (40-50 *S. anguillae* or 80-100 *T. opisthorchis*). As progenetic reproduction can occur in *T. opisthorchis* and *S. anguillae*

(MacFarlane 1945, MacFarlane 1951), whereby trematodes produce eggs while encysted in second intermediate hosts, metacercarial cysts with progenetic eggs were omitted from the experiment. Capsules were administered to anaesthetised fish as for the anthelmintic treatment, with all infected fish maintained in individual tanks on their respective diets post-infection. At the completion of each infection period (5, 15 or 25 days), fish were euthanized and immediately examined for parasites by removing the alimentary canal from oesophagus to anus and splitting it longitudinally. The number of trematodes was noted prior to fixing the parasites in 10% buffered formalin. Worm measurements were made as per the field survey.

5.2.4 Statistical Analyses

Statistical analyses were conducted using SPSS Statistic 15.0 (SPSS 2006). For field and experimental infection data, analyses of variance (ANOVA) were used for most analyses; data were transformed when necessary to meet the assumptions of normality for parametric tests. *Post-hoc* tests were used to assess where differences existed when variance was significant between more than two species. In situations where assumptions for parametric tests could not be met, non-parametric tests (Mann-Whitney and Kruskal-Wallis) were used. In experimental infections, establishment was only assessed at 5 days post-infection between species.

5.2.5 Parasite Flow

Parasite flow diagrams were constructed to assess the potential influence of exotic salmonid presence on *T. opisthorchis* and *S. anguillae* in their native hosts. Although a parasite's fitness can be negatively influenced by the presence of another parasite species (e.g. Holland 1984, Poulin *et al.* 2003), and both trematodes co-infect the same second intermediate and definitive host species, each trematode was evaluated separately as *T. opisthorchis* and *S. anguillae* occupy different parts of an eel's gut (Macfarlane 1952) and their occurrence in fish is positively correlated (Hine & Francis 1980). Therefore it may assumed that *T. opisthorchis* and *S. anguillae* are unlikely to compete for space in the gut, resulting in lower fitness of one species in the other's presence.

Dynamic population models could not be constructed due to lack of data on densities of longfin eels and second intermediate hosts. Densities of brown and rainbow trout were calculated from known salmonid densities in upland lakes (Gauthier 2008), adjusted to the

relative abundance of each salmonid species in field samples (this study). Both the rate at which *P. antipodarum* encounter *T. opisthorchis* eggs or larvae hatched from *S. anguillae* eggs, and the rate at which trematode cercariae encounter second intermediate host fish and successfully establish as metacercarial cysts, were also unknown from the literature. While the abundance of *P. antipodarum* was estimated in this study, it is unlikely to be limiting to either parasite species, as trematodes produce thousands of cercarae whilst in their first intermediate host (McCarthy, Fitzpatrick & Irwin 2002, Karvonen *et al.* 2004).

Common and upland bully could not be distinguished from salmonid gut content analysis, therefore the rate at which definitive hosts acquire infection from each bully species is assumed to be proportional to each bully species' relative abundance in the lake.

Mortality of *T. opisthorchis* in longfin eels could not be parameterised from infection experiments (this study) as worms survived beyond 25 days post-infection and did not show decreasing infection intensity with time. Mortality for this parasite was calculated from a regression of longevity of adult trematodes against their body size (y = 260.63 x - 777.99), based on a compilation of platyhelminth life history data (Trouve *et al.* 1998).

5.3 Results

5.3.1 Field survey

Brown trout (n = 71, length 117-610 mm) and rainbow trout (n = 41, length 87-536 mm) were the only salmonid species captured in Lake Pearson, occurring at a ratio of 2.6:1. Minnow traps captured common bully (n = 290, length 20-114 mm), upland bully (n = 40, length 26-59 mm) and koaro (n = 2, length 39-65 mm) at a ratio of 95:13:1. Fyke nets captured longfin eel in Lake Pearson (n = 16, length 405-830 mm) and Lake Sumner (n = 19, length 403-1055 mm), with eels significantly longer in the latter (one-way ANOVA lake: $F_{1,33}$ = 10.227, P = 0.003).

5.3.1.1 *Telogaster opisthorchis*

Infection prevalence was highest in longfin eel and decreased over winter (81.8-66.7%, respectively), while prevalence increased in both brown (17-67%, respectively) and rainbow trout (22-33%, respectively; Table 5.1). Infection intensity was also greater in longfin eel than salmonids (log transformed, two-way ANOVA species: $F_{2,29}$ = 5.299, P = 0.011, Tukey *post-hoc* test), though it showed no difference between seasons (P = 0.944). Worm size

(surface area) differed between species and seasons (square-root transformed, two-way ANOVA species*season: $F_{2,347}$ = 47.81, P < 0.001), with longfin eels having larger worms that increased in size over winter, while worms in brown trout were small and decreased in size over winter (Figure 5.1). Few *T. opisthorchis* in brown trout (2%) and rainbow trout (0-4%) were mature, while the majority of worms in longfin eels were gravid (80-83%; Table 5.1). Mean number of eggs for gravid worms per season ranged between 228.7 and 564.6 eggs in longfin eels, while fewer eggs were present in brown and rainbow trout (10-13 and 3.5 eggs respectively; Figure 5.1). Eggs were also of greater size in longfin eels than in either salmonid species (pooled for seasons; one-way ANOVA species: $F_{2,744}$ = 49.48, P < 0.001; Figure 5.1).

5.3.1.2 *Stegodexamene anguillae*

Infection prevalence of *S. anguillae* increased in all hosts over winter, with highest prevalence recorded in longfin eel pre- and post-winter (63.6, 100%, respectively; Table 5.1). Infection intensity did not differ between hosts (Kruskal-Wallis X_2 = 3.408, P = 0.182). Worms in longfin eels were larger than worms in either salmonid (log transformed; two-way ANOVA species*season: $F_{2,214}$ = 5.956, P = 0.003, Games-Howell *post-hoc* tests), with worm size increasing in longfin eels and rainbow trout over winter (Figure 5.1). The majority of *S. anguillae* in longfin eels were gravid (82-93%), while few gravid worms were present in salmonids (rainbow trout 3-12%, brown trout 10-14%). Mean number of eggs per gravid worm only differed between longfin eels and rainbow trout (pooled seasons: one-way ANOVA species: $F_{2,87}$ = 4.565, P = 0.013, Games-Howell *post-hoc* test). Egg size also differed between hosts and seasons (two-way ANOVA species*season: $F_{2,392}$ = 12.97, P < 0.001), with the largest eggs occurring in longfin eels pre-winter, and the smallest eggs in rainbow trout (Games-Howell *post-hoc* test). Post-winter, egg size increased in brown trout but showed little change in longfin eel or rainbow trout.

5.3.1.3 Definitive host diet

Bully were the main fish consumed by salmonids, with the percentage of stomachs containing bullies increasing from pre- to post-winter (rainbow trout 43-67%; brown trout 26-53%). Brown trout contained an average of 2.4-2.9 bullies while rainbow trout contained 1.7-4.3 bullies. Koaro were present in less than 3.2-6.7% of brown trout (mean intensity 2-3 koaro/stomach) and 20-7% of rainbow trout (mean intensity 2.3-1 koaro/stomach) between

seasons. Fish were absent from stomachs of longfin eel from Lake Sumner, with the majority of stomachs either empty or containing the snail *P. antipodarum*.

5.3.1.4 Intermediate hosts

Common bully contributed 87% of the total community of second intermediate host species for *T. opisthorchis* and *S. anguillae* in Lake Pearson, with 32.5% infected with *T. opisthorchis* and 25% infected with *S. anguillae* (Table 5.2). Upland bully and koaro comprised 12% and 1% of the second intermediate host community, with *T. opisthorchis* occurring in upland bully, and *S. anguillae* in koaro.

Snails were found in all benthic samples, with an average of 631.8 snails per m^2, of which an average of 50.1% were larger than the minimum of 3.6 mm shell length recorded for infected snails (Macfarlane 1952).

5.3.2 Experimental infection

5.3.2.1 Telogaster opisthorchis

Establishment of *T. opisthorchis* did not differ among host species at five days post-infection (Kruskal-Wallis $X_2 = 1.604$, $P = 0.499$), with many individuals failing to acquire infection (Table 5.3). Worms survived in longfin eels until 25 days post-infection, while no worms were present in salmonids at 15 days post-infection. Interestingly, higher numbers of worms were recovered in longfin eels were positively correlated with time since infection. Worm size (surface area) did not differ between salmonids and longfin eel at five days post infection (ANOVA species: $F_{2,46} = 0.549$, $P = 0.581$; Figure 5.2). No gravid worms were present in brown trout or in longfin eels five days post-infection, while one of the two worms present in rainbow trout contained mature eggs. The percentage of gravid worms in longfin eels increased with infection time, with 66.0% of worms gravid at 25 days post-infection, and egg size did not increase with time since infection (square-root transformed, ANOVA time: $F_{1,450} = 3.287$, $P = 0.070$).

5.3.2.2 Stegodexamene anguillae

Few fish became infected with *S. anguillae*, which was reflected in the poor prevalence and establishment success in all species (Table 5.3). Highest establishment success occurred in longfin eel, with 6.7% of worms establishing at five days, and 18.3% at 15 days post-infection. Worms were found in brown trout and longfin eels at 15 days post-infection, but in rainbow trout only at five days post-infection. Worm size (surface area) showed little variation between host species at five days post-infection, and did not differ between brown trout and longfin eels at 15 days post-infection (P = 0.653; Figure 5.2). Twenty-five percent of worms in longfin eels (n = 8) were gravid five days post-infection, increasing to 60% (n = 15) after 15 days post-infection. A gravid worm was also present in brown trout 15 days post-infection, with no gravid worms observed in rainbow trout. Eggs present in brown trout were larger than those from longfin eels at 15 days post-infection (log-transformed; ANOVA species: $F_{1,38}$ = 18.764, P <0.001).

5.3.3 Parasite Flow

5.3.3.1 Telogaster opisthorchis

Higher rates of parasite establishment and maturation, and lower mortality in longfin eels show that eels are the most successful hosts of *T. opisthorchis*, as indicated by higher parasite abundance in the field (Figure 5.3). Of the two salmonid species present in Lake Pearson, brown trout is likely to have greater influence on *T. opisthorchis* dynamics, because this host has greater host abundance and parasite establishment as reflected by the higher infection levels observed in the field. The majority of infections from second intermediate to definitive hosts originate from common bully, as this host is seven times more abundant than upland bully. Mean predation rates on bullies indicate that rainbow trout must encounter more trematodes than brown trout, but the lower establishment rate of *T. opisthorchis* in rainbow trout results in fewer parasites in this salmonid host. However, as the relative density of this host in relation to brown trout is unknown, it is difficult to determine how the contrasting parasite fitness rates between native and exotic hosts will manifest in altered parasite dynamics.

5.3.3.2 Stegodexamene anguillae

Higher *S. anguillae* establishment rates in longfin eels than the salmonids are also reflected in higher parasite abundance in the native host (Figure 5.3). Field surveys suggest that this parasite can mature in both salmonid species (as verified experimentally in brown trout), although poor survival of worms in salmonids means they are unlikely to contribute significantly to the parasite population. Worms establish at a lower rate in rainbow trout than brown trout, but rainbow trout consume more second intermediate hosts than brown trout. Thus, rainbow trout encounter more metacercarial cysts, resulting in higher parasite abundance in this host. Common bullies are the major source of *S. anguillae* infection for salmonids, due to the parasite's high abundance in common bullies, this host's high relative abundance, and high predation rates by the definitive hosts. Koaro had similar infection abundance to common bully, but koaro has low relative abundance and few are eaten by definitive hosts.

5.4 Discussion

We used a combination of field surveys and experimental infections to determine the likely relative influence of brown and rainbow trout on the host-parasite dynamics of two native trematode species. I also evaluated field and experimental results in relation to likely parasite flows through the system. In general, field surveys demonstrated that longfin eels were superior hosts of both trematodes, though some similarities were observed in *S. anguillae* infections between eels and salmonids. Experimental infections showed that both trematode species had poorer establishment and survival in salmonids, though some worms matured and attained similar sizes to those in eels prior to being lost. Overall, both field surveys and experimental infections indicated that salmonids are unlikely to increase native parasite flow to native hosts by amplifying infection rates.

Of the two salmonid species in Lake Pearson, brown trout is likely to make the greater contribution to the parasite populations, due to its higher relative host density, infection intensity and establishment rate. Rainbow trout are more likely to act as a parasite sink because not only do they consume more intermediate host fish, but trematodes in rainbow trout have poorer establishment and survival compared to both brown trout and longfin eel. Poorer establishment and survival of trematodes in salmonids compared with longfin eel appear to be the main reasons why salmonids are inferior hosts, as trematodes which do persist in salmonids achieve similar sizes to those in eels.

The spillback of infection to native hosts may be unlikely, but any potential for salmonids to dilute infection in native hosts cannot be inferred solely from poor exotic host competency for native parasites. Relative densities of exotic and native hosts strongly influence whether native parasite dynamics will be altered by the presence of an exotic host (see Chapter 4). Whilst the densities of longfin eels in Lake Pearson are unknown, previous studies of shared native parasites from native–exotic host complexes allow us to consider how exotic salmonids may influence parasite dynamics under given relative host densities. For example, if the relative density of salmonids to longfin eels is low, infection in native hosts may remain unchanged, as the density of salmonids would be too small for the poor parasite competency of salmonids to influence the overall parasite transmission dynamics. Alternatively, equal or greater density of salmonids to longfin eels may reduce infection in native hosts, as the poor competency of exotic salmonids for native parasites decreases the overall egg output from definitive hosts, potentially reducing infection prevalence and intensity in intermediate hosts, and resulting in reduced exposure to infection in definitive hosts.

Infection dilution in native hosts, driven by the poor host competency of an exotic host for a shared native parasite, may only occur if infection to native hosts becomes limiting. Although salmonids encounter second intermediate hosts infected with parasites that have higher fitness in longfin eels, it is common for a large proportion of infected second intermediate hosts not to encounter their definitive hosts, due to the effects of natural mortality or parasite induced mortality for example (Poulin 2007). Hence, the consumption of second intermediate hosts by salmonids that were unlikely to encounter longfin eels may not alter parasite transmission to the native host. However, if infection from the second intermediate host population is limiting, either through naturally low host abundance prior to salmonid introduction, or driven by resource competition from salmonids, eels may experience reduced infection. Infection rates in intermediate hosts may also remain unchanged if the lowered infection intensity of trematodes supported by eels can still provide sufficient parasite eggs to maintain infection in the first intermediate host. Additionally, trematodes reproduce asexually in their first intermediate host, producing thousands of cercariae to increase their transmission to second intermediate hosts; the effect of a reduction in egg output from definitive hosts may be trivial when compared with the chance of cercariae encountering second intermediate hosts.

Although salmonids may be altering native host parasite dynamics, they may also benefit native parasites by acting as an alternative host for parasites that normally infect eels.

Widespread population declines due to commercial harvests, habitat loss and impeded migration (Jellyman 2007), have reduced the densities of definitive hosts available to both *T. opisthorchis* and *S. anguillae.* While salmonids may be less competent hosts for native trematodes, they may contribute to the persistence of these parasites in some localities, especially if salmonids have both high relative densities and encounter rates with infected second intermediate hosts. Salmonids may also alter seasonal patterns of infection prevalence and intensity in native hosts, because trematode infections increase in salmonids over winter, while simultaneously decreasing in longfin eel. This may be related to different infection exposure rates to metacercarial cysts associated with seasonal changes in diet, with small fish species making larger contributions to salmonid diets in winter months (McCarter 1986), while shortfin eels have reduced activity and feeding rates over the same period (Ryan 1984).

The use of dynamic population models would have been beneficial to examine the influence of salmonids on the dynamics of *T. opisthorchis* and *S. anguillae* in their native hosts. As dynamic population models can only provide realistic simulations of the flow of parasites if parameterised correctly, it is important to obtain the best estimates of population parameters in order for robust models to be constructed. A number of parameters have been identified that need detailed assessment before constructing realistic population models of this system. First, in order to relate the importance of each host species to the flow of parasites, host densities are required, especially when multiple hosts are available at various lifecycle stages. In systems such as Lake Pearson, where there are multiple fish species with varying body size and habitat use, a single fishing technique that captures all species is often unavailable, and therefore only relative abundances of fish captured by each fishing method can be compared. Additionally, passive techniques such as fyke nets and minnow traps only provide estimates of catch per unit effort, rather than density. In this study system, salmonid densities can be estimated from comprehensive acoustic assessments of salmonid densities in upland South Island lakes (Gauthier 2008), corrected for the relative proportion of brown and rainbow trout captured by seine netting. Similar assessments for longfin eels, bullies and koaro in upland lakes are currently not available due to the difficulty in assessing the populations of these species, though mark-recapture techniques may be useful in obtaining host density estimates in future studies.

Second, assessment of infection in hosts and the diet of definitive hosts need to account for differences associated with seasons, not just species. As mentioned earlier, seasonal differences in the consumption of second intermediate hosts may alter infection intensities in definitive hosts. In Lake Pearson, fish populations were only sampled pre- and

post-winter, which only captured over-winter variability in infection. Trends in infection prevalence and intensities in longfin eels and salmonids suggest that differences between native and exotic hosts may be more pronounced in other seasons. Therefore sampling of fish populations must encompass multiple seasons in order to provide realistic levels of prevalence and infection intensity for population simulations.

