MANAGING AQUATIC NON-NATIVE SPECIES

The role of biosecurity

Lucy Grace Anderson

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School of Biology
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DECLARATION

The candidate confirms that the work submitted is her own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

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Chapter Two is based on a jointly authored publication:

Anderson, L.G., White, P.C.L., Stebbing, P.D., Stentiford G.D., Dunn. A.M. (2014) Biosecurity and vector behaviour: evaluating the potential threat posed by anglers and canoeists as pathways for the spread of invasive non-native species and pathogens. *PLOS ONE*, **9, e92788.**

LA formulated the idea, conducted the research, analysed the data and wrote the manuscript. PW, AD and PS assisted with idea formulation and contributed to writing the manuscript. GS contributed to writing the manuscript.

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LA and PS formulated the idea and AD assisted with idea formulation. LA, PR and PS conducted the experiment, LA analysed results and wrote the manuscript. PR, PS and AD contributed to writing the manuscript. Ellis Bradbury, Hannah Lintott, Ammar Kahla and Georgina Rimmer provided technical assistance with the research.

Chapter Four: LA formulated the idea. Steve Rocliffe (SR) and PS assisted with idea formulation. LA and SR conducted the experiment. LA analysed results and wrote the manuscript. SR, AD and PS contributed to writing the manuscript.

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ABSTRACT

Across the globe, invasive non-native species (INNS) are a major ecological and economic problem with freshwater environments particularly susceptible to their impacts. Preventing their introduction and spread is considered the most environmentally desirable and cost-effective form of management by the Convention on Biological Diversity, and is advocated in new EU INNS legislation which comes into force in 2015. Biosecurity – a term describing actions taken to prevent the introduction and spread of unwanted organisms – is central to this preventative approach. This PhD combines ecological and social research to identify human-mediated pathways for the spread of INNS in freshwater environments; to examine the effectiveness of biosecurity measures; and to identify how biosecurity awareness and compliance could be improved. Initial questionnaire research revealed that recreational water users in the UK are potential vectors for INNS due to their movement patterns and low biosecurity compliance. A survival experiment showed that many aquatic INNS threatening the UK can survive in damp conditions for 16 days but demonstrated that hot water (45℃, 15 mins) is an effective biosecurity control measure, causing 99% mortality in many high risk INNS within 1 hour. As a result of a long-term biosecurity campaign, New Zealand water users had high biosecurity awareness and compliance compared to the UK. The development of regional partnerships and the support of national legislation were key components of the country's streamlined approach to biosecurity. Invasive non-native crayfish had a significantly lower diversity and prevalence of parasites than native crayfish in the UK, supporting the concept of enemy release. Finally, a global meta-analysis revealed that recreational activities also act as vectors for the introduction of INNS in terrestrial and marine environments and require biosecurity measures of a similar magnitude. The results provide an evidence base from which to develop freshwater biosecurity strategies in the UK and wider Europe.

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1 GENERAL INTRODUCTION

Invasive non-native species (INNS) are one of the greatest threats to biodiversity on earth. They have devastated native wildlife and habitats on all continents (Mack *et al.*, 2000), causing widespread species extinctions (Clavero and Garciaberthou, 2005). Although their impacts have affected terrestrial and marine environments, freshwater environments are particularly susceptible to biological invasions because they are exposed to multiple introduction pathways including ship ballast release, fish stocking and the deliberate release of ornamental plants and animals (Strayer, 2010); while the movement of recreational boats and angling equipment between catchments facilitates secondary spread (Kelly *et al.*, 2013b). INNS have contributed to the higher rate of biodiversity loss in freshwater systems (76%) relative to marine and terrestrial systems (39% each) over the past 40 years (WWF, 2014; Collen *et al.*, 2014).

As the eradication of established INNS is costly and not always possible, preventing the introduction and spread of INNS by managing their pathways of introduction is universally accepted as the most effective control measure (Caffrey *et al.*, 2014; Roy *et al.*, 2014; Convention on Biological Diversity, 2006). Consequently, effective pathway management is listed as a key target of the Convention on Biological Diversity's Aichi Targets for 2020 (Secretariat of the Convention on Biological Diversity, 2011), as well as forming a major component of new EU INNS legislation which comes into force in January 2015 (European Commission, 2013). Biosecurity – a term used to describe actions taken to prevent the introduction and spread of unwanted organisms – is central to pathway management. Although it is a relatively new concept in Europe, improving biosecurity awareness and biosecurity compliance will be key to meeting the objectives of new EU legislation (Caffrey *et al.*, 2014). In the first part of this introduction, I will give a background to invasive non-native species and their impacts in freshwater habitats and how parasites and pathogens can influence invasion success. I will go on to discuss INNS management and the role of biosecurity in preventing freshwater invasions before outlining the specific aims and focus of this thesis.

1.1 A Background to Invasive Non-Native Species

Throughout history, the geographical and taxonomic movement of organisms has mirrored trends in human trade and transport (Hulme, 2009). The end of the Middle Ages saw the first step-change in the movement of species, as human migrations, trade and the dawn of agriculture led to the movement of domestic animals and crops (Preston et al., 2004; Hulme, 2009). Later – as indicated in the manifests of Columbus' second voyage – Europeans transported Old World species to their New World settlements (Di Castri, 1989). However, over the past 25-50 years, globalisation has prompted a second and more significant step-change in biological invasions (Hulme, 2009). An acceleration in international trade, transport and tourism has caused an unprecedented increase in the translocation of species to new biogeographic ranges (Hulme, 2006; Mooney and Cleland, 2001; Ricciardi, 2007; Hulme, 2009).

1.1.1 Stages of the invasion process

Although the number of species being moved beyond their native ranges is increasing exponentially, the majority of introduced species die in transit, or shortly after release and therefore pose no threat to the ecosystem that they have been introduced into (Kolar and Lodge, 2001). In order for an introduced species to become invasive, it must progress through successive stages of the invasion process: transport, introduction, establishment and spread (Figure 1.1) (Blackburn *et al.*, 2011). At each stage, organisms must overcome a series of environmental, reproductive and dispersal-related hurdles and it is estimated that as few as ten percent will survive each one (Williamson and Fitter, 1996).

Propagule pressure – the number and frequency of individuals arriving in a recipient region – is a key determinant of invasion success (Lockwood *et al.*, 2005; Simberloff, 2009). High propagule pressure enhances establishment success by increasing the likelihood that the introduced individuals will overcome the environmental stochasticity associated with small founder populations and survive (Simberloff, 2009). Globalisation has increased the number of opportunities for non-native propagules to be transported, but preventative measures such as biosecurity (see 1.3.1) can act against propagule pressure to reduce the number of individuals released and the frequency of releases, reducing the probability of establishment (Kolar and Lodge, 2001).