In this study it is likely that snail densities in Lake Pearson were incorrectly estimated by only sampling shallow waters (<1m depth), as a more comprehensive survey of Lake Pearson that sampled invertebrate communities in summer and winter at varying lake depths (2-17m) estimated a mean *P. antipodarum* density of 1101 individuals/m^2 (Timms 1983), almost twice that obtained in the present study. However, as parasite transmission to and from the first intermediate host is very difficult to measure in the field, a single transmission parameter that encompasses both host density and parasite transmission could be substituted to represent this lifecycle stage, for the purposes of modelling. This transmission parameter would need to reflect the likelihood that each second intermediate host species will become infected, with results from infection prevalence and intensity observed from field surveys in the present study and by MacFarlane (1952) suggesting that benthic dwelling bullies tend to be more commonly infected than pelagic koaro. Although the assessment of trematode infection in koaro was made from a very small sample size, error in the estimated infection of koaro is unlikely to significantly influence parasite dynamics because relatively rare host species usually play minor roles in transmission (see Chapter 4).

Understanding how exotic species may influence native parasite dynamics may not be as simple as sampling hosts in the field or experimentally determining parasite fitness, as field surveys and experimental infections do not take into account the influence of host densities on parasite dynamics. Furthermore, it may be unwise to generalise trends from previous studies of the influence of exotic species on native parasites because they may falsely indicate that an exotic species will be a competent host for a native parasite. This study emphasises the need to evaluate on a case by case basis the potential impacts of native parasite acquisition by exotic species on native host-parasite dynamics.

Table 5.1. Seasonal infection prevalence, intensity and reproductive status of the trematodes *Telogaster opisthorchis* and *Stegodexamene anguillae* naturally infecting exotic salmonids Lake Pearson and native longfin eel in Lake Sumner.

Parasite Species	Host	No. of hosts	Season	Prevalence %	Infection intensity (mean ± *SE*)	No. of parasites	Gravid %
T. opisthorchis	BT	32	Pre-winter	22	10.57 ± 6.19	52	2
		15	Post-winter	33	10.20 ± 5.09	48	2
	RT	30	Pre-winter	17	4.00 ± 1.48	19	0
		12	Post-winter	67	5.14 ± 2.09	55	4
	LE	10	Pre-winter	82	62.5 ± 27.9	150	80
		3	Post-winter	67	25 ± 17	29	83
S. anguillae	BT	32	Pre-winter	9	1.67 ± 0.33	9	10
		15	Post-winter	53	3.43 ± 1.00	19	14
	RT	30	Pre-winter	17	7.4 ± 4.11	30	3
		12	Post-winter	37	2.71 ± 0.57	39	13
	LE	10	Pre-winter	64	18.4 ± 5.8	84	82
		3	Post-winter	100	7 ± 3.5	39	93

Note: BT – brown trout, RT – rainbow trout, LE – longfin eel (Lake Sumner).

Table 5.2. Prevalence and infection intensity *T. opisthorchis* and *S. anguillae* metacercariae in second intermediate hosts from Lake Pearson.

Parasite Species		No. of hosts	Prevalence %	Infection intensity (mean ± *SE*)
T. opisthorchis	Common bully	40	33	1.31 ± 0.17
	Upland bully	3	67	3 ± 0
	Koaro	2	0	-
S. anguillae	Common bully	40	25	2.5 ± 0.5
	Upland bully	3	0	-
	Koaro	2	50	2

Table 5.3. Prevalence, infection intensity, size and reproductive status of the trematodes *Telogaster opisthorchis* and *Stegodexamene anguillae* in experimentally infected exotic brown trout, rainbow trout and native longfin eel.

Parasite species	Host	No. of hosts	Day	Prevalence %	Establishment % (mean ± SE)	Infection intensity (mean ± SE)	No. of parasites	Gravid %	Egg numbers (mean ± SE)	Egg size µm (mean ± SE)
T. opisthorchis	BT	5	5	60	3.71 ± 2.4	4.33 ± 2.4	13	0	-	-
		5	15	0	-	-	-	-	-	-
	RT	5	5	20	0.42 ± 0.4	2	2	50	8	3251 ±1660
		5	15	0	-	-	-	-	-	-
	LF	4	5	40	8.04 ± 6.5	17.5 ± 11.5	36	0	-	-
		5	15	40	19.72 ± 13.2	47 ± 16	96	26.04	27.96 ± 4.2	3628 ± 119
		4	25	40	26.75 ± 18.9	53.5 ± 13.5	106	66.04	42.89 ± 4.7	3915 ± 84
S. anguillae	BT	5	5	20	0.67	1	1	0	-	-
		5	15	20	1.33	2	2	50	11	84581 ± 7044
	RT	5	5	20	0.47	1	1	0	-	-
		4	15	0	-	-	-	-	-	-
	LF	4	5	25	6.67	8	8	25	7.5 ± 4.5	25895 ± 5096
		5	15	40	18.33 ± 24.4	11 ± 10	15	60	10.88 ± 1.5	59348 ± 1694
		3	25	0	-	-	-	-	-	-

Note: BT – brown trout, RT – rainbow trout, LE – longfin eel

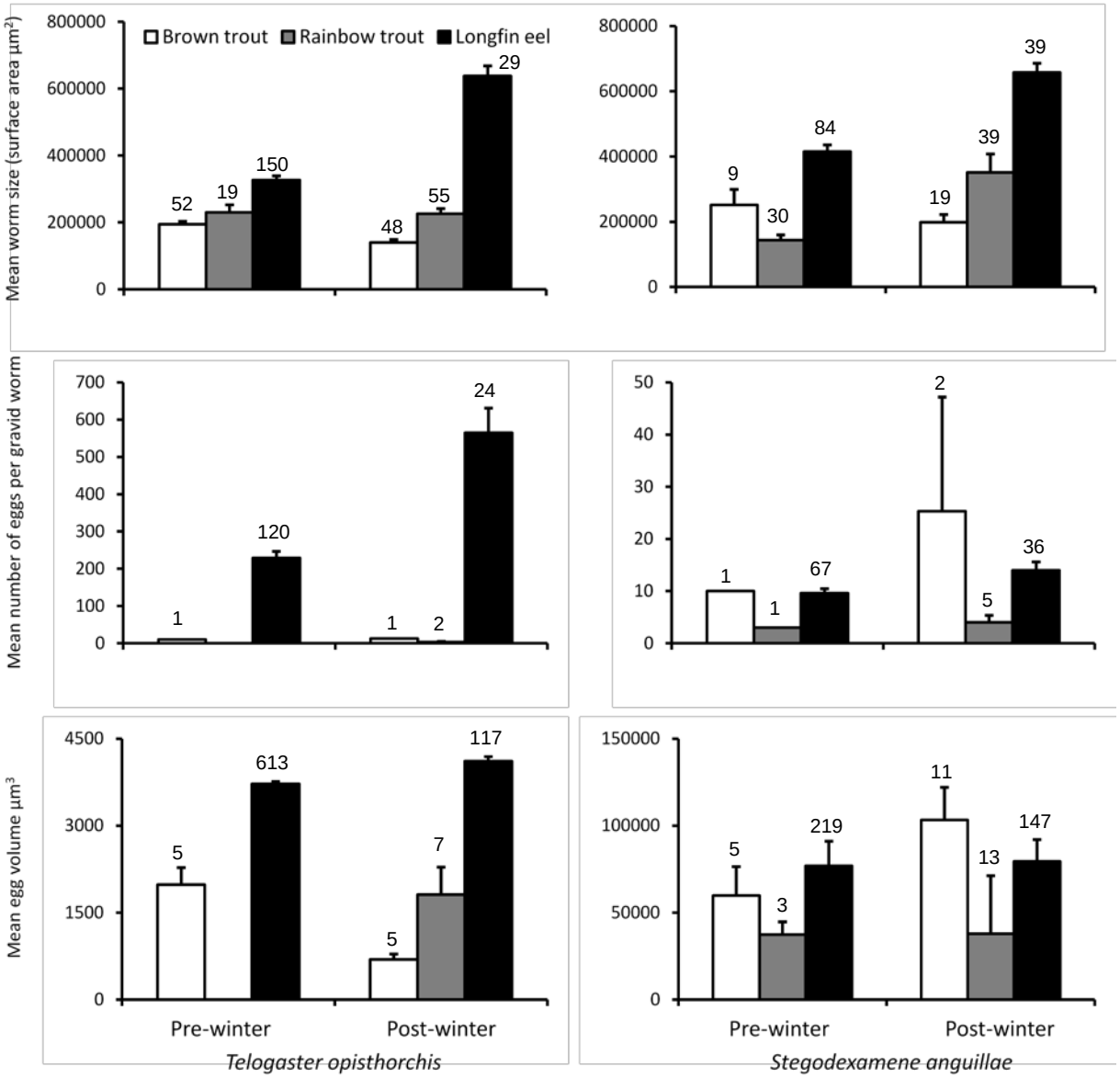


Figure 5.1. Mean worm size, number of eggs and egg volume of the trematodes *Telogaster opisthorchis* and *Stegodexamene anguillae* naturally infecting exotic salmonids in Lake Pearson and native longfin eels in Lake Sumner. Error bars indicate standard errors, with values above error bars indicating sample size.

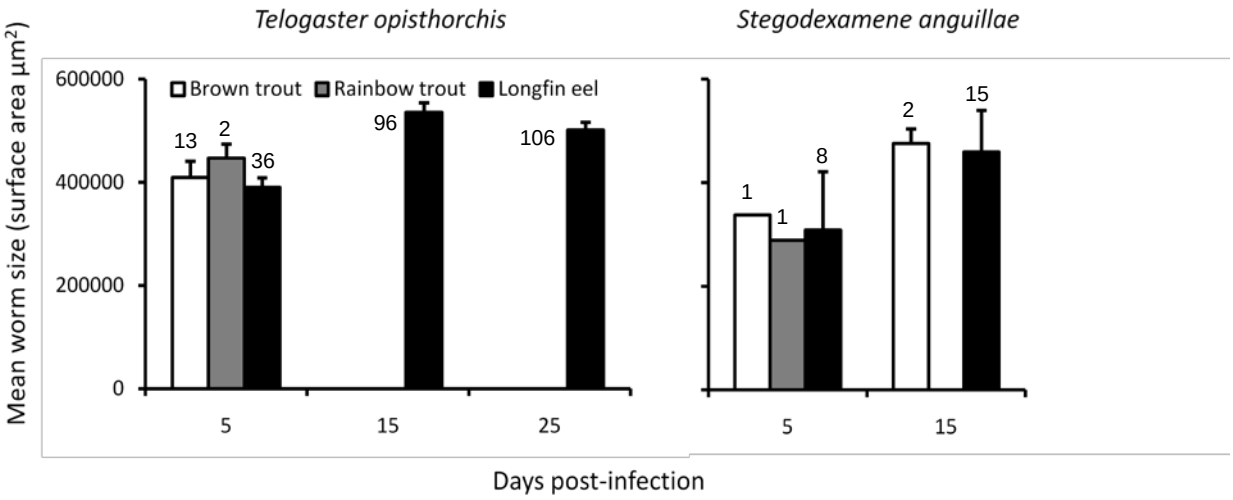
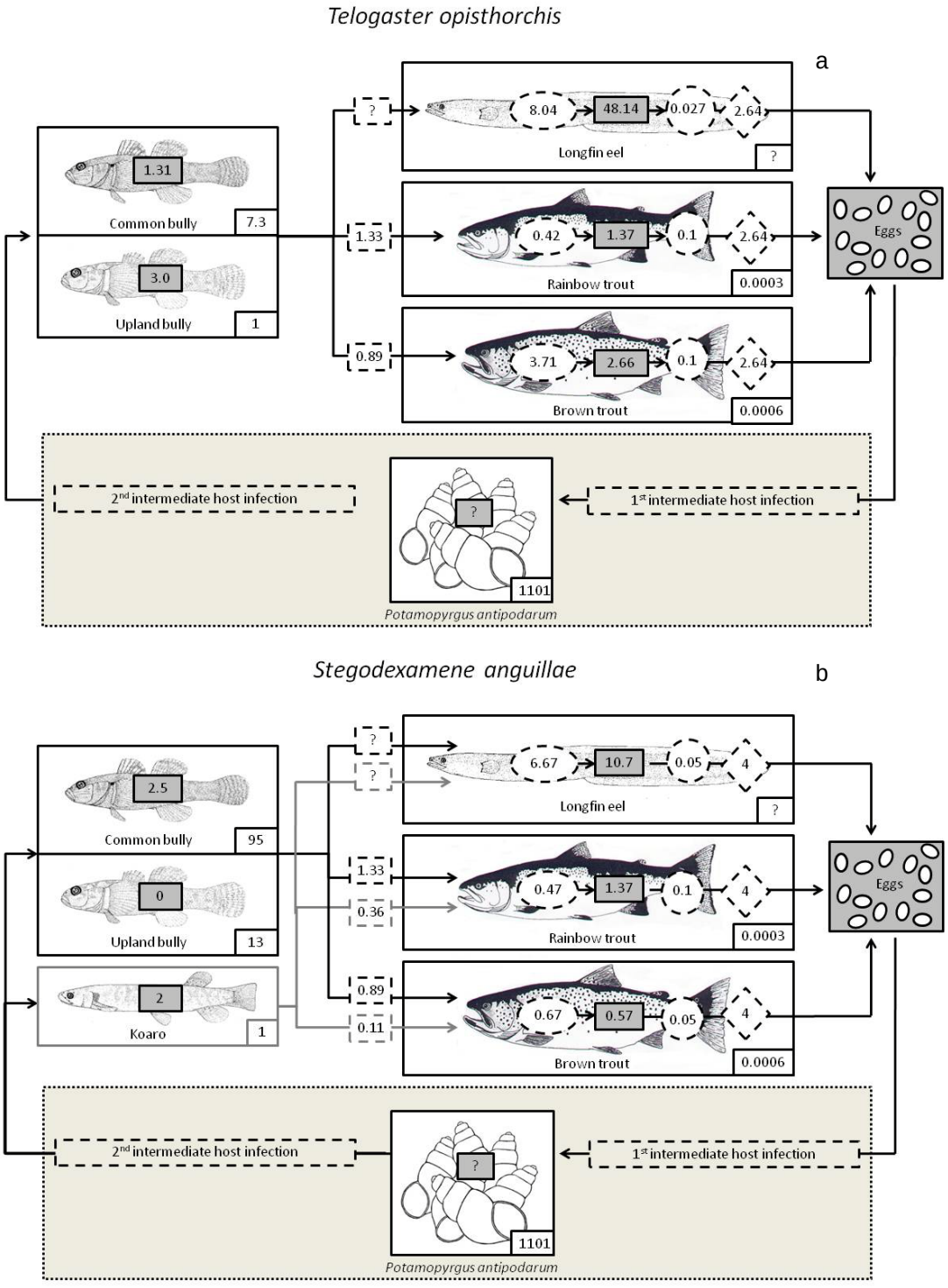


Figure 5.2. Mean worm size of the trematodes *Telogaster opisthorchis* and *Stegodexamene anguillae* in experimentally infected exotic brown trout, rainbow trout and native longfin eels. Error bars indicate standard errors, with values above error bars indicating sample size.


Telogaster opisthorchis
a
?
8.04
48.14
0.027
2.64
Longfin eel
?
1.31
Common bully
7.3
1.33
0.42
1.37
0.1
2.64
Eggs
Rainbow trout
0.0003
3.0
Upland bully
1
0.89
3.71
2.66
0.1
2.64
Brown trout
0.0006
2nd intermediate host infection
?
1101
1st intermediate host infection
Potamopyrgus antipodarum
Stegodexamene anguillae
b
?
?
6.67
10.7
0.05
4
Longfin eel
?
2.5
Common bully
95
1.33
0.36
0.47
1.37
0.1
4
Eggs
Rainbow trout
0.0003
0
Upland bully
13
2
Koaro
1
0.89
0.11
0.67
0.57
0.05
4
Brown trout
0.0006
2nd intermediate host infection
?
1101
1st intermediate host infection
Potamopyrgus antipodarum

	Infection abundance in host (parasites per host)		Bully consumption by definitive host (mean bullies per stomach)
	Host abundance: snail and trout (m^{-2}), Bully and koaro (relative ratio)		Koaro consumption by definitive host (mean koaro per stomach)
	Transmission to and from first intermediate host		Establishment success in definitive host
	Parasite flow		Maturation in definitive host (day^{-1})
	Mortality in definitive host (day^{-1})	?	Parameter value unknown

Figure 5.3. Flowchart summarising the circulation and transmission dynamics of trematodes, (a) *Telogaster opisthorchis* and (b) *Stegodexamene anguillae*, in native and exotic hosts in Lake Pearson.

CHAPTER 6

Can we predict whether an exotic freshwater fish will acquire native parasites?

Telogaster opisthorchis, a native trematode acquired by exotic fish in New Zealand

6.1 Introduction

Disease threats mediated by exotic host species frequently involve introduced parasites (e.g. Cunningham, Daszak & Rodriguez 2003, Tompkins, White & Boots 2003). However, as exotic species often lose their original parasites during translocation(MacLeod *et al.* 2010), their parasite burdens may be dominated by native parasites acquired in their new locality rather than those retained from their area of origin (Torchin *et al.* 2003, Kelly *et al.* 2009b). For example, such is the case for the many exotic fish species that have been translocated globally as parasite-free eggs or juveniles (e.g. *Oncorhynchus mykiss*; Ortubay *et al.* 1994, *Salmo trutta*; Hine, Jones & Diggles 2000). Additionally, disease threats mediated by exotic species are therefore likely to involve altered native host-parasite dynamics, which potentially result in the spillback (Daszak, Cunningham & Hyatt 2000, Kelly *et al.* 2009b) or dilution of native infection (Telfer *et al.* 2005, Thieltges *et al.* 2009).

While native parasites can dominate the parasite communities of exotic host species, both the number and type (e.g. endo- or ecto-parasites) of native parasite species acquired differ among exotic hosts (Kelly *et al.* 2009b, Poulin *et al.*). Two sets of factors may potentially influence such acquisition. First, several biological and geographical traits have been shown to influence parasite richness in fish species, including host size, diet, density and geographical range (Bell & Burt 1991, Poulin 1997, Sasal, Morand & Guegan 1997, Morand *et al.* 2000). Such factors, that have influenced the acquisition and accumulation of parasite species by particular host lineages over evolutionary time, may also be important in determining the shorter time-scale acquisition of native parasites by exotic host species. Second, traits determining the success of exotic free-living species after introduction to new regions have also been identified in the literature; in the case of fish, these include habitat, migratory behaviour and phylogeny, among others (Kolar & Lodge 2001). Understanding the importance of these factors is likely essential for predicting and managing disease emergence. However, although such factors may also influence the richness of native parasites acquired by exotic fish, the latter are frequently excluded from studies of the determinants of parasite richess, as short-term geographic and historical influences can obscure the effect of basic biological traits on rates of parasite acquisition (Luque & Poulin 2007). When parasite richness studies have included exotic fish, distinctions between traits influencing native and exotic hosts have not been made (e.g. Price & Clancy 1983).

In order to predict which exotic fish species are likely to pose disease-mediated threats to sympatric native populations, it is necessary to identify which traits increase the probability of exotic fish acquiring native parasites. This question is addressed here through the meta-

analysis of a database compiled from the literature. Specifically, I use an information-theoretic approach based on model selection to investigate the influence of a series of biological and geographical traits reported to affect the invasion success of exotic species and/or the parasite species richness of freshwater fish.

6.2 Methods

6.2.1 Data collection

Searches of both literature databases and the internet were performed to obtain freshwater fish parasite checklists, i.e. lists of the parasite species found in each fish species in a given area. Checklists were selected based on the following criteria: (1) they were published in English or Spanish, (2) the majority of parasites recorded were identified to species level, and (3) supporting reference material was obtainable. Fish-parasite checklists from 10 geographical areas met these criteria and were searched for parasite species present in the exotic freshwater fish present (or those with a freshwater phase): Argentina (Patagonia only Ortubay *et al.* 1994), Australia (Beumer *et al.* 1982), Canada (Margolis & Arthur 1979, McDonald & Margolis 1995), Chile (Olmos & Munoz 2006), Czech Republic and Slovak Republic (Moravec 2001), Hawaii (Font 2003), Ireland (Holland & Kennedy 1997), Puerto Rico (Bunkley-Williams & Williams 1994), Mexico (Salgado-Maldonado 2006) and New Zealand (Hine, Jones & Diggles 2000).

All native parasite taxa in exotic fish (present in the exotic fish's introduced range only) were identified; to avoid incorrectly classifying the origin of a parasite species, only parasites with full taxonomic names were included. Parasites classified as introduced (present in the exotic fish's original range only), or of unknown /cryptic origin (distribution of parasite could not be verified from literature) were excluded. Parasites present in both the original and introduced range of an exotic fish were also excluded from analyses, as were those with worldwide distributions (to avoid incorrectly classifying parasites that might infect exotic fish in their original ranges but have not been detected due to low sampling effort). Furthermore, only monogeneans, trematodes, cestodes, nematodes, acanthocephalans and copepods were included in analyses as other taxa, such as protozoa or myxosporea, were seldom sampled or infrequently reported.

The total number of native parasite species acquired by an exotic fish in each of its introduced ranges was determined. Although Poulin (2004) recommends distinguishing between endo- and ecto-parasites because different host traits are likely to influence whether

parasites with direct or complex lifecycles are acquired by a fish, endo- and ecto-parasites were not separately analysed as relatively few ectoparasites were reported.

Sampling effort strongly influences estimates of parasite richness (Walther *et al.* 1995), but freshwater fish parasite checklists seldom report the number of hosts examined or the duration (e.g. seasons or years) of sampling. An index of study effort was calculated to account for the effects of unequal sampling on parasite richness, by assessing the number of publications for each exotic fish species per geographical region. The country of introduction, the exotic fish's Latin name and all known synonyms extracted from FishBase (Froese & Pauly 2010) were used as keywords in a search of the Zoological Record (1864-), with the upper date limit set as the checklist publication year. This measure of study effort provides an estimate of the research activity targeting a given fish species, and thus of the relative number of individual fish examined for parasites (Luque & Poulin 2007). As study efforts of zero were recorded during this period for some species, study effort was $\log x + 1$ transformed; zero values indicate that some fish species have received little research attention in their country of introduction.

6.2.2 Predictors of parasite richness

A series of traits that relate to the exotic fish's biological characteristics, or their places of introduction, were selected for analysis as outlined below. Although traits that may influence parasite richness were selected on the basis of the current understanding of the evolution of parasite faunas (Poulin 1997), it is acknowledged that native parasite richness in exotic fish may be influenced by additional traits not included in this study. With the exception of phylogenetic relatedness, all traits were parameterised from data available from FishBase (Froese & Pauly 2010).

6.2.2.1 Biological traits

Six biological traits (host size [total length], host age, host diet, trophic level, phylogenetic relatedness and migratory behaviour) were identified from previous studies as potential determinants of parasite richness. Host size in particular is frequently invoked as a determinant of parasite richness in fish (e.g. Price & Clancy 1983, Guegan *et al.* 1992, Poulin 1997, Lo, Morand & Galzin 1998), though this relationship may not be the same for endo- and ecto-parasites (Sasal, Morand & Guegan 1997). Host age may also influence parasite

richness because long-lived hosts accumulate more parasites (Morand 2000); however, there may be collinearity between host size and host age, as large hosts tend to be long-lived. Host age and size were quantified as the maximum reported age (years), and LENGTH-LENGTH relationship tables (Froese & Pauly 2010) were used to calculate the maximum total length (cm) when standard or fork length was reported.

Host diet has also been shown to influence parasite richness, especially for endoparasites that rely on trophic transmission for completion of their lifecycles; it may be of less importance to ectoparasites relying on direct transmission between host individuals. The diet of each exotic fish species was categorised as detritivore (detritus only), planktivore (phytoplankton only), zooplanktivore (zooplankton, or phyto- and zooplankton), omnivore (plant and invertebrates/fish), insectivore (invertebrates only), carnivore (invertebrates and fish) or piscivore (predominantly fish). Species consuming plants and detritus were considered omnivores, given that they could obtain parasites (e.g. trematode metacercarial cysts) from tissues of decaying animals. Fish consuming phytoplankton and zooplankton were classified as zooplanktivores to reflect the presence of animals in the diet. Although host diet is known to change with host age, host diet was classified from adult fish only because juvenile diet was seldom reported. The trophic level of a host is an alternative measure of the influence of diet, and has also been shown to be positively correlated with parasite richness (Luque & Poulin 2008). Trophic level was estimated from the number of energy transfer steps separating a fish species from basal resources.

Phylogenetic relatedness of an exotic fish to the native fish fauna may alter the probability of native parasite acquisition by exotic fish, since parasites adapted to a specific native fish genus or family may be more likely to be acquired by an exotic fish of the same genus or family than by more distantly related fishes (Freeland 1983). Phylogenetic relatedness was quantified as the number of taxonomical steps between an exotic species and the closest native species in the introduced country's fish fauna (exotic fish and closest native species are in the same genus = 1 step, same family = 2 steps, same order = 3 steps).

Migratory patterns of exotic fish also have the potential to influence native parasite acquisition, as fish moving between different freshwater habitats (potamodromous species) are likely to encounter different suites of parasites associated with different populations or species of native fish. Fish species that have a marine life history phase may acquire fewer native parasites as these hosts are only present in freshwater habitats for a portion of their life. This is especially true for anadromous species that utilise freshwater habitats for breeding only, and may not feed in freshwater environments, thus reducing their encounter rates with

trophically transmitted parasites. In contrast, the migration patterns of catadromous species, which spend the majority of their lives in freshwater environments, should have a lesser impact on the acquisition of native parasites as catadromous species only enter marine environments for breeding. Here, I separated exotic fish species into four categories: non-migratory, potamodromous, anadromous, or catadromous.

6.2.2.2 Geographic factors

I identified four factors (latitude of introduced region, latitudinal difference between original and introduced regions, time since introduction, richness of native fish fauna) associated with the country of introduction that may influence native parasite acquisition. The difference between the mean latitude of an exotic fish's original distribution and that of the region of introduction was calculated. Freshwater fish from temperate regions have been shown to have greater parasite richness than those from tropical regions when the effects of host size and sampling effort are taken into account (Poulin 2001), suggesting that fish introduced to mid-latitude regions may acquire more native parasites due to the greater availability of parasite species.

Local parasite richness is often positively correlated with local host richness (Watters 1992, Krasnov *et al.* 2004, Thieltges *et al.* in press); therefore, the number of native hosts present in a country may also influence the size of the pool of native parasites available for acquisition by exotic hosts. The probability of an exotic species acquiring native parasites may also increase with the length of time the exotic species has been in a new habitat. Time since introduction was calculated as the difference between the year of introduction and publication date of the parasite checklist.

6.2.3 Statistical approach

Total number of native parasites acquired was analysed using a Generalised Linear Mixed Model (GLMM), within a quasi-Akaike information criterion (QAIC) and model averaging framework (Bolker *et al.* 2009). The QAIC was selected rather than an Akaike information criterion (AIC), because the quasipoisson distribution in the former takes into account the over-dispersed nature of the response variable (number of native parasites acquired). As collinearity amongst explanatory variables can cause problems with model selection and parameter estimation (Freckleton 2010), potential collinearity between

positively correlated predictors was investigated through preliminary data exploration. One of two positively correlated predictors (e.g. host size and host age; host diet and trophic level; introduced latitude and richness of native fish fauna) was removed from the global set to generate a sub-set of variables that could potentially influence parasite species richness in exotic fish species. Preliminary data exploration revealed that difference in migratory behaviour, and latitude differences between original and introduced locations did not have a strong influence on parasite richness, so these factors were excluded from further analysis to avoid over-parameterising the model. The final set of explanatory variables included host length, trophic level, time since introduction, phylogenetic relatedness and richness of the native fish fauna. The final dataset included only fish species for which data for all explanatory variables were available because missing data prevents model averaging.

The global model included study effort as a random effect, to control for the influence of differential sampling on estimates of parasite richness. Fish species was also included as a random effect as some exotic fish species were introduced to multiple countries, and each introduction event was treated as a separate data point. No interaction terms were included in the global model as strong interactions between any of the explanatory values were not hypothesised. The global model that included the final set of explanatory variables was fitted in the package lme4 (Bates & Maechler 2009) of the program *R* (R Development Core Team 2010). Using the *R* package MuMIn functions (Barton 2010), a set of all possible sub-models was created from the global model. The quasi-likelihood information criterion (QAICc) was calculated by hand, and was used in conjunction with model averaging ("zero" method) to rank all sub-models within 4 $QAIC_C$ of the best model. The model-averaged parameter estimates, standard errors (SE), 95% confidence intervals and relative importance of each explanatory variable are reported for the top models.

6.3 Results

The final data set comprised 39 exotic freshwater fish introductions involving 26 different fish species in the Cypriniformes (six introductions) Cyprinodontiformes (three), Perciformes (14) and Salmoniformes (16; Table 6.1). Exotic fish acquired on average 2.4 (range 1-8) native parasites per fish per introduced locality, of which the majority were endoparasites (86.6 %).

From the global model, a total of 32 possible models were produced from the data dredging function in R, of which 14 top models were found to be within 4 QAIC of the top

model (Table 6.2). All predictor variables were included in at least one top model. Host length was included in all 14 top models, while time since introduction was included in half of all top models. All other predictor variables were included in six or fewer top models. Ninety-five percent confidence intervals for all predictor variables included zero, suggesting that no predictors included in the analyses explained a significant proportion of the variance in parasite richness (Table 6.3). R-squared values, calculated for each predictor variable, showed that a weak relationship existed only between the number of native parasites acquired and host size or time since introduction (Figure 6.1).

6.4 Discussion

Although numerous studies have reported that parasite species richness is influenced by a number of traits associated with the host's biology or geography (e.g. Watters 1992, Poulin 1997, Morand *et al.* 2000, Poulin 2001), this study found that none of these traits was a strong predictor of the number of native parasites acquired by exotic fish. Traits known to influence invasive species establishment also had little power to predict whether exotic species subsequently acquire native parasites upon introduction to a new area. At best, both host size and time since introduction have weak influences on native parasite acquisition, with some evidence to suggest that larger-bodied species or those introduced earlier have more native parasites than recently introduced or smaller-bodied species. Neither of these relationships, if they hold, is unexpected. Host size is often considered a major determinant of parasite species richness in native fish (e.g. Price & Clancy 1983, Guegan *et al.* 1992, Lo, Morand & Galzin 1998), while the time since introduction has also been shown to affect parasite acquisition in fish when the time scale considered is relatively long (Guégan & Kennedy 1993). For exotic plants as well, time since introduction has a strong influence on pathogen acquisition, with species introduced 400 years ago acquiring over six times more pathogens than those introduced 40 years ago (Mitchell *et al.* 2010).

Diet and/or trophic level are often invoked as determinants of parasite species richness, especially for endoparasites that rely on trophic transmission. More specifically, Chen (2008) has demonstrated that host species with high parasite richness are characterised by having wider diet ranges or occupying food chain positions that are either close to many prey species or that allow them to accumulate parasites from lower trophic levels. Although the majority of native parasites acquired by exotic fish in this study were endoparasites, neither trophic level nor diet was shown to be a strong determinant of parasite richness of

exotic fish. Similarly, the presence of closely related species did not show a clear relationship with parasite acquisition, although host phylogenetic relatedness often constrains the spectrum of host species that a parasite can infect (Mitchell *et al.* 2010). For instance, both *Carassius gibelio* and *Thymallus baicalensis* introduced to the Czech and Slovak Republics, where native *Carassius* sp. and *Thymallus* sp. are present, acquired only one native parasite species each, while exotic fish with no close phylogenetic affinities to any species in the native fish communities showed highly variable native parasite acquisition.

The results of this study suggest that some predictors of parasite richness in native communities may become less important for exotic species. For example, while parasite species richness may be related to the diversity of native hosts, which itself is correlated to the size of available habitat (Thieltges *et al.* in press), an exotic species may occupy only a small portion of this habitat and interact with only a fraction of the native fish fauna. This restricts the potential pool of parasites that an exotic species may encounter to its relative overlap with native species, thus making the overall number of native fish present or habitat size poor predictors of the potential to acquire native parasites. A measure of the relative area invaded or the proportion of native fish actually encountered by an exotic fish species (both currently unavailable) may reveal stronger correlations between these variables and native parasite acquisition

It is of course possible that other biological or geographical traits not considered here may determine native parasite acquisition by exotic hosts. To account for this potential bias, a wide set of biological and geographical traits were included in the initial model set, with traits subsequently removed from the global model based on collinearity. There is also a limit to the number of predictor traits that can be included in GLMM's for a model to be fit, so only the traits with greatest influence on parasite richness in exotic fish (based on univariate exploratory analyses) were incorporated in the global model.

Alternatively, the results may suggest that the process of becoming a host for a new parasite depends on a complex set of interactions involving aspects of host biology, and spatial and temporal scales that cannot be measured by a few generalised traits. For instance, the compatibility of a parasite for a novel host at the physiological or immunological levels is probably independent of that host's size, its latitude of origin, or how long it has been introduced to the parasite's area. In both the exotic species' original region and in the native parasite's region, parasites have evolved adaptations over very long time scales that allow them to infect locally available hosts. However, when exotic species are introduced to new localities, the evolutionary timescale for such adaptations to develop is relatively short. Host-

parasite encounter may be strongly dependent on host ecology, but host-parasite compatibility allowing a parasite to establish inside a novel host will either require numerous generations to evolve, or already exists independently of the ecological factors included in the present analysis, which makes it unpredictable using the ecological variables.

Finally, exotic hosts may not be suitable hosts for all native parasites that they encounter, even if those parasites briefly survive inside exotic hosts. This would further reduce the chance of exotic fish being reported as hosts for certain native parasites, although sympatric native fish act as sources for a variety of native parasites. Examples of this can be seen in New Zealand streams, where brown trout are host to only two of seven native parasites present in sympatric native fish species (Kelly *et al.* 2009a), and in Italy where exotic fish populations harbour none of the 10 parasite species found in sympatric native fish (Galli *et al.* 2005). The likelihood that an exotic fish will acquire native parasites may also be a function of the proportion of generalist parasite species available in a locality, since these are more likely to be acquired by novel hosts (Poulin & Mouillot 2003, Kelly *et al.* 2009b). If this is the case, disease threats mediated by the acquisition of native parasites by exotic hosts are likely to involve generalist parasites, while modified host-parasite dynamics resulting in parasite dilution could involve either generalist or specialist parasite species.

Table 6.1. The number of native parasites acquired, and host and geographical characteristics for each exotic fish species per country of introduction.