After initial introduction, another important factor determining establishment success is the suitability of the recipient environment and its similarity (e.g. habitat, climate) to the source region (Kolar and Lodge, 2001), something that may be influenced by climate change (Liu *et al.*, 2011). According to Elton's (1958) niche hypothesis, communities with high species diversity – and therefore few unexploited resources – should be more resistant to invasions (Shea and Chesson, 2002). Anthropogenically disturbed habitats are therefore considered to be more vulnerable to invasions because INNS can often fill the space (in terms of biomass, or niche competition) created by disturbance more quickly than native species due to superior rates of reproduction, or adaptation (Mack *et al.*, 2000; Britton-Simmons and Abbott, 2008; Jauni *et al.*, 2014).
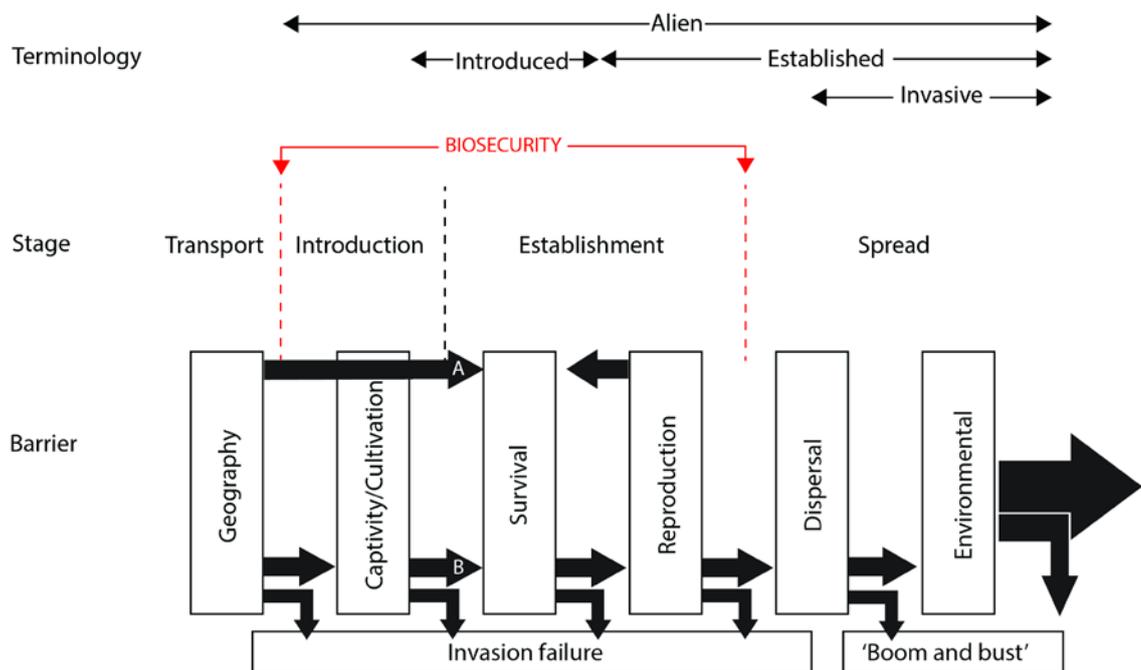
FIGURE 1.1 STAGES OF THE INVASION PROCESS, ADAPTED FROM BLACKBURN ET AL. (2010)

The diagram shows the stages of the invasion process that species need to pass through in order to become 'invasive' in a natural environment. As few as 10% are thought to survive each stage (Williamson and Fitter, 1996). The escape of American signal crayfish (*Pacifastacus leniusculus*) from aquaculture farms in the UK is a classic example of an INNS which escaped from captivity/cultivation (denoted by the letter B). Other freshwater INNS, such as the killer shrimp (*Dikerogammarus villosus*), most likely arrived as stowaways and would have skipped the captivity stage (denoted by the letter A). Biosecurity measures are designed to increase the likelihood of invasion failure between each stage of the process i.e. preventing INNS on transport vectors from reaching the introduction stage, and preventing established INNS from dispersing further (secondary spread).

1.1.2 Terminology

As invasive species research has grown, terminology to describe invasive non-native species (synonyms commonly include alien species, non-indigenous species, exotic

species or introduced species) has been used inconsistently (Blackburn *et al.*, 2011). Throughout this PhD, I use invasive non-native species to describe species which have been introduced either intentionally or unintentionally by human action and which have established, reproduced and spread at multiple sites within their introduced range (Blackburn *et al.*, 2011). Some organisations make reference to the impacts of INNS within their definitions. For example, the Convention on Biological Diversity describe invasive species as *"species whose introduction and/or spread outside their natural past or present distribution threatens biological diversity"* and the GB Non-Native Species Secretariat define INNS as *"any non-native animal or plant that has the ability to spread causing damage to the environment, the economy, our health and the way we live".* These definitions are somewhat contentious because i) quantitative information on the impacts of many established INNS is still lacking (Jeschke *et al.*, 2014); ii) it is possible for species to have negative impacts before they have become 'invasive' per se (Blackburn *et al.*, 2011; Jeschke *et al.*, 2014); and iii) it may be possible, though unlikely, for established species to be benign (Blackburn *et al.*, 2011).

1.1.3 Invasions in freshwater environments

Despite making up only 0.8 percent of the earth's surface, the world's freshwater ecosystems support six percent of global biodiversity and are a vital resource for human wellbeing (Dudgeon *et al.*, 2006). Yet biodiversity is declining in freshwater ecosystems at a far greater rate than in terrestrial and marine ecosystems, and INNS are one of the leading causes (Dudgeon *et al.*, 2006; WWF, 2014).

The Millennium Ecosystem Assessment (2005) revealed that freshwater ecosystems had experienced a high impact from INNS over the past century and that these impacts were continuing to rise rapidly relative to other ecosystems. In 2014, the World Wildlife Fund (WWF)'s Living Planet Report reflected these results, reporting that freshwater biodiversity declined by 76% between 1970 and 2010, compared to declines of 39% in both marine and terrestrial environments over the same time period (WWF, 2014). Invasive non-native species were reported as one of the major contributors to freshwater biodiversity loss (WWF, 2014; Collen *et al.*, 2014).

Freshwater ecosystems are particularly vulnerable to the introduction of INNS because of the breadth of introduction pathways that they are exposed to (Strayer, 2010). Processes such as fish stocking, the redirection of water supplies, ship ballast release, the transfer of recreational angling and boating gear between sites and the release of exotic and ornamental plant and animal species facilitate the introduction,

and secondary spread of INNS (Rahel, 2007; Havell and Shurin, 2011). Recreational activities involving the movement of boats, or angling and watersports equipment also increase the connectivity of freshwater catchments, aiding the dispersal of non-native species, as well as aquatic pathogens, once they have been introduced (Taugbøl *et al.*, 1993; Rahel, 2007; Kelly *et al.*, 2013b).

As well as being exposed to multiple transport pathways, the small volume and high connectivity of freshwater ecosystems relative to marine and terrestrial systems, put them under pressure from pollution, agricultural run-off, flow regulation and water abstraction (Dudgeon *et al.*, 2006). These environmental stressors may reduce the resilience of the native species living with them to further threats, such as invasion by non-native species (Strayer, 2010).

Freshwater invasions have been particularly prevalent in Europe. For example, the past two centuries have seen the total surface area of river catchments connected to the River Rhine increase by a factor of 21.6 as a result of canal construction (Leuven *et al.*, 2009). This increase in connectivity has led to the mixing of previously separate species causing the average number of aquatic invasions to rise from <1 to 13 per decade between 1800 and 2005 (Leuven *et al.*, 2009). Non-native species (primarily from the USA and the Ponto-Caspian region of Eastern Europe) now comprise at over 11 percent of species richness and dominate benthic communities (Leuven *et al.*, 2009). The River Thames in the UK is another heavily invaded European freshwater catchment. Over 96 established INNS have been reported in the Thames, with more than half of them introduced in the past 50 years due to a major increase in shipping traffic (Jackson and Grey, 2012).

1.1.4 Impacts of invasive non-native species

In an analysis of 170 extinct species on the IUCN Red List for which the causes of extinction were available, INNS were cited as contributing to 54% of extinctions and being the primary cause of over 20% of extinctions (Clavero and García-Berthou, 2005). A recent global synthesis revealed that a higher proportion of freshwater species are threatened with extinction from impacts that include INNS than their terrestrial counterparts (32% in freshwater habitats vs. 24% in terrestrial) (Collen *et al.*, 2014). For example in freshwater environments, the spread of the non-native fungal pathogen *Batrachochytrium dendrobatidis* has caused the decline or extinction of over 200 species of frogs (Skerratt *et al.*, 2007), and the introduction of invasive

non-native crayfish in Europe has been responsible for population declines of up to 80% in native crayfish species (Richman *et al.*, 2015).

The ecological impacts of INNS on native species include predation, competition for resources, habitat alteration (Mack *et al.*, 2000), and the introduction of novel pathogens which may result in the emergence of an infectious disease (Dunn, 2009; Dunn and Hatcher, 2015; Tompkins *et al.*, 2011). As a freshwater example, where invasive signal crayfish (*Pacifastacus leniusculus*) have established in the UK, they have altered community structure by removing fish eggs and invertebrates (Peay *et al.*, 2010; Setzer *et al.*, 2011; Guan, 1998; Nilsson *et al.*, 2012); caused habitat degradation and potential flooding by burrowing into river banks (Guan, 2010); out-competed native crayfish populations for food and habitat space (Dunn *et al.*, 2009; Vorburger and Ribi, 1999) and introduced the highly pathogenic crayfish plague (Alderman, 1993).

The ecological impacts of invasive non-native species result in significant economic impacts (Vilà *et al.*, 2010a). Management and mitigation cost an estimated €12 billion/year in Europe (Shine *et al.*, 2009), £1.7 billion/year in the UK (Williams *et al.*, 2010), US$120 billion/year in the USA (Pimentel *et al.*, 2005), and AUD $7 billion/year in Australia (CSIRO, 2009). These costs largely result from the downstream consequences (e.g. remediation and control) that INNS can have on the provision of ecosystem services which are classified into supporting services (major ecosystem resources e.g. soil, nutrients, primary production); provisioning services (e.g. fuel, fibre, food); regulating services (e.g. flood prevention, disease control); and cultural services (e.g. recreation, eco-tourism) (Vilà *et al.*, 2010a).

Freshwater INNS can affect a broad suite of ecosystem services by altering the hydrology, biogeochemical cycling, and biotic composition of ecosystems (Strayer, 2010). For example, by fouling the pipes of water treatment plants and hydroelectric power stations, the zebra mussel (*Dreissena polymorpha*) has modified supporting, regulating and provisioning services in aquatic ecosystems (Vilà *et al.*, 2010b), causing significant economic impacts in both the USA (Pejchar and Mooney, 2009) and the UK (Williams *et al.*, 2010). Other freshwater INNS such as floating pennywort (*Hydrocotyle ranunculoides*) have impacted tourism and recreation (cultural services) by blocking waterways, preventing angling and boat access (Williams *et al.*, 2010).