Country	Host	Native parasites	Study effort (log transformed)	Host characteristics		Geographical characteristics		
				Total Length (cm)	Trophic level	Time since introduction (years)	Native fish species richness	Phylogenetic relatedness (steps)
Argentina	*Oncorhynchus mykiss*	8	1.000	120	4.42	90	485	4
	Salmo salar	1	1.176	150	4.43	90	485	4
	Salmo trutta	2	0.778	150	3.16	90	485	4
	Salvelinus fontinalis	3	0.903	95	3.14	90	485	4
	Salvelinus namaycush	1	0.000	150	4.29	90	485	4
Australia	*Carassius auratus*	1	0.954	59	2.00	107	319	4
	Oncorhynchus mykiss	1	1.204	120	4.42	89	319	4
	Salmo trutta	2	1.653	150	3.16	119	319	4
Canada	*Cyprinus carpio*	7	1.301	126	2.96	164	206	2
	Salmo trutta	4	1.623	150	3.16	90	206	2
Chile	*Oncorhynchus mykiss*	4	1.863	120	4.42	101	44	4

	Salmo trutta	4	1.491	150	3.16	101	44	4
Czech and Slovak Republics	*Carassius gibelio*	1	0.903	45	2.54	29	76	1
	Ctenopharyngodon idella	4	0.778	150	2.00	40	76	2
	Lepomis gibbosus	5	1.041	40	3.12	72	76	3
	Oncorhynchus mykiss	3	1.322	120	4.42	110	76	2
	Pseudorasbora parva	2	0.778	11	3.04	36	76	2
	Salvelinus fontinalis	4	1.079	95	3.14	111	76	2
	Thymallus baicalensis	1	0.699	35	3.53	42	76	1
Hawaii	*Poecilia reticulate*	1	0.954	8	3.20	103	7	4
	Xiphophorus helleri	1	0.699	14	3.19	81	7	4
Ireland	*Oncorhynchus mykiss*	3	1.146	120	4.42	97	21	2
Mexico	*Oreochromis aureus*	6	1.279	46	2.07	40	511	2
	Oreochromis mossambicus	4	1.146	47	2.00	40	511	2
	Oreochromis niloticus niloticus	2	1.114	76	2.00	40	511	2
	Oreochromis urolepis hornorum	1	0.778	31	2.00	26	511	2
	Tilapia zillii	1	0.778	52	2.00	59	511	2
New Zealand	*Carassius auratus*	2	0.954	59	2.00	134	40	4
	Oncorhynchus tshawytscha	2	1.875	150	4.40	124	40	4
	Perca fluviatilis	2	1.000	72	4.35	133	40	3
	Salmo trutta	5	2.053	150	3.16	133	40	4

Puerto Rico	*Lepomis auritus*	1	0.000	31	3.12	37	22	3
	Lepomis macrochirus	1	0.000	41	3.18	79	22	3
	Lepomis microlophus	1	0.000	43	3.39	37	22	3
	Micropterus coosae	1	0.000	47	3.60	36	22	3
	Oreochromis aureus	1	0.000	46	2.07	23	22	3
	Oreochromis mossambicus	2	0.477	47	2.00	36	22	3
	Poecilia reticulate	2	0.301	8	3.20	59	22	3
	Tilapia rendalli	1	0.000	45	2.18	31	22	3

Table 6.2. The 14 top-ranked models sorted by corrected quasi-Akaike information criterion (QAICc), with model deviance, difference in QAICc from the best model ($\Delta QAIC_C$) and model weights ($QAIC_W$). Models within 4 QAICc of the top model were considered in the top model set.

Response	Model	Deviance	$QAIC_C$	$\Delta QAIC_C$	$QAIC_W$
Parasites	Total length + Time since introduction	35.88	114.68	117.56	0.17
	Total length + Phylogenetic relatedness + Time since introduction	35.01	114.24	118.12	0.13
	Total length + Time since introduction + Trophic level	35.13	114.59	118.47	0.11
	Total length + Phylogenetic relatedness	36.27	115.79	118.68	0.10
	Total length + Trophic level	36.34	115.99	118.87	0.09
	Total length + Phylogenetic relatedness + Trophic level	35.53	115.70	119.58	0.06
	Total length + Native fish species richness + Phylogenetic relatedness	35.63	115.98	119.86	0.06
	Total length + Phylogenetic relatedness + Time since introduction + Trophic level	34.58	115.04	120.10	0.05
	Total length	37.71	117.83	119.89	0.05
	Total length + Native fish species richness + Time since introduction	35.72	116.23	120.11	0.05
	Total length + Native fish species richness + Phylogenetic relatedness + Time since introduction	34.72	115.42	120.49	0.04
	Total length + Native fish species richness + Trophic level	36.14	117.41	121.29	0.03
	Total length + Native fish species richness	37.21	118.42	121.30	0.03
	Total length + Native fish species richness + Time since introduction + Trophic level	35.06	116.40	121.46	0.02

Table 6.3. Standardized coefficients of model predictors for number of native parasites acquired by exotic freshwater fish, after model averaging of 14 top candidate models (see Table 6.2).

Response	Predictor variable	∑	Estimate	SE	95% CI
Parasite	Intercept		0.787	0.473	-0.455 to 1.402
	Total length	1.00	0.006	0.003	-0.003 to 0.008
	Time since introduction	0.58	-0.001	0.001	-0.0008 to 0.002
	Phylogenetic relatedness	0.44	-0.058	0.070	-0.067 to 0.208
	Trophic level	0.37	-0.051	0.067	-0.065 to 0.200
	Native fish species richness	0.22	6.889E-05	0.00015	-0.0001 to 0.0004

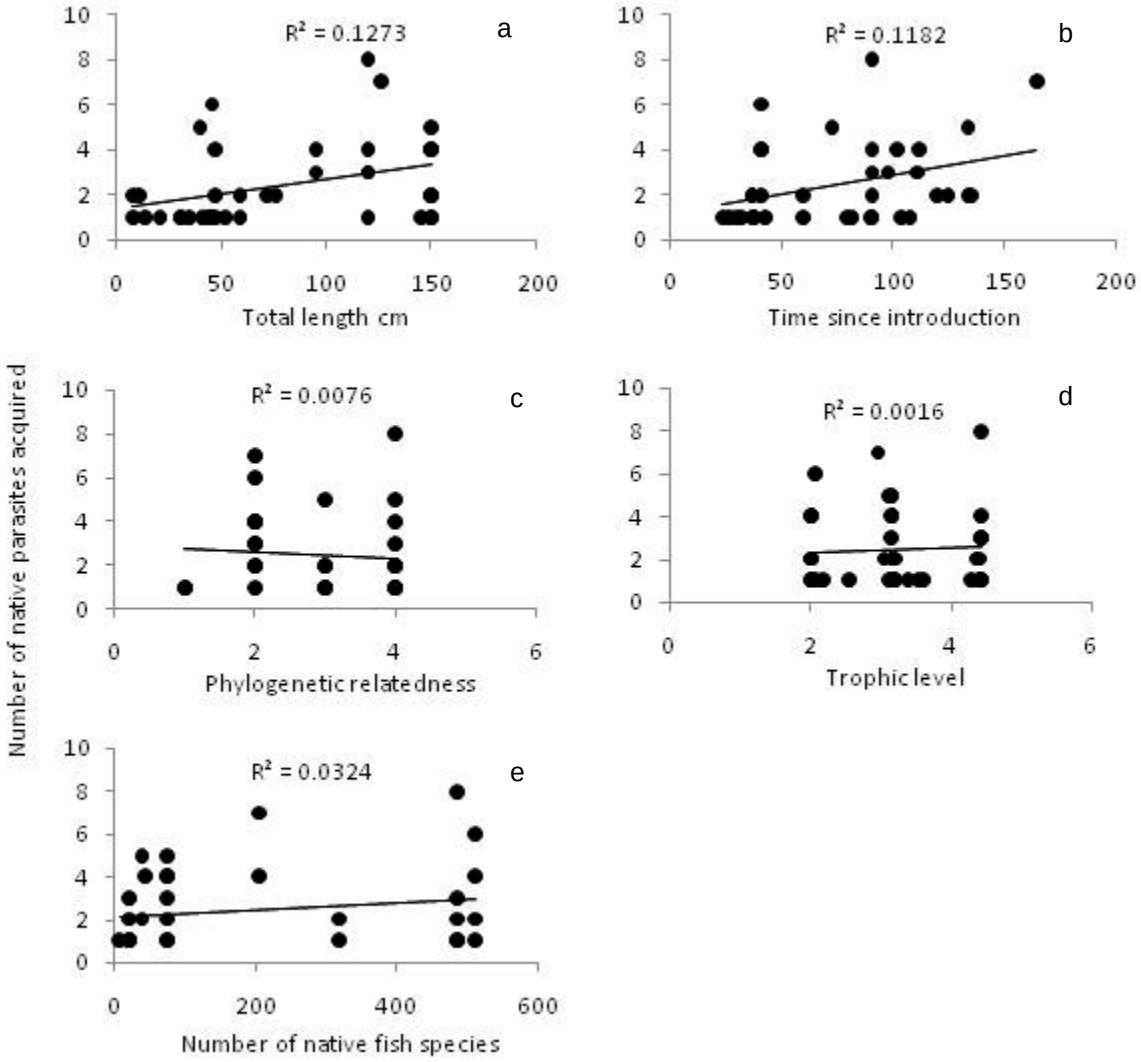


Figure 6.1. The relationships between the number of native parasites acquired by exotic host species and (a) total length, (b) time since introduction, (c) phylogenetic relatedness, (d) trophic level and (e) the richness of the native fish fauna. R^2 values represent the proportion of the variance explained by each predictor.

CHAPTER 7

General Discussion

7.1 What determines whether an exotic fish will acquire a native parasite?

Exotic species frequently have a detrimental influence on native species through a variety of mechanisms, including disease (Roelke-Parker *et al.* 1996, Van Riper, Van Riper & Hansen 2002, Tompkins, White & Boots 2003). Thus knowing which host traits facilitate native parasite acquisition is useful not only in predicting which, and understanding why, certain exotic species acquire more native parasite than others, but also in assessing the risks associated with exotic species range expansion or the proposed introduction of new species. However, the results of my meta-analysis suggest that traits known to influence parasite richness in native fish (e.g. Bell & Burt 1991, Poulin 1997, Sasal, Morand & Guegan 1997, Morand *et al.* 2000) or invasion success of exotic species (e.g. Kolar & Lodge 2001) are not reliable predictors of native parasite acquisition by exotic fish (Chapter 6). Instead, it is more likely that complex interactions between a variety of biological, geographical and historical factors govern parasite acquisition by exotic species, making it difficult to predict whether native parasites will be acquired. Despite this uncertainty as to which traits influence native parasite acquisition, exotic species are nevertheless increasingly reported as hosts of native parasites (e.g. Torchin *et al.* 2003, Kelly *et al.* 2009b), and thus have the potential to alter native host-parasite dynamics.

7.2 What determines whether native host-parasite dynamics will be altered by an exotic fish?

My thesis has examined two key factors that strongly influence whether the dynamics of native parasites will be affected by exotic fish. On one hand, the competency of exotic fish for native parasites is an important determinant of whether native parasite populations are likely to increase or decrease (Figure 7.1). On the other hand, the relative abundance of the exotic species determines whether its competency for a native parasite will actually translate into altered native host-parasite dynamics, with highly abundant exotic species more likely to induce changes in native parasite dynamics (Figure 7.1). The studies of *Acanthocephalus galaxii* in New Zealand (Chapter 3) and of *Acanthocephalus tumescens* in Argentina (Chapter 4), suggest that the importance of exotic species competency for a native parasite diminishes to almost nothing when exotic species are relatively rare in the community.

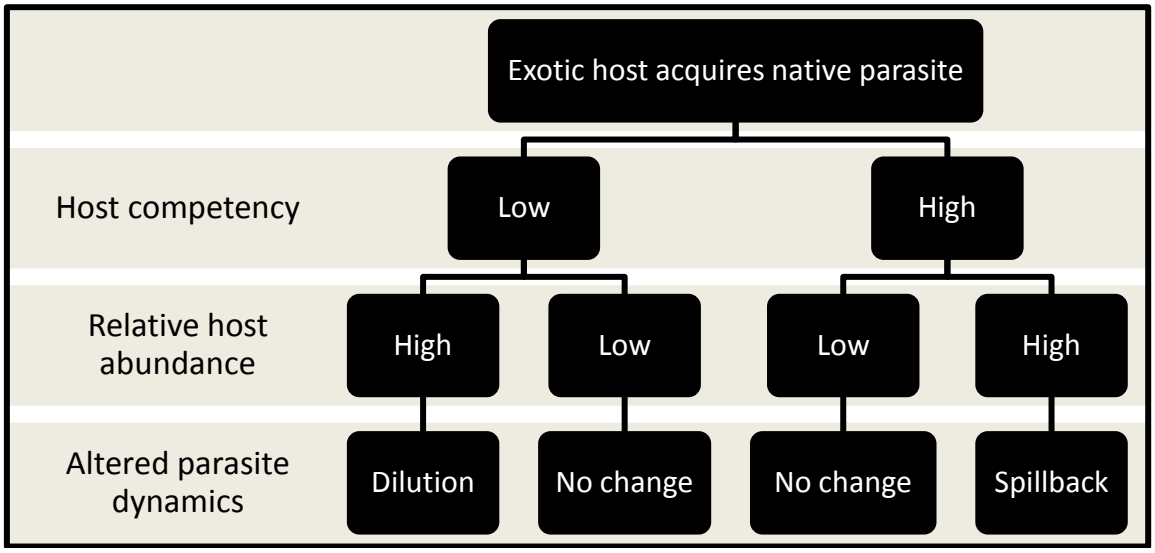


Figure 7.1. Influence of exotic host competency and relative abundance on native host parasite dynamics.

This thesis also highlights that, in some instances, exotic species may be able to override the influence of low host abundance, or competency, to alter native host-parasite dynamics through other mechanisms. Brown trout are known to change the behaviour of native fish, resulting in altered activity patterns, habitat use and foraging (see Townsend 2003, McIntosh *et al.* 2010 for examples). The *A. galaxii* study suggests that changes in native host behaviour that alter their encounter rates with native parasites provide a mechanism for relatively rare exotic hosts to modulate parasite burdens in native hosts. Behavioural changes in native hosts also allow exotic species to manipulate the dynamics of native parasites that the exotic species themselves may not actually encounter or for which they cannot act as a host, as seen in the Otago streams survey (Chapter 2).

This research also emphasises the benefits of using a multi-pronged approach of field surveys, experimental infections and dynamic population modelling to assess the influence of exotic species on native parasite population dynamics (Chapters 3, 4 and 5). While field-based observations are essential to determine which parasites have been acquired by exotic species; differences in parasite fitness parameters between native and exotic hosts observed only from the field may not provide a true reflection of the parasite's overall performance in a host. Experimental infections overcome some of the potential limitations of field observations, by controlling for the dose and timing of infection; yet, even this approach fails to account for the dynamics of the whole host/parasite system. Dynamic population modelling can allow researchers to understand how differences in host competency and abundance may manifest

themselves in community dynamics, and in the case of *A. galaxii*, modelling allows one to derive hypotheses regarding the most likely mechanisms behind infection dilution in native hosts. Furthermore, dynamic population models can also provide predictions about the consequences of altered species compositions (e.g. invasions, extinctions) and assist in identifying the most influential parameter(s) in a system. However, a modelling approach on its own is somewhat constrained by the availability of quality data to parameterise models (e.g. long-term field data sets, experimental infections), in order for population simulations to depict 'real world' dynamics. In the case of *Telogaster opisthorchis* and *Stegodexamene anguillae* populations in Lake Pearson (Chapter 5), the absence of accurate host density information currently restricts the use of modelling to assess the influence of exotic salmonid hosts on the dynamics of these native trematodes.

7.3 Future research directions

Although it appears that the combined influences of exotic host competency and relative host abundance prevented spillback from occurring in the systems studied here, the absence of spillback may also be a consequence of the study focusing on fish populations in relatively pristine upland environments. Anthropogenic stressors (e.g. agricultural pollutants) may lower the immunity of freshwater organisms to parasitism (Sures 2006) and pollutants may accumulate in lowland waterways. Thus, fish from degraded lowland environments may harbour greater parasite burdens than those from less contaminated upland regions. In New Zealand, the interaction between pollutants and host immunity could potentially result in lowland trout being more competent hosts in which native parasites survive to maturity because of suppressed host immunity. Native fish in lowland environments may also have lower resistance to native parasites and their survival may be impacted by amplified parasite infections generated from exotic hosts. Extending field surveys to incorporate a wider geographic range may reveal a greater potential for spillback for those native parasites already identified as being acquired by salmonids.

The findings of this thesis may also be strengthened by assessing the dynamics of native parasites from systems not yet invaded by exotic salmonids. While modelling is useful in hypothesising the dynamics of native parasite populations in the absence of exotic fish, extending field surveys to incorporate systems containing only native fish would allow model predictions to be verified. Locating comparable trout-free systems, in New Zealand, to assess native parasite dynamics is becoming increasingly difficult, however, both because of the

ability of trout to self-colonise new regions, and through illegal introductions of fish to waterways that were previously protected from trout invasion by natural barriers.

Finally, the meta-analysis presented in this thesis focused on the host traits that may determine the number of native parasites an exotic fish acquires. Although none of the traits examined strongly influenced native parasite acquisition, exotic fish are host to numerous native parasites for which they have varying competency. Investigations of which host traits influence whether an exotic species will be a competent native parasite host, and whether certain parasite types are more likely to achieve greater fitness in exotic hosts, would be useful in developing a predictive model to assess whether highly abundant exotic species are likely to spillback or dilute infection in native hosts

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APPENDIX 1

Parasite spillback: A neglected concept in invasion ecology?

CONCEPTS & SYNTHESIS
EMPHASIZING NEW IDEAS TO STIMULATE RESEARCH IN ECOLOGY

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Parasite spillback: A neglected concept in invasion ecology?

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Abstract. While there is good evidence linking animal introductions to impacts on native communities via disease emergence, our understanding of how such impacts occur is incomplete. Invasion ecologists have focused on the disease risks to native communities through "spillover" of infectious agents introduced with nonindigenous hosts, while overlooking a potentially more common mechanism of impact, that of "parasite spillback." We hypothesize that parasite spillback could occur when a nonindigenous species is a competent host for a native parasite, with the presence of the additional host increasing disease impacts in native species. Despite its lack of formalization in all recent reviews of the role of parasites in species introductions, aspects of the invasion process actually favor parasite spillback over spillover. We specifically review the animal-parasite literature and show that native species (arthropods, parasitoids, protozoa, and helminths) account for 67% of the parasite fauna of nonindigenous animals from a range of taxonomic groups. We show that nonindigenous species can be highly competent hosts for such parasites and provide evidence that infection by native parasites does spillback from nonindigenous species to native host species, with effects at both the host individual and population scale. We conclude by calling for greater recognition of parasite spillback as a potential threat to native species, discuss possible reasons for its neglect by invasion ecologists, and identify future research directions.