1.1.5 Invasive non-native species affecting the UK

Seven out of ten of the UK Environment Agency's 'most wanted' INNS are freshwater species and include the American signal crayfish (*Pacifastacus leniusculus*), killer shrimp (*Dikerogammarus villosus*), topmouth gudgeon (*Pseudorasbora parva*), American mink (*Neovison vison*) floating pennywort (*Hydrocotyle ranunculoides*), parrot's feather (*Myriophyllum aquaticum*), and water primrose (*Ludwigia grandiflora*), (Environment Agency, 2011). However, since this list was compiled the quagga mussel – considered the highest risk invasive non-native species in Great Britain (Roy *et al.*, 2014)– has also been introduced to the UK and would be a likely addition. Unlike terrestrial and marine environments, which are increasingly threatened by INNS originating in Asia, most of the INNS threatening UK freshwater environments are predicted to originate from the Ponto-Caspian region of Eastern Europe (Roy *et al.*, 2014; Gallardo and Aldridge, 2013a).
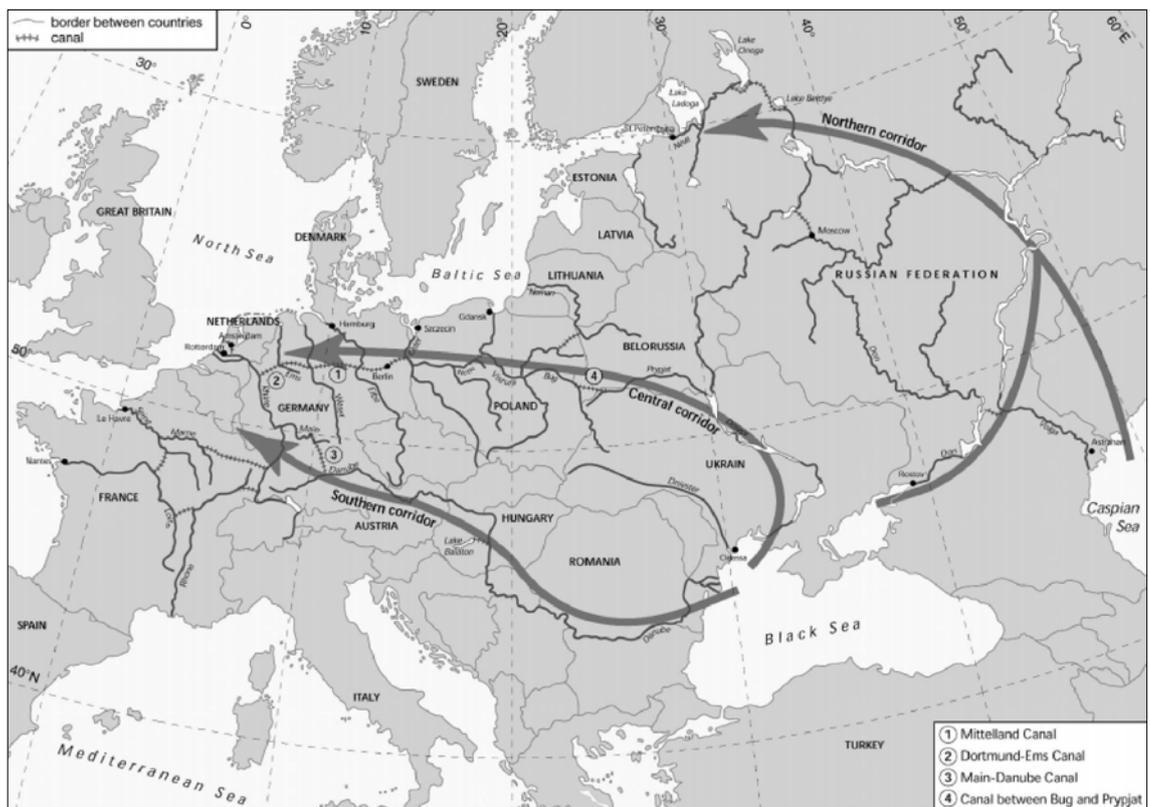
FIGURE 1.2 MAP SHOWING THE WESTWARD MIGRATION CORRIDORS OF PONTO-CASPIAN SPECIES IN EUROPE, REPRODUCED FROM BIJ DE VAATE ET AL. (2002).

The westward range expansion of Ponto-Caspian species has largely been facilitated by the interconnection of waterbodies through the construction of canals (Bij de Vaate *et al.*, 2002). Three inland migration corridors have been identified (Figure 1.2): the Northern Corridor which connects River Volga → Lake Beloye→ Lake Onega→ Lake Ladoga→ River Neva → Baltic Sea; the Central Corridor which connects River Dnieper → River Vistula → River Oder → River Elbe → River Rhine; and the Southern Corridor which connects the River Danube to the River Rhine (Bij de Vaate *et al.*, 2002).

Shipping has been identified as the most common pathway by which Ponto-Caspian species are initially introduced from Western Europe to the UK, with ballast water and, to a lesser extent, hull fouling identified as the most likely mechanisms (Godard *et al.*, 2012). As such, the Netherlands is the most likely origin of Ponto-Caspian arriving into the UK as it is the European country that exchanges the greatest volume of shipping trade with the UK (Gallardo and Aldridge, 2014). Recreational activities including angling and recreational boating are thought to be the most common pathways for the secondary spread of Ponto-Caspian species, once they have established in the UK (Godard *et al.*, 2012).

In line with predictions, increasing numbers of Ponto-Caspian species have been reported in the UK over the past decade (Keller *et al.*, 2009; Jackson and Grey, 2012; Gallardo and Aldridge, 2013a; Gallardo and Aldridge, 2013b). Of particular note are the killer shrimp (*Dikerogammarus villosus*), zebra mussel (*Dreissena polymorpha)* and quagga mussel (*Dreissena rostriformis bugensis*), which are considered among the worst invasive non-native species in Europe due to their abilities to alter entire invertebrate communities (shrimp) and to obstruct pipes supplying water treatment works with major economic consequences (mussels) (Madgwick and Aldridge, 2011; GB Non Native Species Secretariat, 2014b; Roy *et al.*, 2014; Bacela-Spychalska *et al.*, 2013; Williams *et al.*, 2010). The introduction of multiple species from the same origin causes particular concern because the species can interact synergistically, facilitating future invasions and potentially causing an 'invasional meltdown' (Simberloff and Holle, 1999; Gallardo and Aldridge, 2014). Such an invasional meltdown followed the introduction of the zebra mussel into the Great Lakes, North America (Ricciardi, 2001). By providing habitat complexity, shelter and food, the mussels are reported to have facilitated the introduction of a further 14 INNS including plants, invertebrates and fish (Ricciardi, 2001).

1.1.6 Invasive non-native crayfish in the UK

Over one third of the world's crayfish species are threatened with extinction (Richman *et al.*, 2015), largely because of displacement by non-native invasive crayfish species and the introduction of crayfish plague (Richman *et al.*, 2015; Holdich *et al.*, 2010). Compared to other non-native freshwater species, non-native crayfish have an unusually high 'success' rate, i.e. of the ten species that have been introduced into Europe, at least nine have become established (Holdich *et al.*, 2010). Native crayfish are considered to be keystone species in freshwater ecosystems, acting as indicators of water quality, controllers of trophic food webs and ecological engineers (Reynolds *et al.*, 2013). As such, they are important study organisms with which to learn about the dynamics of biological invasions so that evidence-based conservation plans can be developed (Richman *et al.*, 2015). The UK's only native species of crayfish is the white-clawed crayfish (*Austropotamobius pallipes*). It is now listed as 'endangered' on the IUCN red list having experienced declines of 50% - 80% over the past ten years (Füreder *et al.*, 2010). The invasive signal crayfish (*Pacifastacus leniusculus*) is also widespread in the UK and several other invasive non-native species of crayfish (e.g. *Orconectes virilis* and *Procambarus clarkii*) are also present in more localised populations (Ahern *et al.*, 2008; Ellis *et al.*, 2012; Holdich *et al.*, 2010; Holdich, 2007).

The American signal crayfish (*Pacifastacus leniusculus)*, is native to British Columbia, Canada, and western states of the USA (Bondar *et al.*, 2005), however it is now more abundant than native crayfish in much of Europe (Holdich *et al.*, 2010). It was originally imported to the UK from California for aquaculture in the 1970s and 1980s (Holdich *et al.*, 2010). Alderman (1993) suggested that although the crayfish were supposedly imported via quarantine in Sweden to prevent disease introduction, this was not always carried out and that some imports came directly from California with no disease screening. Once introduced to aquaculture farms, they escaped into nearby watercourses where they established and dispersed (Holdich *et al.*, 2014).

Signal crayfish have a faster growth rate, broader environmental tolerance and higher fecundity than native white clawed crayfish (Bubb *et al.*, 2004). They are also resistant carriers of the oomycete *Aphanomyces astaci*, the causal agent of crayfish plague (Unestam, 1969), an acutely pathogenic disease in native white-clawed crayfish (Unestam and Weiss, 1970), responsible for widespread local extinctions (Edgerton *et al.*, 2004). However, signal crayfish may also be associated with the introduction of

other sub-lethal pathogens (Longshaw, 2011; Longshaw *et al.*, 2012b; Dunn *et al.*, 2008).

1.2 Pathogens, parasites and invasive non-native species

On the IUCN's representative list of '100 of the World's Worst Alien Species' (IUCN Invasive Species Specialist Group, 2014), the introduction of disease to wildlife or humans is listed as a major impact of 25% of species (Dunn and Hatcher, 2015; Hatcher *et al.*, 2012). INNS which act as host species include the rainbow trout (*Oncorhynchus mykiss*), a reservoir for *Myxobolus cerebalis*, the causative agent of whirling disease in salmonid fish, and the mosquito fish *Gambusia affinis*, an impact of which is the introduction of helminths (Hatcher *et al.*, 2012). Seven species on the IUCN list are both INNS *and* parasites including *Aphanomyces astaci* the causative agent of crayfish plague (Alderman *et al.*, 1984) and *Batrachochytrium dendrobatidis* the causative agent of chytridiomycosis, a fungal infection which has devastated amphibian populations in at least 37 countries across of the continents they inhabit (Kriger and Hero, 2009). Closer to home, a recent horizon-scanning exercise identified the salmon fluke *Gyrodactylus salaris* as one of the 30 highest risk INNS to threaten the UK (Roy *et al.*, 2014). *G. salaris* is already considered to be the most important exotic fish-disease threat to the UK (Peeler *et al.*, 2004) having caused economic losses of approximately €4.3 billion to Norway since its arrival 30 years ago (Anon, 2008). These examples demonstrate the intrinsic link between biological invasions, parasitic infections and the emergence of disease (Dunn and Hatcher, 2015; Hatcher *et al.*, 2012). It is unsurprising then, that there is overlap between their pathways of transmission (Hatcher *et al.*, 2012; Dunn and Hatcher, 2015). Although the primary focus of this PhD is the spread of free-living INNS, I recognise that aquatic parasites form a sub-set of aquatic INNS as they can either be co-introduced with INNS (e.g. the American bullfrog, a reservoir of *Batrachochytrium dendrobatidis)*, or introduced as resistant live-stages which can survive outside the host (for example *A. astaci* spores can survive outside the host for 16 days (Oidtmann, 2000); *B. dendrobatidis* for 7 days (Johnson and Speare, 2003); and *G.salaris* for 2-5 days (Olstad *et al.*, 2006)*.

Much like biological invasions, the characteristics of freshwater habitats and the environmental stressors afflicting them can also influence the emergence of infectious diseases (Okamura and Feist, 2011a). The small volume and high connectivity of freshwater environments compared to terrestrial or marine environments can increase the likelihood of an introduced pathogen coming into contact with a host

(Okamura and Feist, 2011a). The deliberate stocking of non-native species for aquaculture and sport fishing is also common in freshwater environments and these introduced species may act as vectors for disease introduction (Okamura and Feist, 2011a). Additionally, the environmental stressors already described as reducing the resilience of native species to invasion (e.g. agricultural runoff, pollution, habitat loss through water abstraction (Dudgeon *et al.*, 2006)) may also reduce the immunity of host organisms, thus increasing the risk of disease introduction (Johnson and Paull, 2011; Okamura and Feist, 2011a).

1.2.1 The influence of pathogens and parasites on the invasion process

As well as sometimes being INNS themselves, and following similar transmission pathways, parasites and pathogens can facilitate or limit invasions, and have a positive or negative impact on native species (Dunn *et al.*, 2012). They can be involved in determining the success of the initial stages of an invasion in one of three ways.

First, novel pathogens can be introduced to native populations by introduced INNS (a concept termed parasite '*spill over*'), potentially resulting in an emerging infectious disease that poses a significant threat to biodiversity conservation (Tompkins *et al.*, 2011; Hatcher *et al.*, 2012). A classic example of this is the introduction of squirrel pox virus to red squirrel (*Sciurus vulgaris*) populations in the UK. Grey squirrels (*Sciurus carolinensis*) were introduced to the UK in the late 19th and early 20th Century and, in addition to out-competing native red squirrels for resources, they introduced squirrel pox, a virus that they had resistance to but that red squirrels did not, accelerating the decline of red squirrels (Rushton *et al.*, 2006). Although models predict that the virus should burn out in pure red squirrel populations, in mixed populations the resistant greys act as a reservoir hosts, maintaining parasite transmission to the red squirrel (Rushton *et al.*, 2006). Similarly, crayfish plague, caused by the oomycete pathogen *Aphanomyces astaci*, was introduced into European white-clawed crayfish (*A. pallipes*) populations by the invasive signal crayfish (*P. leniusculus*), an asymptomatic reservoir host, resulting in widespread local extinctions of the native species (Holdich and Pöckl, 2007).

Secondly, INNS can acquire native parasites in their introduced range (Kelly *et al.*, 2009a). INNS may act as new hosts for native parasites which can result in one of two outcomes. If the INNS are competent hosts for the pathogen in question, they may act as a reservoir for the pathogen and the infection may '*spill back*' from INNS

populations into native populations, increasing the overall prevalence of infection (Strauss *et al.*, 2012; Poulin *et al.*, 2011; Kelly *et al.*, 2009a), particularly if the disease is density-dependent disease (de Castro and M. Bolker, 2005; Poulin *et al.*, 2011). In contrast, if the INNS is a less competent host than the native species, it may acquire the pathogen but not develop a transmissible infection, therefore acting as a sink and diluting prevalence of infection, potentially benefiting the native host (Dunn and Hatcher, 2011; Kelly *et al.*, 2009a). For example, native roundhead galaxias fish (*Galaxias anomalus*) in New Zealand are infected with fewer helminths where they co-occur with introduced brown trout (*Salmo trutta*) (Kelly *et al.*, 2009b).

Finally, INNS can lose parasites during the invasion process, a concept termed 'enemy release' (Keane and Crawley, 2002). The invasion success of many host species is often attributed to the release from the regulatory effects of parasites (Keane and Crawley, 2002; Shea and Chesson, 2002), which may allow hosts to achieve unnaturally high population densities and explain their subsequent success (MacLeod *et al.*, 2010). Parasites may be lost because of stochastic and selective pressures during the invasion process (Dunn, 2009). They include i) only a small number of uninfected individuals surviving the invasion process; ii) selective pressures in the introduced habitat favouring fitter (i.e. uninfected/resistant) individuals; iii) reduced transmission opportunities due to a low density founder population, or absence of an intermediate host; iv) the founding population of an INNS could be an uninfected life history stage (e.g. marine larvae (Torchin *et al.*, 2002)) (Dunn, 2009).