Key words: enemy release; infectious disease; invasion ecology; nonindigenous species; parasite; parasite-spillback hypothesis; spillover of infectious agents.

Introduction

The introduction and spread of nonindigenous species (NIS) threatens native biodiversity and the ecosystem function of terrestrial, marine, and freshwater communities globally (Jenkins 2003, Clavero and Garcia-Berthou 2005). While predation and competition have traditionally been considered the key mechanisms underlying NIS impacts (D'Antonio and Kark 2002, Sax et al. 2002), there is now also good evidence for disease impacts (Daszak et al. 2000, Cleaveland et al. 2002, Ladeau et al. 2007). In particular, many studies have documented how infectious agents introduced with NIS can affect native communities, especially when introduced hosts act as reservoirs from which infection can "spill over" to native species. While spillover has received considerable attention (Cleaveland et al. 2002, Prenter et al. 2004), another mechanism of potential importance in NIS-driven disease impacts has not been formalized in invasion ecology. In addition to introducing nonindigenous agents, NIS may also act as new hosts for native infectious agents, from which infection may "spill back" to native fauna (Fig. 1; Daszak et al. 2000, Tompkins and Poulin 2006).

While NIS often leave behind, or lose, their natural parasites (Dobson and May 1986, Torchin et al. 2003, Tompkins and Poulin 2006), they tend to acquire generalist parasites from the local fauna (Poulin and Mouillot 2003). Hence, NIS have the potential to impact disease in native fauna via the spillback of native parasites. Theoretical treatments of predator–prey and biological control systems already recognize dynamics that are analogous to parasite spillback. Modeling studies predict that native prey populations may be at risk of local extinction when native predator populations are enhanced via consumption of NIS prey (Noonburg and Byers 2005). Similarly, introduced biological-control agents are often exploited by native natural enemies whose enhanced numerical response could indirectly affect nontarget native species (Pearson

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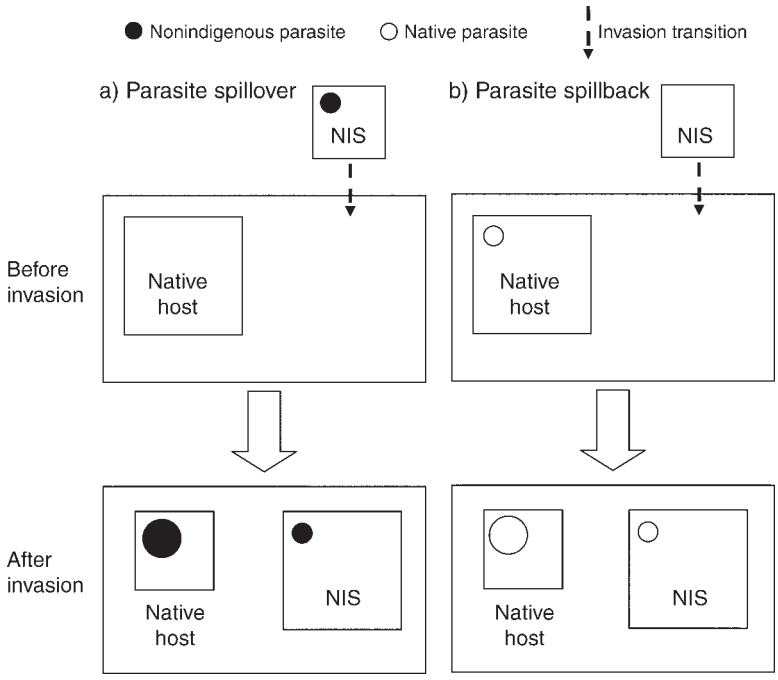

FIG. 1. Hypothetical examples of (a) parasite spillover and (b) parasite spillback, illustrating the fundamental differences between the two processes. The large rectangle represents the native environment. The size of the circle relative to host box represents infection burden. The size of the host box represents population size/density. In panel (a), infection of a native host population by a nonindigenous (NIS) parasite occurs after the introduction of that parasite's NIS host. Infection of the native host is maintained by the NIS host, which acts as a reservoir of infection. Infection of the native host by the NIS parasite causes reduced population size, compared to preinvasion levels. Note that the native host may or may not possess native parasites. In panel (b), the NIS acquires a native parasite that is already present in a native host population. The infection burden of the native parasite in the native host is increased by the NIS, which acts as a new reservoir of infection. Elevated infection in the native host causes reduced population size, compared to preinvasion levels.

Notes: Increased infection burden in a native host population in the presence of an NIS reservoir is not solely reliant on that reservoir having a larger population size; this is because the NIS may actually be a more suitable host for native parasites (see *Are NIS competent hosts for native parasites?*). Note also that the NIS host may or may not lose parasites from its native range upon introduction to a new region.

and Callaway 2005). For example, the enhancement of native hyperparasitoid populations upon introduction of parasitoids for biological control, could lead to suppression of native parasitoid populations (e.g., Heimpel et al. 2004). Parasite spillback is thus one form of "apparent competition," where two or more species that do not compete directly for resources, share a natural enemy whose numerical response is predicted to have reciprocal negative population effects (Holt 1977, Holt and Lawton 1993). However, despite a vast literature on apparent competition, parasite spillback has been either overlooked or never considered in recent reviews of the role of parasites in species introductions and as threats to native populations (Prenter et al. 2004, de Castro and Bolker 2005, Hatcher et al. 2006, Pedersen et al. 2007). There are two possible reasons for this. First, studies of NIS impacts have generally focused on introduced parasites, assuming that native parasites are unimportant. Second, native parasites are unimportant, with the potential for impacts on native fauna being rare.

Here we present evidence supporting parasite spillback as a potentially common impact of NIS on native animals, discuss the likely reasons for its prior neglect, and identify avenues for future research. Our aim is to raise awareness and stimulate research effort into what is likely an important, but currently untested, concept in invasion ecology

THE POTENTIAL FOR PARASITE SPILLBACK

The acquisition of native parasites by nonindigenous species (NIS) is the essential precursor for spillback to occur. As noted above, data already show how NIS can acquire native parasites upon introduction or invasion. For example, in their review of "enemy release" by NIS (in this case, the loss of parasites), Torchin et al. (2003) reported that while an average of three helminth parasites were co-introduced with NIS, an average of four helminth species were acquired in the newly colonized region. Moreover, in a meta-analysis of the parasitoids of 87 NIS insect herbivores, an average of 4 native parasitoids were acquired in introduced regions, with over 25% of hosts acquiring >10 parasitoid species (Cornell and Hawkins 1993). To further investigate the extent of this phenomenon in introduced animals, with

the aim of characterizing how broad or narrow the potential for spillback impacts of NIS is, we searched the ISI Web of Science (WOS) database (see Appendix A for a full description of the methods).

Data were obtained from comprehensive parasite faunal surveys for 40 animal NIS, in which a mean of 6.3 native parasite species were acquired per host, with native parasites dominating (67.0%) the total parasite fauna of each nonindigenous animal host (see Appendix B for the list of studies). Of the NIS, 70% acquired $\geq$4 native parasites, and 15% acquired >10 native parasites (Fig. 2). At least 38 of the 40 NIS hosts acquired parasites that were generalists, being reported from more than one native host species. NIS hosts were diverse, and comprised aquatic and terrestrial invertebrates, fish, amphibians, reptiles, birds, and mammals. The types of parasite acquired also were diverse, including helminths, arthropods, protozoa, and parasitoids. While NIS acquired native viruses and bacteria (see Appendix C), the reports probably represent a fraction of the actual acquisition since the literature was often unclear with respect to virus or bacterial status. Indeed, numerous studies documenting the sharing of these and other parasites between native wildlife and NIS were excluded from our main analysis due to uncertain parasite origin (see Appendix D for the full list of studies). The limited availability of data on shared parasites partly biases our selection of NIS, and precludes accurate estimates of the total number of native parasites acquired per NIS. Nonetheless, the breadth of taxonomic coverage, for both animal host and parasite type, is sufficiently comprehensive to illustrate that animal NIS commonly acquire native parasites.

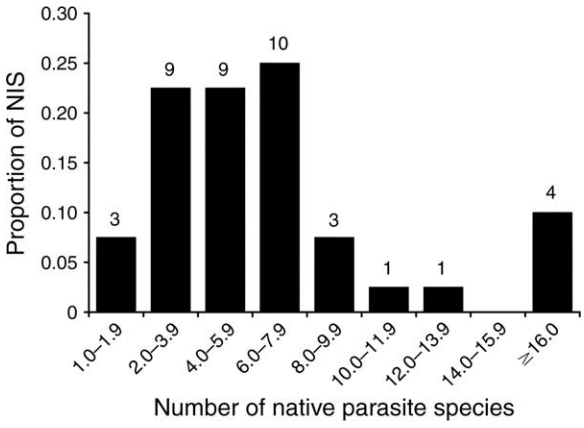

FIG. 2. Frequency distribution of native parasites acquired by the 40 nonindigenous species (NIS) hosts identified in our literature review. The number of parasites acquired is not discrete since there were several NIS for which multiple studies were conducted in different invaded regions. In such cases, the number of parasites acquired was the mean number across multiple studies. The actual number of studies is indicated above each bar.

Are NIS Competent Hosts for Native Parasites?

The acquisition of a native parasite by a nonindigenous species (NIS) will not automatically lead to the spillback of infection to native fauna; the NIS also needs to be a competent host for the parasite, and be capable of disseminating the parasite's infective stages (i.e., be a "reservoir" host in which the parasite can persist and reproduce; Tompkins et al. 2000*b*, Holt et al. 2003, de Castro and Bolker 2005). If this were not the case, the NIS would be a sink of parasite infection that could potentially act to reduce infection levels in the native fauna ("dilution" effect; Heimpel et al. 2003, Keesing et al. 2006).

There is evidence from a range of studies that NIS are not only often competent hosts for native parasites, but can also be better than the original native hosts (i.e., the parasite's basic reproductive rate, R_0, is greater when infecting them). For example, Ross River virus, a mosquito-borne virus native to Australia, is acquired by horses and other domestic stock from native marsupials (Russell 2002), with seroprevalence in horses even exceeding that of native marsupials (Vale et al. 1991, Kay et al. 2007). Another example is eastern equine encephalitis virus (EEEV), a virus native to North America, occurring principally in an enzootic cycle involving mosquito vectors and a variety of native avian hosts, which is often fatal in a range of mammalian and avian species (Komar et al. 1999, Nolen-Walston et al. 2007). In a comparison of reservoir competence of introduced European Starlings (*Sturnis vulgaris*) vs. 11 native bird species, starlings had higher intensities and durations of viraemia, and infected a higher proportion of mosquito vectors than did native birds (Komar et al. 1999). As a result, even though they suffered higher mortality from infection, starlings were twice as competent as reservoirs of infection as any native species.

There are also cases where fitness-associated traits of native macroparasties are higher in NIS vs. native hosts. For instance, in many introduced salmonid fish, native macroparasites occur in equal or higher abundance (percentage of fish infected, mean number of parasites per fish) than in their native fish hosts (Kennedy et al. 1991), with some parasites also being more fecund. This is illustrated by a study on Lake Moreno (Argentina), where four of the five helminth parasites of introduced rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*), both native to North America, are acquired from native fish (Rauque et al. 2003; Appendix B). In a comprehensive assessment of host infection by the native intestinal worm *Acanthocephalus tumescens*, both salmonids had similar prevalences (33–50%) but generally lower infection intensities, harboring <9 worms per host, compared with 10–27 worms in three native fish species. Collectively, the salmonids represent a small fraction (2.9%) of the total fish abundance in the lake. However, as a greater percentage of *A. tumescens* mature in the salmonids than in any native fish, the two

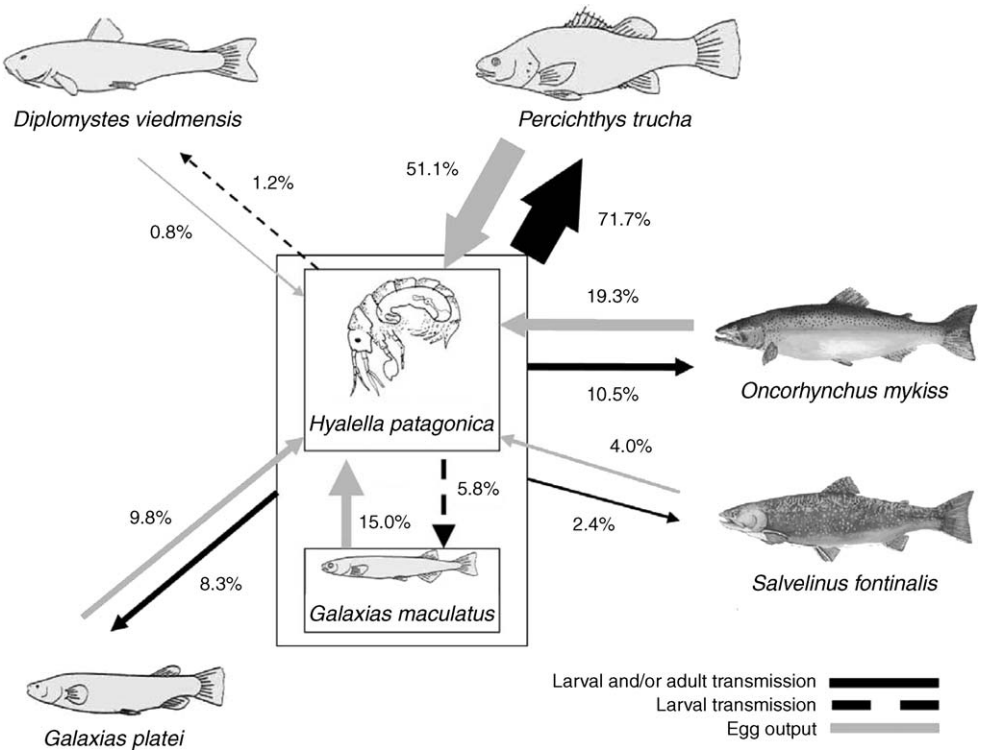

FIG. 3. The relative flow rates of infective stages of the worm *Acanthocephalus tumescens* in native and introduced fish hosts in Lake Moreno, Argentina. Eggs of the adult parasite, which are shed from a definitive fish host, are eaten by the amphipod *Hyalella patagonia*, in which they develop as larval worms. Infected amphipods are eaten by a definitive fish host in which the parasite develops to an adult. Fish become infected with parasite larvae (larval transmission) by feeding on *H. patagonia* or through consumption of adult worms (adult transmission) infecting small fish such as *Galaxias maculatus*. Arrow thickness indicates the relative importance of each fish host for parasite larval/adult transmission, or parasite egg output. The introduced salmonids *Oncorhynchus mykiss* and *Salvelinus fontinalis* are infected by a small proportion of worms (transmission) but account for a high proportion of worm egg output (see *Are NIS competent hosts for native parasites?*). This figure was reproduced from Rauque et al. (2003) by permission of *Folia Parasitologica*.

NIS account for almost a quarter of the parasite's total egg production (Rauque et al. 2003; Fig. 3).

There are thus some compelling examples demonstrating how NIS can be competent hosts for native parasites, from which spillback to native hosts could potentially occur. However, the relative frequency with which NIS are competent hosts, as opposed to "sink" hosts, still needs to be determined.

CAN INFECTION SPILL BACK FROM NIS TO NATIVE HOSTS?

The parasite-spillback hypothesis is consistent with the endemic-pathogen hypothesis proposed for recent emerging infectious diseases of wildlife (Rachowicz et al. 2005). Many wildlife populations may have endured a stable co-existence with endemic diseases, but the disequilibrium often associated with perturbations, such as species introductions, can promote disease emergence (Rachowicz et al. 2005). Interestingly, several viral diseases that are prevalent in domestic cats and dogs have putatively spilled back to wild canids and felids, despite an historic presence in the latter hosts (Steinel et al. 2001, Fiorello et al. 2006, McCarthy et al. 2007). In addition, bacterial haemoplasmas, long associated with domestic cats, are highly prevalent among native wild felids (Willi et al. 2007). For example, studies of domestic cats and dogs in South America indicate that they are important reservoirs for spillover of the apicomplexan *Toxoplasma gondii* (the aetiological agent of Toxoplasmosis) to native wildlife (Fiorello et al. 2004), with the increased ubiquity of *T. gondii* in native felids being attributed to the introduction and increase in abundance of domestic cats since the 16th century (Lehmann et al. 2006). However, recent phylogeographic studies indicate that *T. gondii* has a South American origin in wild native felids (Lehmann et al. 2006, Dubey et al. 2007). If this is indeed the case, then these striking infection patterns are due to spillback, not spillover.

There is little evidence, however, with which to estimate the frequency at which the spillback of infection from competent NIS to native hosts actually occurs, since obtaining such evidence would normally require an experimental approach. As spillback has not received that kind of attention, here we are restricted to presenting evidence that strongly implies (as with the *T. gondii* example above) that such transmission does

occur. Another compelling case study is provided by the introduction of the muskoxen subspecies *Ovibos moschatus wardi* to northwestern America, and its subsequent acquisition of the lungworm *Protostrongylus stilesi*. After the extirpation of native muskoxen *O. m. moschatus* from this region, *O. m. wardi* was introduced from East Greenland to the Arctic coastal plain in 1900 (Hoberg et al. 2002). *O. m. wardi* occupies a growing range eastward to the Yukon and Northwest Territory and most likely acquired *P. stilesi* from native thinhorned Dall's sheep (*Ovis dalli dalli*). Although the parasite is common in Dall's sheep (see Kutz et al. 2001), a new geographic record in the sheep population of Northwest Territory is coincident with the maximum eastward range expansion of *O. m. wardi* (Hoberg et al. 2002). Thus, it is most likely that the muskoxen transmitted the parasite, initially picked up from the sheep, to this population (Hoberg et al. 2002).