Biogeographical studies (those comparing the diversity of parasites in the native and invasive range of a species) have reported that introduced animals and plants may escape up to 75% of the parasite and pathogen species in their native range (Torchin and Mitchell, 2004). However such studies may over-represent the effects of enemy release if they do compare the invasive population with the specific source population from which it was founded (Colautti *et al.*, 2004; Colautti *et al.*, 2005) as there may be genetic heterogeneity in different native populations which could influence their resistance to parasites as well as spatial heterogeneity in parasite prevalence (MacLeod *et al.*, 2010). Community studies (those comparing the diversity of parasites in native and invasive conspecifics in the introduced range) have often contradicted the Enemy Release Hypothesis, showing similar levels of parasites in both species (Colautti *et al.*, 2004).

Once a non-native species has established in its introduced range, parasites can influence competitive, predatory and other interactions between the native and invasive non-native species (Dunn *et al.*, 2012). These indirect effects include density-mediated effects (parasite-induced reductions in survival and reproduction, reducing host population density e.g. the aforementioned squirrel pox (Rushton *et al.*, 2006)) and trait-mediated effects (parasite-induced changes in host behaviour or morphology affecting competitive or predatory interactions) (Dunn *et al.*, 2012). Trait-mediated affects can influence the competitive ability of either the native or invasive non-native species. For example, the predatory strength of native white-clawed crayfish (*A. pallipes*) declined by 30% in individuals infected by the microsporidian parasite *T. contejeani* , reducing its ability to compete with the introduced signal crayfish (*P. leniusculus*) (Haddaway *et al.*, 2012). In contrast, the predatory strength of the freshwater invader *Gammarus pulex* increased by 30% when it was infected by the acanthocephalan parasite *Echinorhyncus truttae,* exacerbating its impacts on the invaded community (Dick *et al.*, 2010).

1.3 Managing Biological Invasions

The Convention on Biological Diversity endorses a three stage approach to INNS management: prevention (including horizon scanning, risk assessment, surveillance and biosecurity); early detection and rapid eradication; containment and long-term control (Convention on Biological Diversity, 2006). However, the complete eradication of established INNS is not always possible and is often considered controversial, and mitigation is often difficult and expensive (Mack *et al.*, 2000; Kolar and Lodge, 2001).

Economic analyses of INNS in the UK revealed that the cost of invasive non-native species management accelerates exponentially the longer the species is in the country (Williams *et al.*, 2010), while research in the USA has estimated that every dollar spent in early control and prevention returns an average of US$17 in prevented expenditure (Caplat and Coutts, 2011). Prevention – through effective pathway management – is now universally accepted as the most successful, environmentally desirable and cost-effective INNS control strategy (Caffrey *et al.*, 2014; Sambrook *et al.*, 2014; Roy *et al.*, 2014). To this end, the Convention on Biological Diversity (CBD) Aichi Biodiversity Targets for 2020 (Secretariat of the Convention on Biological Diversity, 2011), and the new EU Regulation on the Prevention and Management of the Introduction and Spread of Invasive Alien Species (European Commission, 2013) which comes into force in

2015, both focus on identifying and managing the pathways and vectors by which INNS can be introduced and spread.

> *"Target 9: By 2020, invasive alien species and pathways are identified and prioritized, priority species are controlled or eradicated, and measures are in place to manage pathways to prevent their introduction and establishment"* (CBD 2010).

Pathways are the processes that result in the introduction of non-native species from one location to another (Hulme *et al.*, 2008). They can be broadly grouped into six categories: release, escape, contaminant, stowaway, corridor and unaided (Table 1.1) (Hulme *et al.*, 2008; Convention on Biological Diversity, 2014).

TABLE 1.1 A SIMPLIFIED FRAMEWORK TO CATEGORIZE PATHWAYS OF INITIAL INTRODUCTION OF ALIEN SPECIES INTO A NEW REGION, ADAPTED FROM HULME (2009).

Initial introduction into region	Pathway	Description	Freshwater example	Regulation
Commodity	Release	Intentional introduction as a commodity for release	Introduction of sport fish, introduction of bait fish in USA great lakes.	Responsibility: applicant. Regulatory tools: permits, national legislation.
	Escape	Intentional introduction as a commodity but escapes unintentionally	Escape of American signal crayfish (*Pacifastacus leniusculus*) from aquaculture facilities in UK.	Responsibility: importer. Regulatory tools: Screening, risk analysis and national legislation.
	Contaminant	Unintentional introduction with a specific commodity	Parasites and commensals on traded crustacea or fish. E.g. *Gyrodactylus salaris* salmon ectoparasite (future UK threat)	Responsibility: exporter. Regulatory tools: Quarantine procedures and international legislation.
Vector	Stowaway	Unintentional introduction attached to or within a transport vector	Hull fouling, entanglement in boating/watersports equipment, ballast water. E.g. zebra mussel (*Dreissena polymorpha)* and killer shrimp (*Dikerogammarus villosus)*	Responsibility: carrier. Regulatory tools: biosecurity, border inspections, national and international regulation.
	Corridor	Unintentional introduction via human infrastructure linking previously unconnected regions	The movement of Ponto-Caspian species such as the killer shrimp (*Dikerogammarus villosus)* via canals and canal transport.	Responsibility: developer. Regulatory tools: environmental impact laws and international regulation.
Dispersal	Unaided	Unintentional introduction through natural dispersal across political boundaries	Potentially any aquatic INNS in a river which crosses borders.	Responsibility: polluter. Regulatory tools: "Polluter pays" fines supported by international regulation.

Note: The table describes the pathways by which non-native species can be either intentionally or unintentionally introduced into a region through direct or indirect human activity. An example of each pathway in a freshwater environment is provided and the different regulatory approaches used to control each pathway are summarised. A similar categorization of pathways was adopted by the Convention on Biological Diversity in June 2014.

1.3.1 Biosecurity

Within this PhD, I focus on the role of biosecurity in preventing the introduction, and secondary spread of INNS (primarily stowaways – an increasingly common pathway of introduction (Roy *et al.*, 2014)) along freshwater pathways. Biosecurity is defined as "*protection against the incursion or escape of potentially harmful or undesirable organisms*" (OED Online, 2014) and refers to the actions taken to reduce the risk of introducing or spreading INNS (GB Non Native Species Secretariat, 2014a). Biosecurity measures aim to reduce propagule pressure – a key determinant of invasion success –by minimising the likelihood of propagules entering transport vectors; intercepting and/or destroying the propagules at the point of introduction (e.g. border); and preventing propagules that are introduced from spreading further (Chapple *et al.*, 2013). On a small scale, such actions include the cleaning of visitors' walking boots to prevent the introduction of non-native seeds into protected areas or pristine environments, such as polar regions (Chown *et al.*, 2012; Huiskes *et al.*, 2014; Ware *et al.*, 2012). On a larger scale, biosecurity actions include the open-ocean exchange of cargo-ship ballast water to prevent near-shore NNS from being introduced into a recipient port (Gray *et al.*, 2007), or the checking and fumigating of tourist luggage at the border when air craft passengers enter a country (Tatem and Hay, 2007; Liebhold *et al.*, 2006). Biosecurity measures are designed to control a whole assemblage of INNS simultaneously, making them one of the most effective strategies to minimise new INNS introductions, spread and impacts (Briski *et al.*, 2012; Chan *et al.*, 2012; Caffrey *et al.*, 2014; Hulme, 2009; Hulme *et al.*, 2008; Convention on Biological Diversity, 2014).

Until recently, biosecurity was a term used primarily to describe actions taken to prevent the introduction of disease in crops or livestock (Meyerson and Reaser, 2002). This is reflected in the fact that biosecurity policies and guidelines are widespread in the prevention of emerging diseases in humans (World Health Organisation, 2014), aquatic animals (Oidtmann *et al.*, 2011) and agriculture (Food and Agriculture Organisation of the UN, 2014) while biosecurity policies to prevent threats to wildlife are currently fragmented and lack international cooperation (Dunn and Hatcher, 2015).

1.3.2 Recreational water users

Anglers and pleasure boaters are a significant pathway for the spread of INNS, having been responsible for much of the spread of freshwater INNS in Europe (Gallardo and

Aldridge, 2013b), the USA and New Zealand (deWinton *et al.*, 2009). Moreover, many of the Ponto-Caspian species recently reported in the UK and Europe were first discovered in popular watersports sites (Madgwick and Aldridge, 2011; Bacela-Spychalska *et al.*, 2013). This is because many INNS can survive for days in the anchor wells, bilges or hulls of boats, on trailers, or on damp equipment used by water users, allowing them to "hitchhike" between sites (Anderson *et al.*, 2014a; Bothwell *et al.*, 2009; Keller *et al.*, 2009; Keller *et al.*, 2007; Rothlisberger *et al.*, 2010).

The risk that recreational water users pose to the introduction and spread of aquatic non-native species pathogens has received considerable attention in the USA. This has largely been prompted by the ecological and economic impacts that they have had in the Great Lakes (Rothlisberger *et al.*, 2010; Vander Zanden *et al.*, 2010). Researchers in the USA have investigated the level of awareness that different groups of recreational water users, primarily anglers and pleasure boaters, have of aquatic invasive plants and animals (Lindgren, 2006; Gates *et al.*, 2009); the distances travelled by recreational water users (Johnson *et al.*, 2001; Buchan *et al.*, 1999; Gates *et al.*, 2009; Coetzee *et al.*, 2008); and the sourcing and disposal of live bait (Kilian *et al.*, 2012; Ludwig Jr and Leitch, 1996). The results of these studies indicated that anglers and boaters were travelling hundreds of kilometres between sites (Gates *et al.*, 2009) and were frequently transporting muddy waders (Gates *et al.*, 2009), the remnants of invasive aquatic plants on their boats (Johnson *et al.*, 2001; Rothlisberger *et al.*, 2010) and buckets containing live bait and water collected from another catchment within the time frame that pathogens could remain viable (Oidtmann *et al.*, 2002; Ludwig Jr and Leitch, 1996; Kilian *et al.*, 2012). Moreover, in Maryland, USA, over 60% of anglers who used live bait admitted releasing unused bait, which was frequently non-native crayfish, into the water body at the end of their angling trip (Kilian *et al.*, 2012). These results highlight the sizeable biosecurity threat that uninformed water users may pose to biodiversity conservation. The need to improve biosecurity practices of high risk groups such as recreational water users through the development of awareness-raising programmes is recognised as an important component of the new EU invasive non-native species legislation (European Commission, 2013; Caffrey *et al.*, 2014; Beninde *et al.*, 2014).

1.3.3 Freshwater biosecurity in New Zealand

New Zealand is widely recognised as a leading example of aquatic biosecurity best-practice (Chapple *et al.*, 2013; Caffrey *et al.*, 2014; Sambrook *et al.*, 2014). Its comprehensive biosecurity strategies are coordinated by a dedicated team in government (Ministry of Primary Industries (MPI)) and supported by unified legislation: the Biosecurity Act 1993 which covers threats posed by pests and unwanted organisms which may cause harm to the economy and public health, as well as the environment (Ministry of Primary Industries, 1993). Under the Act it is an offence to knowingly spread an unwanted organism with penalties of up to 5 years imprisonment and/or a fine of up to NZD $100,000 (Meyerson and Reaser, 2002; Chapple *et al.*, 2013). In recognition of the role that biosecurity can play in biodiversity conservation, and to meet the requirements of new EU INNS legislation, leading researchers, policy makers and practitioners advocate building evidence-based biosecurity programmes in Europe based on New Zealand's best practice (Caffrey *et al.*, 2014; Sambrook *et al.*, 2014).

Efforts to raise biosecurity awareness in New Zealand were catalyzed in 2004 by the discovery of the invasive non-native diatom *Didymosphenia geminata* (hereafter didymo) in New Zealand's Lower Wairau River (Kilroy and Unwin, 2011). As a result of its prolific reproduction, didymo has unusually high biomass and forms dense brown mats on the surface of invaded rivers, smothering rocks and submerged plants (Bothwell *et al.*, 2009). Didymo blooms have had negative impacts on the abundance and diversity of invertebrates in benthic communities in New Zealand rivers as well as reducing the aesthetic value of rivers, restricting recreational activities and causing major economic losses (Beville *et al.*, 2012; Kilroy *et al.*, 2009). The economic cost of didymo was estimated at NZD $127.8 million between 2006 and 2011 and is expected to reach between NZD $210.6 and NZD $854.8 million between 2011 and 2020 (Deloitte, 2011).

MPI quickly identified that human actions posed the biggest threat to the containment of didymo after it was first discovered in an area of the South Island which attracts fishing enthusiasts from across the world (Kilroy and Unwin, 2011). In response, the *Check Clean Dry* campaign was quickly launched with the primary goal of improving biosecurity awareness and action among the public in order to slow the spread (Ministry of Primary Industries, 2009). Because of the unsightly nature of didymo, it

received widespread, and long term national media attention, which is likely to have played a major part in increasing public awareness (Gozlan *et al.*, 2013).