Two more case studies that imply spillback transmission in animal parasite systems are described in the following section. Generally, however, it can be argued that the spillback of native parasites from competent NIS hosts to native fauna is likely to be common, since NIS often possess particular demographic traits that can facilitate the spread of infection. For example, NIS often undergo rapid population growth and achieve high densities relative to ecologically similar native species (e.g., Elton 1958, Ricciardi and Maclsaac 2000, Kelly et al. 2006), with many also spreading rapidly and achieving wide distributions due to their broad environmental tolerance and an association with human dispersal vectors (Kolar and Lodge 2002, Fevre et al. 2006, Muirhead et al. 2006). Indeed, these demographic characteristics have been invoked to explain why some NIS are important dispersers of previously introduced pathogens in certain regions (e.g., the role of the North American Bullfrog in chytrid fungus dissemination globally; Garner et al. 2006). The aforementioned demographic characteristics could be particularly important in facilitating the spillback of acquired native viruses and bacteria (see Appendix C). For example, because most microparasites are directly transmitted, the additional presence of a competent NIS host would increase the threshold density necessary for microparasite persistence (de Castro and Bolker 2005, Pederson et al. 2007).

Can Spillback Impact Native Hosts?

Theoretical support that parasite spillback could impact native hosts already comes from multiple-host shared-parasite systems where local extinction of a focal host is predicted when interspecific transmission equals or exceeds intraspecific transmission, and when parasite virulence in a focal host is higher than in a second host (Hatcher et al. 2006). However, robust empirical data supporting spillback impacts on native animal host populations are lacking.

An empirical example from a plant-pathogen system clearly illustrates the dynamics by which parasite spillback could also impact native animal populations. Malmstrom et al. (2005) investigated the role of introduced annual grasses on the incidence of barley and cereal yellow dwarf viruses (B/CYDVs) in perennial native grasses in California, USA, and the preference and fecundity of the aphid vector of the viruses on the different grass host species. In a field experiment, viral incidence in the native plant more than doubled when grown in sympatry vs. allopatry with the nonindigenous species (NIS) of grass, while aphid densities were significantly higher in plots that included the NIS grass. Laboratory trials showed that aphids preferred feeding on NIS grasses, and had significantly higher fecundity when feeding on the latter hosts. Although the study of Malmstrom et al. (2005) was unclear with respect to virus origin, and did not specifically link amplified infection to negative population impacts on native grasses, it provides a confirmation that parasite spillback can potentially impact native host populations.

Our review of the animal parasite literature uncovered two examples that, in addition to providing further support for the spillback of infection from NIS, also strongly imply that subsequent impact can occur. The first example is Settle and Wilson's classic study of apparent competition in an insect herbivore system (Settle and Wilson 1990). Field observations showed that population declines of the native grape leaf hopper in Californian vineyards were coincident with the introduction and spread of the variegated leaf hopper. Eggs of the grape leaf hopper were naturally parasitized by the hymenopteran *Anagrus epos*, which kills its host upon emergence. Although the variegated leaf hopper was susceptible to *A. epos* attack, it suffered lower rates of parasitism and was less preferred than the native host. Evidence for a parasite-mediated indirect effect of the invader came from observations of positive correlations between its relative density and the proportion of infected native grape leaf hoppers, and between the invader's density and the ratio of the number of parasitoids per native host individual. Crucially, the invader contributed to ~50% of the parasitoid population only when it became the dominant (e.g., 85%) leaf hopper species. This study illustrates how interactions between NIS density, differences in host susceptibility to parasitism, and changes in infection intensity of native hosts, can lead to declines in native host populations.

The second example is the displacement of the native house gecko *Lepidodactylus lugubris,* from across the Pacific Islands, by the introduced *Hemidactylus frenatus*, a native of Southeast Asia (Case et al. 1994). To investigate whether the pattern observed is mediated by parasitism, Hanley et al. (1995) surveyed helminths and protozoa of both hosts across islands where they occurred in both allopatry and sympatry. We obtained the Hanley dataset to determine if any native parasites were acquired by the NIS host, assuming that a parasite

of the NIS was acquired if it was absent in the native range of *H. frenatus*, present in *L. lugubris* on uninvaded islands, and present in both host species when they occurred in sympatry (K. Hanley, *personal communication*). Under this assumption, the invading *H. frenatus* acquired one protozoan (*Eimeria furmani*), two nematodes (*Hedruris* spp.; *Physaloptera* spp.), and one cestode (*Cylindrotaenia*) from *L. lugubris*. Interestingly, increasing intensity of infection of *Cylindrotaenia* was negatively correlated with body condition in the native gecko. *Cylindrotaenia* also was significantly more prevalent in *L. lugubris* populations when they occurred in sympatry vs. allopatry with *H. frenatus* (13% and 5%, respectively; Hanley et al. 1995). These observations suggest a negative population effect on the native gecko through parasite spillback (Hanley et al. 1995).

These two examples demonstrate that spillback effects can likely impact native animal species. However, a definitive experimental test is still required, and further evidence from multiple systems is needed to determine how widespread and common (and hence important) a mechanism it is. After identifying a system in which parasite spillback is potentially having host community-scale effects, generally achievable through a combined field survey, laboratory trial, and mathematical modeling approach (see Tompkins et al. (2000*a*, *b*) for a parasite-mediated apparent-competition example), proof of concept would require replicated controlled manipulations at the population scale. If experimental reductions in the parasite burdens of NIS hosts alone led to a decrease in infection in a native species, and accompanying increases in both individual fitness and population size of that species, parasite spillback as a mechanism of NIS impact would be proven.

Acquired Parasites and Complex Interactions

In addition to directly amplifying infection of native parasites by acting as a definitive host (spillback), nonindigeneous species (NIS) could also potentially increase infection by native parasites in native hosts by fulfilling other roles in the parasite life cycle, such as that of intermediate host or vector. Such an interaction could be termed "trophically mediated" spillback, with the NIS becoming part of a sequence of energy transfers among multiple hosts. In addition to a parasite numerical response to the presence of a new host, such spillback could also potentially be driven by differences in the behavior or ecology of NIS vs. native intermediate hosts or vectors. This is illustrated in a long-term study by Strecker (2006) on the impact of introduced African cichlid fish *Oreochromis* on endemic *Cyprinodon* species in Lake Chichancanab, Mexico. Cichlids invaded the lake in 1988 and their population size grew rapidly. However, in the years following invasion, six of the seven *Cyprinodon* species declined dramatically and one species, *C. simus*, became locally extinct. Since *Oreochromis* are detritivore–planktivores, this pattern was not due to predation. Of particular interest were the temporal dynamics of parasitism in the endemic fish. The fish are intermediate hosts for transmission of the native trematode *Crassiphiala cf. bulboglossa* to piscivorous definitive bird hosts. Preinvasion data showed that six of the seven species were infected at low prevalence (0–25%) by the trematode but prevalence reached 90–100% by 6–7 years after *Oreochromis* invasion, during which time the invader became a new intermediate host of the parasite. Furthermore, a decline in body size from pre to post cichlid invasion in one endemic fish, *Cyprinodon maya*, was associated with increased parasitism, with predation of *Oreochromis* by the bird hosts augmenting the flow of infective stages from NIS intermediate, to native definitive, to native intermediate host (Strecker 2006). Increased bird predation on the NIS was driven by the invader's larger size, greater population abundance, and use of open-water habitats as compared to the native fish.

In multiple-host shared-parasite systems involving vectors, asymmetries in host and vector competence, and host susceptibility to parasitism, can affect parasite transmission and persistence (Hatcher et al. 2006). Thus, further complexities might be expected in cases where several introduced species fulfill different roles in the life cycle of a shared native parasite. For example, EEEV (eastern equine encephalitis virus) was one of four native viruses isolated from the invasive Asian tiger mosquito *Aedes albopictus* in North America (Mitchell et al. 1992). Patterns of *A. albopictus* infection by EEEV, together with high competence of the vector, suggest that it may be responsible for recent epizootics (Mitchell et al. 1992). The high reservoir competence of NIS starlings for EEEV, described earlier (Komar et al. 1999), suggests a potential interaction with *A. albopictus* or other introduced vectors that would amplify the virus. For instance, *A. albopictus* is a highly competent vector when exposed to viremic starlings (Komar et al. 1999), while another rapidly spreading Asian mosquito, *Ochlerotatus japonicus japonicus*, also is a competent vector of EEEV (Sardelis et al. 2002). Such situations have the potential to increase the spread and impact of a native parasite, perhaps paralleling the invasional-meltdown hypothesis proposed to explain the impacts of many NIS (Simberloff 2006).

Discussion

Current assessment

Here we extend the evidence demonstrating that nonindigenous species (NIS) do generally acquire native parasites during or after introduction or invasion, illustrating that there is great potential for parasite spillback from NIS to impact native communities. We then demonstrate that NIS can be highly competent hosts for native parasites, and present evidence that infection by native parasites does spill back from NIS to native host species, with subsequent effects at both the host individual and population scale. Hence, although still requiring further study to confirm the role of this

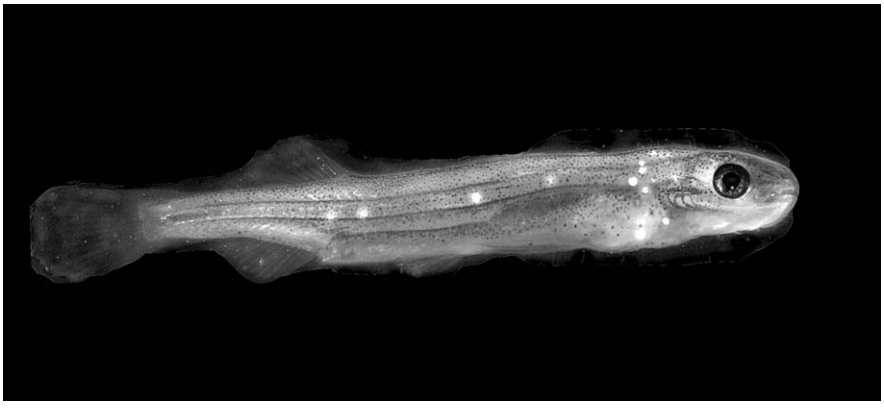

PLATE 1. Larval *Galaxias anomalus*, a native of New Zealand, parasitized by cysts of the trematode *Telogaster opisthorchis*. Could introduced salmonid fish be competent hosts that spill back infection to the detriment of the native species? Photo credit: D. W. Kelly.

mechanism as an impact of NIS on native fauna, and to understand the extent to which the potential for such impacts is actually achieved in the wild, it is likely to be an important mechanism.

So, why has spillback largely been overlooked in the invasion ecology literature? The animal-parasite literature reviewed here indicates that studies of NIS impacts generally assume that native parasites are unimportant. This is illustrated in the study of Smith and Carpenter (2006), investigating threats to the native endemic deer mouse *Peromyscus maniculatus*, on California's Channel Islands (USA), from parasites co-introduced with the nonindigenous black rat *Rattus rattus*. Taking advantage of a patchwork of invaded and noninvaded islands, Smith and Carpenter ascertained that rats introduced the nematode *Trichuris muris* to endemic deer mice through spillover, and discussed the conservation implications. However, closer inspection of their data set reveals that another nematode, *Protospirura peromysci*, was probably acquired by rats from the native mice, because it was absent in global surveys of black rat parasites (Smith and Carpenter 2006: Appendix S1), present in mainland North American deermouse populations, and, in the only two islands in which both hosts were sympatric, reached higher prevalence in rats than the parasite assumed to have spilled over.

The occurrence of spillback is also likely underestimated through failure to identify the status of shared parasites (i.e., native vs. exotic). This issue is highlighted in our review, where many shared parasites had unknown or uncertain status (see Appendix D). Factors hampering identification of parasite status include a lack of parasite faunal surveys of native hosts (particularly records prior to the introduction of NIS), and the presence of shared parasites that were putatively cosmopolitan. Also, parasite faunal lists are often incomplete due to many endangered wildlife species being difficult to sample (Steinel et al. 2001). For example, in Australia, a country renowned for rodent introductions, there is also a diverse assemblage of native rodents, many of which have become extinct or are endangered (Warner 1998). However, while extensive parasite faunal lists have been compiled for introduced rodents (typically after major disease and population epidemics), few studies have focused on native rodents, and it is almost impossible to determine whether parasites are native or introduced (Warner 1998). Other studies appear to have made erroneous assumptions on the status of shared parasites. This problem was recognized by Torchin et al. (2002), who noted that the colonization of more abundant NIS by previously rare native parasites could lead to apparently novel epizootics that are erroneously ascribed to spillover of a co-introduced parasite. In addition to potential impacts being overlooked, the distinction between spillover and spillback is also important for their management. When such impacts are driven by parasite spillover, control of infection in the NIS reservoir host should normally be sufficient to manage disease impacts (e.g., control of canine distemper in lions through domestic dog culls; Cleaveland et al. 2002). However, when such impacts are driven by parasite spillback, control of infection in both native and NIS hosts would likely be required (e.g., control of bovine tuberculosis usually requires management of both domestic stock and wildlife reservoirs; Corner 2006).

Most dramatic outbreaks of disease in native species have been ascribed to spillover infections, where native hosts are exposed to novel infectious agents (Daszak et al. 2000). However, we have shown that, due to uncertainty in parasite origin, several such disease outbreaks may have been caused by parasite spillback. Parasite spillback may have important but less obvious impacts at the population and community scale, since it is well established that parasites causing subtle effects can often regulate host populations (Hudson et al. 1998, Albon et al. 2002, Tompkins et al. 2002). Although such effects are hard to detect, as noted above, they generally require an experimental approach to elucidate.

Future research directions

As is evident from the discussion above, research on parasite spillback is required to provide the definitive proof first that the presence of competent NIS hosts can increase the infection burdens of native parasites in native hosts, and second that such raised levels of infection can impact native populations and communities. In addition, uncertainties over parasite origin demonstrate a need for the resolution of parasite status in studies of shared parasites of native hosts and NIS in general. Such resolution will allow a clearer understanding of the potential of parasite spillback, a more complete understanding of the mechanisms by which parasite interactions associated with NIS impact native fauna, and will influence future conservation management decisions (Rachowicz et al. 2005).

The examples discussed above provide some level of guidance on the native communities in which parasite spillback impacts of NIS are likely occurring, and hence are good candidates for our proposed experimental approach to test the phenomenon. For example, in the freshwater fish community of Lake Moreno in Argentina described above (see *Are NIS competent hosts for native parasites?*), the observation that the remaining individuals of the native fish thought most affected by salmonid introductions, the catfish *Diplomystes viedmensis*, carry the highest abundance of the shared parasite *Acanthosephalus tumescens* of any host (Rauque et al. 2003), is highly suggestive of parasite-spillback effects. Indeed, in other systems where salmonids have been introduced, there may be similar opportunities to test parasite spillback (see Plate 1). Several studies in our review also report the sharing of native parasites among multiple nonindigenous hosts in a particular site or region (e.g., fish, Rauque et al. 2003; deer, Richardson and Demarais 1992; marine invertebrates, Krakau et al. 2006, Thieltges et al. 2006; birds, Miles et al. 1971). For example, in the Wadden Sea, near the coasts of The Netherlands and Germany, all five species of parasite reported in the invading Pacific oyster *Crassostrea gigas* (native to North America) are shared with the native blue mussel *Mytilus edulis* and the common periwinkle *Littorina littorea* (Aguirre-Macedo and Kennedy 1999, Thieltges et al. 2006). Of the parasites acquired, the copepod *Mytilicola intestinalis* and the shell-boring polychaete *Polydora ciliata* appear the most likely candidates for spillback of infection. *M. intestinalis* infection was responsible for historical population declines in native mussels and attains a prevalence of over 50% in *C. gigas*, whereas *P. ciliata* occurs at higher prevalence in *C. gigas* than in *M. edulis* or *L. littorea* where all three hosts are sympatric (Aguirre-Macedo and Kennedy 1999, Thieltges et al. 2006). Interestingly, the slipper limpet *Crepidula fornicata* (native to the Northwest Atlantic coast) is another NIS that has acquired *P. ciliata* in areas of sympatry with *C. gigas* and the native hosts. Both the slipper limpet and the Pacific oyster are increasing in abundance, and spillback of infection could have dramatic consequences because both parasites influence population viability of the native hosts (Thieltges et al. 2006, Buschbaum et al. 2007). Given that the amplifying effect of reservoir hosts may be greater in multiple-host shared-parasite systems (Keesing et al. 2006), such systems are thus perhaps the most suitable candidates for testing the parasite-spillback-from-NIS hypothesis.

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APPENDIX A

Methods used in the ISI Web of Science literature search (*Ecological Archives* E090-142-A1).

APPENDIX B

Studies documenting native parasites that are shared between native and nonindigenous hosts (*Ecological Archives* E090-142-A2).

APPENDIX C

Studies documenting native viruses and bacteria that are shared between native and nonindigenous hosts (*Ecological Archives* E090-142-A3).

APPENDIX D

Parasites of unknown/unspecified origin that are shared between native and nonindigenous hosts (*Ecological Archives* E090-142-A4).

APPENDIX 2

Biological invasions and the dynamics of endemic diseases in freshwater ecosystems

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Biological invasions and the dynamics of endemic diseases in freshwater ecosystems

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SUMMARY

1. Biological invasions, still occurring worldwide at an alarming rate, are widely acknowledged as threats to the integrity and functioning of ecosystems. In addition to introducing disease, biological invasions have also been linked to sudden increases in the incidence or severity of previously existing diseases. We review and illustrate the potential direct and indirect impacts of introduced species on the dynamics of endemic parasites in freshwater ecosystems.
2. Introduced species may trigger and sustain disease emergence by acting as competent hosts for endemic parasites in which infection is amplified and then 'spilled back' to native hosts. In contrast, if introduced species are not suitable hosts for endemic parasites but become infected anyway, they may act as sinks for parasites and thus dilute disease risk for native hosts.
3. Another mechanism by which introduced species can influence endemic parasitic diseases is by altering the relative abundance of one of the parasite's hosts in ways that could either enhance or reduce disease transmission to other native hosts in the parasite's life cycle.
4. Introduced species may also alter disease incidence and severity in native hosts through trait-mediated indirect effects. For example, the introduced species could change the exposure or susceptibility of native hosts to infection by causing alterations in their behaviour or immunocompetence. Also, by directly changing physicochemical conditions and modifying environmental stressors introduced species may indirectly affect native host exposure and/or resistance to disease.
5. A survey of parasites infecting introduced freshwater fish in four distinct geographical areas revealed that use of non-indigenous hosts by endemic parasites is widespread, mostly involving parasites transmitted via the food chain.
6. We conclude by presenting a framework, based on risk assessment, for the prediction and possible mitigation of the impact of introduced species on endemic diseases and by calling for greater recognition of the potential role of invasive species as triggers of endemic disease emergence.

Keywords: dilution effect, introduced species, parasite spillback, risk assessment, trait-mediated indirect effects

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Introduction

Biological invasions represent a major component of global change, imposing huge economic costs to society (Perrings *et al*., 2005; Pimentel, Zuniga & Morrison, 2005; Hulme *et al*., 2009). The globalisation of modern transport systems ensures that the rate at which invasions occur will remain high (Cohen & Carlton, 1998; Ruiz *et al*., 2000; Drake & Lodge, 2004; Perrings *et al*., 2005). The introductions of non-indigenous species (NIS) pose threats to the integrity and functioning of ecosystems, being (after habitat destruction) the second most important proximate cause of native biodiversity loss worldwide (Wilcove *et al*., 1998; Grosholz, 2002; Clavero & Garcia-Berthou, 2005; Molnar *et al*., 2008). At the same time, emerging infectious diseases and the parasites that cause them are reported with increasing frequency from a wide range of systems, and they too threaten biodiversity and ecosystem functioning (Daszak, Cunningham & Hyatt, 2000; Smith, Acevedo-Whitehouse & Pedersen, 2009). An emerging infectious disease is one that appears for the first time in a population, or a previously existing disease that suddenly increases in incidence or geographic range, or that manifests itself in a new way (Daszak *et al*., 2000).

Biological invasions and emerging infectious diseases are not necessarily independent of each other. The former may trigger the latter and together they may have additive or synergistic effects on ecosystems (Fèvre *et al*., 2006; Brook, Sodhi & Bradshaw, 2008). The most obvious way in which biological invasion can be linked with emerging infectious disease is when NIS introduce and transmit novel parasites to native species (Daszak *et al*., 2000; Taraschewski, 2006; Dunn, 2009). For example, the only freshwater crayfish native to the British Isles, *Austropotamobius pallipes* Lereboullet, once widespread, has suffered several local extinctions since the 1980s, and its geographical distribution is now greatly restricted (Holdich & Reeve, 1991). Its decline appears to have been mediated by the oomycete *Aphanomyces astaci* Schikora introduced in the 1970s along with the invasive American crayfish *Pacifastacus leniusculus* Dana; although asymptomatic in its original host, the fungus causes mortality in native European crayfish (Holdich & Reeve, 1991; Kozubikova *et al*., 2009). Similarly, populations of the native European eel, *Anguilla anguilla* L., have declined markedly following the introduction to Europe of the eel-specific parasitic nematode *Anguillicola crassus* Kuwahara, Niimi & Hagaki, along with its original host from East Asia, via importation of live eels to Germany in the early 1980s (Taraschewski, 2006). Although overfishing and other causes have doubtless played large roles, the severe pathology induced by the nematode in its new European host and its rapid spread are probably key contributing factors (Taraschewski, 2006). Introductions of novel parasites along with their non-indigenous hosts may not be as common as one might think, however, since empirical evidence indicates that during introduction NIS tend to lose most of the parasites they had in their region of origin (Torchin *et al*., 2003).

Interactions between NIS and parasites of native host species may therefore be of greater importance, or at least they may be relevant to a greater proportion of biological invasions. On the one hand, parasitism in native species may facilitate the invasion process by making native species more susceptible to predation or competition from NIS (Prenter *et al*., 2004; Dunn, 2009). In Irish freshwater habitats, for instance, a microsporidian parasite infecting the native amphipod *Gammarus duebeni celticus* Stock & Pinkster reduces its host's capacity to prey on small invasive amphipod species and increases the host's likelihood of being preyed upon by larger invaders (MacNeil *et al*., 2003). On the other hand, under certain circumstances, NIS can directly or indirectly alter the dynamics of endemic parasites, possibly initiating and then sustaining emerging diseases (the 'endemic pathogen' hypothesis of disease emergence; Rachowicz *et al*., 2005). Changes in the environment can affect many steps in the infection process, such as the survival of parasite transmission stages or host resistance, as well as modulating parasite virulence or host recovery rates. The potential of environmental change to alter disease dynamics in the wild has been discussed at length in the context of climate change (Marcogliese, 2001; Harvell *et al*., 2002; Mouritsen & Poulin, 2002; Lafferty, 2009). Similarly, the introduction of NIS may perturb native host–parasite interactions by, for instance, acting as alternative hosts for endemic parasites or by altering the behaviour and subsequent infection risk of native host species. Biological invasions may therefore be an underestimated cause of emerging infectious diseases.

In this review, we explore the potential impacts of introduced species on the dynamics of endemic parasites in freshwater ecosystems. We focus specifically on situations where introduced species have the potential to cause a previously existing endemic parasite to increase in prevalence or severity, thus triggering disease emergence. First, we discuss the different mechanisms by which NIS can influence the dynamics of endemic parasitic diseases. We illustrate each mechanism with case studies from freshwater systems and also consider alternative scenarios where introduced species lead to reductions in infection levels or in their consequences for native hosts. Second, we use published surveys of freshwater fish introductions to provide quantitative estimates of how frequently NIS might serve as alternative hosts of endemic parasites, which is how they affect endemic disease dynamics in most cases. Third, we present a framework for the prediction and possible mitigation of endemic disease emergence because of species introduction. Our overall goal is to expose the under-appreciated but potentially important link between biological invasions and disease emergence in freshwater ecosystems and to address its practical implications.

Impact of invaders on endemic diseases

There are several mechanisms by which NIS might influence endemic diseases. These are not mutually exclusive and they can act in concert in many situations. In addition to acting as hosts of endemic parasites, NIS can increase the severity of endemic diseases by inducing either numerical or functional changes in native species. In other words, introduced species may change, directly or indirectly, the abundance of one or more hosts of endemic parasites in ways that promote parasite transmission or they may induce changes in the behaviour or physiology of native hosts that make them more susceptible to infection. The various scenarios discussed below are illustrated in Fig. 1 using the example of a parasite with a two-host life cycle that is typical of many freshwater parasite taxa such as nematodes, cestodes,

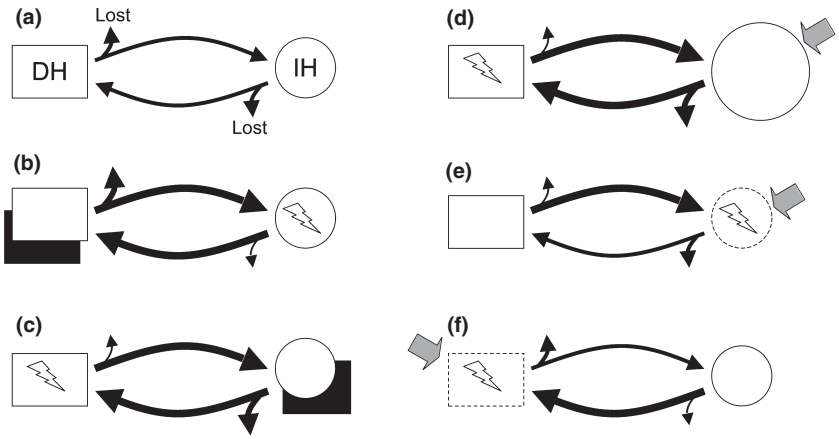

Fig. 1 Summary of possible impacts of NIS on the dynamics of endemic parasitic diseases that might lead to disease emergence. The hypothetical parasite considered here has a two-host life cycle involving a definitive host, DH (white rectangle), and an intermediate host, IH (white circle). During transmission from one host to the other, some parasites are unsuccessful and therefore lost from the system; the thickness of the arrows indicates the relative numbers that are either lost or successfully transmitted. The NIS (black rectangle) can either act as an alternative host for the parasite, or have an indirect effect (shaded arrow) on one of the native hosts. The native host incurring an emergent disease (i.e. an increase in infection rate) is indicated by a lightning bolt. (a) The situation prior to the invasion, providing a benchmark for comparisons. (b) The invader is a suitable alternative definitive host, more parasites therefore reach a definitive host and more infective stages are produced to infect the intermediate host. (c) Same as the previous scenario except that the invader can serve as an alternative intermediate host, leading to greater infection risk for native definitive hosts. (d) The invader indirectly causes the population of native intermediate hosts to increase in size (larger circle), for instance by feeding on their predators or competitors, which leads to reduced losses at that stage of the parasite's life cycle, and greater infection risk for native definitive hosts. (e) The invader indirectly causes intermediate hosts to become more susceptible to infection, for instance by forcing them to change their microhabitat or diet, or via immunosuppression induced by stress. Induced habitat changes may be spatially extensive, such that the native species uses sub-optimal habitats in which infection risk is modified by other stressors. (f) Same as the previous scenario except that the invader indirectly affects the definitive host instead of the intermediate host.

acanthocephalans and myxozoans (scenario a in Fig. 1). Adults or reproductive stages of these parasites typically exploit vertebrate definitive hosts, from which they release eggs or transmission stages that must infect an intermediate host, usually an invertebrate; the life cycle is completed when infected intermediate hosts (or, in the case of myxozoans, further transmission stages released from intermediate hosts) are eaten by a suitable definitive host. The mechanisms discussed later apply equally well to other types of parasites, and equivalent scenarios to those in Fig. 1 could easily be developed for parasites with life cycles involving three or more host species, such as those of many trematodes, or for simple one-host life cycles such as those of most viruses, bacteria, fungi, monogeneans and parasitic copepods.

Parasite spillback: non-indigenous species as hosts of endemic parasites

The most obvious way in which NIS can influence endemic parasite dynamics is by playing an active role in their life cycle and transmission. Following their introduction to a new geographical area, NIS may serve as alternative hosts for endemic parasites, from which infection may 'spill back' to native fauna (Daszak *et al*., 2000; Tompkins & Poulin, 2006; Kelly *et al*., 2009b). It is not unusual for introduced species to acquire parasites from the local fauna. For example, salmonid fishes have been introduced to many parts of the world as fertilised eggs and completely free of the parasites from their area of origin, but following establishment they have accumulated endemic parasites in numbers and taxonomic diversity matching those from their original area (Poulin & Mouillot, 2003). In addition, introduced salmonids often harbour higher abundances of endemic parasites than native host species (Kennedy, Hartvigsen & Halvorsen, 1991). When NIS are competent hosts for endemic parasites (i.e. hosts in which the parasites can develop normally), they may amplify the total number of infective stages to which native hosts are exposed, potentially leading to an emerging disease (scenarios b and c in Fig. 1).

Parasite spillback from NIS to native hosts may be an important but neglected cause of disease emergence. In a review of data available in the literature, Kelly *et al*. (2009b) found that NIS had acquired a mean of 6.3 endemic parasites following their introduction, with 70% acquiring more than four endemic parasites. The non-indigenous taxa in this survey included aquatic and terrestrial invertebrates and vertebrates, while the parasites included protozoa, helminths and arthropods. The literature contains too few rigorous surveys of the frequency at which endemic bacteria and viruses are acquired by NIS to obtain accurate estimates, but we suggest this will be equally common.

A study of the dynamics of fish parasitism in Lake Moreno, Argentina, provides strong evidence for parasite spillback. Two introduced salmonids, rainbow trout (*Oncorhynchus mykiss* Walbaum) and brook trout (*Salvelinus fontinalis* Mitchill), both native to North America, are used as alternative hosts by four endemic parasites acquired from native fish (Rauque, Viozzi & Semenas, 2003). Together, the two introduced salmonids represent only 3% of the total fish abundance in the lake, and yet they now play a very important role in the life cycle and transmission of the endemic acanthocephalan parasite *Acanthocephalus tumescens* von Linstow. Although the parasite does not reach infection intensities as high in the salmonids (<9 worms per fish on average) as in its native hosts (averages in three native fish: 10–27 worms per fish), a higher proportion of female worms reach maturity and produce eggs in the introduced salmonids than in native hosts (Rauque *et al*., 2003). The upshot is that the two salmonids account for approximately a quarter of all parasite eggs produced and released in lake waters. Although it remains to be tested, the boost in parasite reproduction made possible by a relatively small number of exotic hosts should result in greater risk of infection for native species, including both amphipod intermediate hosts and fish definitive hosts.

Studies of recurrent mass mortalities in a variety of waterfowl species in North American freshwaters have identified infection by the trematodes *Cyathocotyle bushiensis* Khan and *Sphaeridiotrema globulus* Rudolphi as the probable cause (Hoeve & Scott, 1988; Herrmann & Sorensen, 2009). Transmission to birds occurs when the introduced gastropod *Bithynia tentaculata* L., an intermediate host for both parasites, is eaten by molluscivorous birds. Although *C. bushiensis* was probably introduced, *S. globulus* occurs in several native gastropod intermediate host species in areas where the invasive *B. tentaculata* is absent (e.g. Roscoe, 1983), and is therefore most likely native. The

extremely high densities at which *B. tentaculata* can occur, coupled with high prevalence and intensity of *S. globulus* infection, and heavy feeding activity on snails by birds, increase the probability that waterfowl will ingest a lethal infection dose (Herrmann & Sorensen, 2009). An almost identical situation exists in Belarus, where the introduced zebra mussel, *Dreissena polymorpha* Pallas, has become disproportionately abundant relative to native molluscs. This introduced bivalve now harbours much higher intensities of infection by endemic trematodes than native molluscan hosts and may act as a source of heavy infections for the waterfowl that prey on molluscs and serve as the trematodes' definitive hosts (Mastitsky & Veres, 2010). These systems show how an abundant NIS acting as an intermediate host can increase infection risk to native definitive hosts (scenario c in Fig. 1).

Another apparent but untested case of spillback has been documented in Lake Chichancanab, Mexico, where African cichlid fish, *Oreochromis* spp., were accidentally introduced two decades ago. Over the following few years, as cichlid abundance rapidly increased, population sizes of five native species of the genus *Cyprinodon* declined dramatically and a sixth one became extinct (Strecker, 2006). The cichlids are detritivore–planktivores, and the decline of native fish was therefore not because of predation by the invasive species. All the fish species, both native and introduced cichlids, serve as second intermediate hosts in the transmission of endemic trematodes to the piscivorous birds used by the parasites as definitive hosts. Pre-invasion data show that *Cyprinodon* fish were infected at low prevalence (<25%) by trematodes, but within 6–7 years of the invasion, prevalence reached 90–100% (Strecker, 2006). In this system, birds preyed heavily on the NIS because of their larger sizes, greater abundance and greater use of open-water habitats compared to native fish. Use of cichlids by the parasites augmented the flow of infection from NIS intermediate hosts to native birds, and back to native fish intermediate hosts (Strecker, 2006). This illustrates how parasite spillback from introduced species could potentially affect all host species in a parasite's life cycle.

One of the main criteria for spillback to occur is that the introduced species must be a competent host for the endemic parasites it acquires. If parasites cannot develop in the NIS but infect it anyway, then it may act as a sink for the parasite population. This would reduce infection levels in native hosts via a 'dilution' effect (Keesing, Holt & Ostfeld, 2006). The introduction of European brown trout, *Salmo trutta* L., to New Zealand has apparently had that effect on native fish species. Although many endemic parasites are found in trout (Dix, 1968), the latter are not suitable hosts since most of these parasites do not reach maturity inside trout. A recent study has found a negative relationship across different streams between intensity of infection by endemic trematode species in two native fish, *Gobiomorphus breviceps* Stokell and *Galaxias anomalus* Stokell, and an index of local trout abundance (Kelly *et al*., 2009c). In other words, in sites where trout are abundant, trematode infections in native fish are less severe. One possible mechanism is that, after leaving their snail first intermediate host, the free-swimming infective stages of trematodes that encounter trout infect this host but fail to complete their development; a greater proportion of infective stages would thus be lost in sites with more abundant trout populations (Kelly *et al*., 2009c). However, modelling and experimental studies are required to confirm that availability of infective stages can be limiting in the parasite life cycle and that their wastage by non-competent hosts (akin to 'lost bites' in vector-borne diseases) does lead to reduced infection in other host species. Other studies have recently provided evidence that invasive snails are often not suitable hosts for the trematode infective stages they encounter, which can lead, at least in mesocosm studies, to a dilution of infection for native snail species (Kopp & Jokela, 2007; Genner, Michel & Todd, 2008).

NIS do not necessarily need to kill endemic parasites post-infection for a dilution effect to occur; they may also cause direct mortality of infective stages by feeding on them or causing transmission failure via physical interference (Thieltges, Jensen & Poulin, 2008). Indeed, numerous freshwater invertebrates can actively feed on the infective stages of a range of parasites (e.g. Christensen, 1979; Achinelly, Micielli & Garcia, 2003) and active filter-feeders, such as the dreissenid mussels invasive throughout North America, may be capable of clearing most free-swimming stages of parasites from the surrounding water (MacIsaac *et al*., 1992; Pace, Findlay & Fischer, 1998; Faust *et al*., 2009). Thus, whether an NIS has the potential for positive (spillback) or negative (dilution) effects on

endemic parasites depends on whether it is a suitable host for infection and development of the parasite, as opposed to a sink that causes the loss of infective stages.

Numerical impacts of non-indigenous species on native hosts

The best-documented impacts of NIS are reductions in the abundance or density of one or more native species, sometimes to the point of extinction (Grosholz, 2002; Clavero & Garcia-Berthou, 2005). If negatively impacted species are essential hosts in the life cycle of an endemic parasite, then the parasite's local population will decline, and its other host species may benefit indirectly from the invasion. However, if instead the NIS causes a reduction in the abundance of a predatory species that kept in check the intermediate host of an endemic parasite, then the opposite could happen: the parasite's transmission rate would increase locally with negative consequences for its other hosts (scenario d in Fig. 1).

The impact of invasive dreissenid mussels (i.e. the zebra mussel, *Dreissena polymorpha* Pallas, and quagga mussel, *D. bugensis* Andrusov) provides a good example. Originally from Ukraine and southern Russia, these mussels were introduced in the 1980s to freshwater systems in North America, where their local densities can now be very high. Among the many reported ecosystem impacts of these NIS, there is clear evidence that the abundance of native macrobenthic invertebrates is higher where the invasive mussels are present than where they are not (Botts, Patterson & Schloesser, 1996; Mayer *et al*., 2000). This is probably caused by the physical habitat provided by clumps of mussels and by their faeces and pseudofaeces (mucus pellets in which unfiltered particles are concentrated) enhancing detritus-based benthic food webs. Invertebrates whose abundance is increased include amphipods and oligochaetes that play major roles as intermediate hosts of myxozoans, acanthocephalans, nematodes and cestodes parasitic in fish (Williams & Jones, 1994). At the same time, the filtering activity of dreissenid mussels can cause substantial decreases in the abundance of small-sized zooplankton (MacIsaac *et al*., 1992; Pace *et al*., 1998). These include the small cyclopoid copepods used exclusively as intermediate hosts by cestodes of the genus *Proteocephalus*, which are very common parasites of North American freshwater fish (McDonald & Margolis, 1995; Scholz, 1999). Although the consequences of increases (or decreases) in intermediate host abundance caused by NIS could well include enhanced (or reduced) transmission rates of parasites back to fish, these remain unexplored to date.

Functional impacts of non-indigenous species on native hosts

To initiate endemic disease emergence, NIS may not have to act as alternative hosts for endemic parasites or even to cause changes in the abundance of native hosts involved in the parasite life cycle. The process could instead involve trait-mediated indirect effects (Werner & Peacor, 2003); NIS might change the exposure or susceptibility of native hosts to infection by causing alterations in their behaviour or immunocompetence (scenarios e and f in Fig. 1). For instance, there is considerable empirical evidence that prey respond to the perceived threat of predation, even predation from novel predators, by changing some aspect of their phenotype such as activity levels, microhabitat choice, prey selection, morphology and life history characters (Boersma, Spaak & De Meester, 1998; Lass & Spaak, 2003; Trussell, Ewanchuk & Matassa, 2006; Wolinska, Loffler & Spaak, 2007; Johansson & Andersson, 2009). Similarly, perceived predation risk can cause physiological stress in prey individuals, measurable as increased levels of stress proteins such as heat-shock proteins or hormones such as cortisol (Kagawa & Mugiya, 2000; Woodley & Peterson, 2003; Slos & Stoks, 2008). Exposure to infection in aquatic organisms depends mostly on diet and microhabitat, since most aquatic parasites are acquired either via ingestion or direct contact with a free-swimming infective stage. Resistance to infection depends on the immunocompetence of the exposed individual and increased stress can depress immunity (Apanius, 1998; Raberg *et al*., 1998; Harris & Bird, 2000). Therefore, if the NIS is a predator or is perceived as such by native species, the latter may experience greater exposure to parasites by shifting to different microhabitats and feeding on different food items, and greater susceptibility to infection because of stress-mediated immunosuppression. In addition, by directly changing physicochemical regimes and modifying environmental stressors, NIS may also indirectly affect host exposure and/or resistance to disease.

These sorts of indirect effects on native freshwater animals have been reported following the introduction of NIS. For instance, the black bullhead *Ameiurus melas* Rafinesque, an ictalurid catfish native to North America, has become one of the most successful exotic fish in European freshwater ecosystems. A series of experiments has shown that the presence of this invader reduces the predation success of *Esox lucius* L. on minnows, not by actively competing with pike for minnows, but simply by interfering with the normal behaviour of pike (Kreutzenberger, Leprieur & Brosse, 2008). Infection levels by parasites normally transmitted trophically from minnows to pike may be reduced as a consequence. However, since pike switch to other food items, they may be exposed to other parasites transmitted via these different preys. In the Laurentian Great Lakes of North America, the large predatory cladoceran *Bythotrephes longimanus* Leydig, introduced from Eurasia in the early 1980s, induces a change in the vertical distribution of its zooplanktonic prey, mostly *Daphnia* spp., causing them to live in deeper and colder waters (Pangle & Peacor, 2006; Pangle, Peacor & Johannsson, 2007). The altered spatial distribution of *Daphnia* induced by a NIS could lead to greater or lower exposure to, and transmission of, parasites (Hall *et al.*, 2005).

Trait-mediated indirect effects also provide alternative hypotheses for the observed pattern in New Zealand of decreasing endemic parasite infection in native freshwater fish with increasing brown trout abundance (see above). Rather than 'parasite dilution', trout may be altering either the behaviour of native fish (on which they prey) or the freshwater environment, in ways that reduce parasite transmission. Behavioural impacts of brown trout on other native species in New Zealand have already been documented; several mayfly species have changed their diel activity patterns and now spend little time grazing on algae during the day in systems where trout have been introduced (McIntosh & Townsend, 1994, 1995). As mayflies and other aquatic insects serve as intermediate hosts for the trematode *Phyllodistomum magnificum* Cribb, which is trophically transmitted to eels, *Anguilla* spp. (Hine, 1978; Cribb, 1987), their altered behaviour in the presence of introduced trout may have consequences for diseases in eels.

Trout invasion highlights another potentially important functional effect of NIS – induced habitat shift – which could interact with other stressors to enhance disease. Populations of non-migratory *Galaxias* fish in New Zealand streams have declined as a result of trout invasion, habitat modification and pollution (McDowall, 2006), but trematode infection may also play a role. Juvenile fish can suffer mortality and severe spinal malformations if infected by free-swimming infective stages (cercariae) of trematodes released by snail intermediate hosts (Kelly *et al.*, 2010). Displaced by trout, the *Galaxias* tend to persist in poor-quality refuges of low flow and higher temperature (Allibone, 2000; Leprieur *et al.*, 2006), marginal habitats that may increase trematode infections by concentrating cercariae, snail intermediate hosts and fish (e.g. Mitchell *et al.*, 2000), with higher temperatures probably promoting snail shedding of cercariae (see Poulin, 2006). Allibone (2000) reported that low flows and high temperatures in streams subjected to water abstraction were associated with high snail densities and a large percentage of malformed adult *Galaxias depressiceps* (McDowall & Wallis, 1996). This example indicates that NIS-driven disease emergence could act in concert with other drivers of environmental change.

There are several additional mechanisms by which NIS could cause disease emergence. First, they could accumulate and biomagnify endemic diseases within the food chain. The zebra mussel invasion of the Great Lakes, and the re-emergence of Type E *Clostridium botulinum* associated with large-scale fish and waterfowl die-offs, provides a good example. Type E botulism is transmitted through diet and proliferates in anaerobic, nutrient-rich sediments. The enormous filtering capacity of zebra mussels has been linked with accumulation of *C. botulinum* and possibly its toxin. It is speculated that waterfowl accumulates *C. botulinum* by preying on invasive round gobies *Neogobius melanostomus* (Pallas), the mussel's main predator (Holeck *et al.*, 2004; Getchell & Bowser, 2006). Second, NIS may enhance disease by altering habitat physicochemistry, illustrated again by zebra mussels. By providing additional nutrients in deposited faeces and pseudofaeces, increasing sediment anoxia because of decomposition of dead mussels and waste and enhancing growth of near-shore benthic algae, mussels create conditions suited to the proliferation of *C. botulinum* (Perez-Fuentetaja *et al.*, 2006; Riley *et al.*, 2008). Because many freshwater NIS, such as crayfish, carp, mitten crabs and beavers, are

ecosystem engineers that cause profound changes in physical habitat, it is probable that the scope for such functional impacts on disease emergence is wide. Indeed, such physicochemical alteration highlights an additional mechanism by which NIS could alter disease, that is, by modifying the strength of environmental stressors of the immune system of native hosts. It is of interest that recent deepwater hypoxic events in parts of the Great Lake Basin have been attributed to zebra mussel invasion (e.g. Kelly, Herborg & MacIsaac, 2009a). Given that immune mechanisms involved in the clearance of pathogens can be highly sensitive to hypoxia (Macey *et al.*, 2008), such hypoxic conditions might lead to the emergence of diseases.

The evidence that functional changes (e.g. microhabitat use, trophic interactions) in native freshwater animals are induced by NIS is very convincing. However, despite the obvious ways in which these changes can influence both the exposure of native animals to parasites and their resistance to infection, there have been no attempts to quantify the indirect impacts of NIS on disease dynamics. This possible link between biological invasion and emerging diseases remains unexplored empirically, possibly because it may appear less important than other causal pathways. Yet, it may be relevant to a large proportion of invasions, and quantifying the strength of these indirect effects of NIS on endemic diseases should be a top priority.

Importance of non-indigenous species as hosts of endemic parasites

Isolated case studies suggest that NIS can indirectly cause the emergence of endemic diseases by either changing the abundance of native species serving as hosts of parasites, or increasing their exposure or susceptibility to infection. There is no global dataset allowing an evaluation of how frequently disease emergence follows biological invasion. However, surveys and checklists of parasites in freshwater animals, especially fish, are available from several parts of the world, providing the basic data necessary to assess how often the conditions for parasite spillback are met following an invasion. Here, we use compilations of host–parasite associations to determine (i) how many endemic parasite species typically exploit an invasive fish species and (ii) what endemic parasite taxa most commonly use an introduced fish species as an alternative host. These are not definitive analyses, but instead a preliminary demonstration of the very real potential for parasite spillback in freshwater habitats.

Data on endemic parasites of introduced freshwater fish species were obtained from checklists for four geographical areas: Canada (Margolis & Arthur, 1979; McDonald & Margolis, 1995), Mexico (Salgado-Maldonado, 2006), the Czech and Slovak Republics (Moravec, 2001) and New Zealand (Hine, Jones & Diggles, 2000). The parasites considered include only monogeneans, trematodes, cestodes, nematodes, acanthocephalans and copepods, other groups being excluded because they were either ignored in most surveys or were rarely found. In addition, we consider only parasites that are either (i) confirmed as species endemic to the area that only started exploiting the introduced fish following its arrival or (ii) suspected by the authors of the checklists of being endemic to the area though confirmation is lacking. All parasite species known to have been introduced, either with the introduced fish on which they are found or via a different route, were excluded. Finally, we only included introduced fish species for which at least one endemic parasite has been reported. Introduced fish with no known endemic parasites in their new

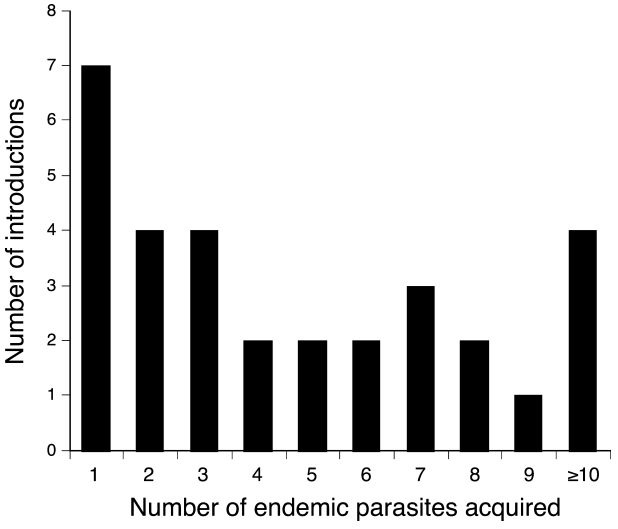

Fig. 2 Frequency distribution of the number of endemic parasite species recorded per species of introduced freshwater fish (n = 31), pooled across four geographical areas. The data include both parasites that are confirmed as endemic species and those that are suspected of being endemic. See text for further details.

area may be 'false negatives': further sampling specifically targeted at finding parasites might reveal that they are indeed used as hosts by endemic parasites.

Well over half of the estimated 50 introduced fish species in the four geographic areas have been found to harbour endemic parasites. Overall, 31 fish introductions, involving mainly Cichlidae, Cyprinidae or Salmonidae, had acquired at least one endemic parasite species, each NIS having acquired between 1 and 15 endemic parasites (mean ± SE: 4.9 ± 0.7 parasite species; see Fig. 2). If only parasites whose endemic status has been independently confirmed are considered, these numbers are reduced by about half (2.3 ± 0.4 parasite species, range 1–9). Overall, the 31 fish introductions led to 151 cases of an endemic parasite using a NIS as an alternative host, with almost half of these cases (i.e. 71) involving parasites confirmed to be endemic. The majority of these were nematodes (23 cases), trematodes (20) or acanthocephalans (14), suggesting that parasites with complex life cycles transmitted via the food chain are more likely to be involved in spillback than those with direct transmission like monogeneans and copepods.

This brief survey suggests that the potential for the amplification of an endemic disease following the use of NIS as alternative hosts, or spillback, is truly considerable for fish parasites. Of course, the widespread use of introduced hosts by endemic parasites does not necessarily imply that spillback (or, for that matter, disease dilution) will ensue; it does, however, set the stage for NIS to alter local parasite dynamics in ways that could lead to disease emergence.

Predicting the risk of emerging endemic disease posed by NIS

The extent of biological invasions is creating major regulatory challenges for government agencies (Hulme *et al.*, 2009). Ecologists need to devise predictive tools for legislators to target prevention and control efforts towards those invasive species most likely to impact ecosystems and the economy, including those probable to initiate emerging diseases. In recent years, ecological risk assessment has become widely used in environmental management and impact assessment (Lackey, 1997; Claassen, 1999). Risk assessment has been used specifically to try to predict which taxa are most likely to successfully invade new geographical areas (Kolar & Lodge, 2002; Landis, 2004). In addition, this approach has previously been applied to the management of diseases in aquatic ecosystems, identifying parasites most likely to spread and have economic impacts (Peeler *et al.*, 2007). Although a lack of data for some critical parameters can limit its full potential, there is little doubt that risk analysis has improved decision making in aquatic animal health management.

In the joint context of biological invasions and infectious diseases, ecological risk assessment could be used to evaluate the likelihood of emerging infectious diseases caused by endemic parasites following the introduction of particular NIS, and to inform decision making and the prioritisation of efforts to eradicate or control invasive species. To achieve this, we would need to build predictive statistical models based on existing data that assign to any given NIS a probability that it will trigger the emergence of an endemic disease; it would then be for decision makers to determine whether the risk is above a threshold (which takes into account not only ecological but also economic factors) for implementation of management actions. Risk (i.e. the probability of an endemic emerging disease following an introduction) can be determined as a function of the number of NIS introduced, using modified logistic regression techniques (see Bender, 1999), or more powerful statistical tools that take into account missing values, uncertainty, nonlinear relationships and higher-order interactions between variables (De'ath, 2002; Elith, Leathwick & Hastie, 2008). Other independent variables probably to matter in such a predictive model include (i) the characteristics of the introduced species, including its phylogenetic affinities and its basic ecological and life history traits, (ii) the characteristics of the native host fauna, such as its diversity and the phylogenetic distance between its component species and the invader and (iii) the properties of the local parasite fauna, including its diversity and its phylogenetic distance to the parasites of the invader in its area of origin. For many biological invasions, data are available for several if not most of these variables. Equipped with models based on these data, it would be relatively easy to quantify the risk of disease emergence associated with a particular NIS after its initial detection in a new habitat. There is an urgent need for this kind of forecasting tool to ensure that the necessary measures to either eliminate the risk of disease or mitigate its consequences are taken before, rather than after, its emergence.

Conclusions

It is clear from our review that empirical evidence supporting NIS as a cause of endemic disease emergence in freshwaters is scant. One of the difficulties in identifying the role of NIS is that parasitism and disease may be driven by multiple stressors, such as climate change, habitat alteration and pollution (Didham *et al*., 2007; Lafferty, 2009), problems that are particularly relevant to freshwaters. However, freshwater systems, when compared to terrestrial habitats, may offer certain advantages in the study of disease emergence. For example, because some NIS are patchily distributed, the often discrete nature of river and lake systems can provide natural spatially replicated experiments that allow a comparison of disease patterns in native hosts in the presence versus absence of an NIS. Lakes and ponds are also more amenable to NIS removal experiments, which would allow temporal assessment of disease dynamics. Such experimental manipulations are vital for demonstrating links between biological invasions and disease emergence. Although many of the direct and indirect interactions leading to disease emergence postulated here have good supporting evidence, conclusive proof is generally still lacking. The challenge is twofold. First, modelling work is required, supported by empirical data from the field and experimental work in the laboratory, to interrogate the complex interactions among parasite fitness and host susceptibility of native and introduced fish and to identify the sets of circumstances under which spillback can be expected to occur. Second, there is a need for well-designed natural field experiments that contrast endemic parasitism of native fish in study sites (e.g. streams or lakes) lacking or containing NIS.

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