As didymo was still absent from the North Island in 2014, the cost benefit is now estimated to be around NZD $7.61 for every $1 spent on the biosecurity campaign, or NZD $168 million in total (Branson and Clough, 2006), demonstrating the sizeable financial benefits associated with investment in biosecurity awareness.

1.3.4 Freshwater biosecurity in the UK

In contrast to New Zealand, biosecurity awareness raising schemes are in their infancy in the UK. The first biosecurity campaign targeting freshwater users – *Check Clean Dry* – was launched by the Department of Environment, Farming and Rural Affairs (Defra), in partnership with the GB Non Native Species Secretariat, in 2010. The campaign, based on New Zealand's, was launched in response to the discovery of the killer shrimp (*Dikerogammarus villosus*), an invasive Ponto-Caspian gammarid shrimp, in the UK. The species was first reported in Cardiff Bay in South Wales and Grafham Water in Cambridgeshire (Madgwick and Aldridge, 2011). Both sites – as well as the sites where the species has been reported in Europe – are popular recreational watersports venues, indicating that recreational water users may play an important role in its introduction and dispersal (Bacela-Spychalska *et al.*, 2013; Madgwick and Aldridge, 2011). The aim of the UK's *Check Clean Dry* campaign was to improve the biosecurity practices of water users in order to prevent the accidental spread of this species, along with other aquatic invasive non-native species and pathogens by checking their equipment for live organisms, cleaning it thoroughly and allowing it to dry after use.

The UK is obligated to improve preventative action under new EU INNS legislation, which comes into force in January 2015 (European Commission, 2013). To date, EU member states have regulated some areas of INNS management (e.g. plant health – EC 29/2000 (European Union, 2014), wildlife trade – EC 338/97 (European Union, 1997) and aquaculture – EC 104/2000 (European Council, 2000)). However, Europe has lacked a comprehensive framework for the management of INNS, despite their impacts costing an estimated €12 billion/year to manage (Shine *et al.*, 2009). As such INNS management initiatives have been disparate across member states, and often reactive – attempting to mitigate/eradicate INNS which have already arrived (Caffrey *et al.*, 2014). The new EU legislation aims to promote consistent INNS management across EU member states and focuses on a preventative approach to INNS

management (Beninde *et al.*, 2014). Researchers and practitioners from across Europe recommend that strategies to improve biosecurity awareness-raising and compliance should form a key part of each EU member state's response to the legislation (Caffrey *et al.*, 2014).

1.4 Research Approach

Throughout this PhD, I have combined social and ecological research to investigate how to reduce the unintentional spread of INNS along freshwater pathways. Engaging with the public and stakeholders is central to the success of environmental awareness initiatives (Bremner and Park, 2007; García-Llorente *et al.*, 2008; Baruch-Mordo *et al.*, 2011). As such, promoting the engagement of stakeholder groups, and promoting awareness of the threats of INNS and mechanisms by which they are introduced are included in the CBD's guiding principles for the prevention, introduction and mitigation of impacts of INNS (Convention on Biological Diversity, 2006). Evidence suggests that when public attitudes are not considered in INNS management programmes, the consequences can be far reaching (Gozlan *et al.*, 2013). For example, when a pike (*Esox lucius*) eradication programme proceeded in California without adequate public consultation, it resulted in lawsuits being taken out against the regulatory authorities responsible (Lee, 2001). Nevertheless, social research into the effectiveness of INNS has often been overlooked (García-Llorente *et al.*, 2011; García-Llorente *et al.*, 2008; Sharp *et al.*, 2011; Schüttler *et al.*, 2011), and existing social research into INNS has often focused on public attitudes towards culling or eradication programmes (e.g. Ford-Thompson *et al.*, 2012; Bremner and Park, 2007) rather than education programmes.

Although less controversial than eradication, high levels of public compliance are still critical to the success of biosecurity programmes. If public behaviour is to be changed (for example to adopt biosecurity actions after certain recreational activities), it is essential that the public understand the problem and are motivated to contribute to the solution (Caffrey *et al.*, 2014; Baruch-Mordo *et al.*, 2011). Moreover, biosecurity measures can cost individuals and organisations considerable time and money (for example through the installation of a wash down station for a yacht marina). Public engagement is therefore essential to determine whether proposed biosecurity measures which prove effective in the laboratory would be feasible – and complied with – in practice.

Social research is also required to assess the risk posed by different transport pathways, particularly those – such as recreational watersports – which are not officially regulated, and therefore lack official movement data (Clarke Murray *et al.*, 2011). Questionnaire research allows a detailed understanding of the vectors, movement patterns and control measures of different groups to be collated.

1.4.1 Research objectives

My primary research aim was to assess the role of human-mediated INNS dispersal in freshwater environments, and identify how such unintentional spread could be reduced through effective biosecurity. The specific aims were to:

- Quantify the movement patterns, biosecurity awareness and biosecurity compliance among recreational water users as a pathway for the spread of INNS in the UK.
- Quantify the effectiveness of biosecurity treatments at preventing the accidental spread of freshwater INNS.
- Identify how best to raise biosecurity awareness and promote pro-environmental behaviour change among individuals and organisations.
- Compare parasite and pathogen profiles in native and invasive non-native species and explore their implications for invasion success.
- Quantitatively review the role that recreational activities play in the introduction of INNS in freshwater, terrestrial and marine environments.

In *Chapter Two*, I used questionnaire research to quantify the movement patterns of recreational water users in the UK, to investigate their awareness of, and compliance with, biosecurity measures and to determine whether any particular groups of water users (e.g. competitors vs. leisure users, or game anglers vs. coarse anglers) posed a higher biosecurity risk than others. Managing the pathways of stowaway organisms requires an in depth knowledge of the spatial dynamics of vectors, the importance of different vectors and the preventative actions being employed (Hulme, 2009). Although recreational water users are commonly cited as a pathway for the dispersal of freshwater INNS in the US and New Zealand (Gates *et al.*, 2009; Rothlisberger *et al.*, 2010; Ludwig Jr and Leitch, 1996; Keller *et al.*, 2007), the potential role they play in the introduction and dispersal of INNS in the UK has received less attention.

In *Chapter Three*, I examined the effectiveness of different biosecurity treatments (hot water (45°C for 15 minutes); drying; hot water AND drying) as biosecurity treatments

to prevent the spread of four invasive non-native plants and three Ponto-Caspian animals all of which are listed as high impact invaders by the UK Technical Advisory Group for the EU Water Framework Directive. To be recommended for public use, a biosecurity treatment must be effective, environmentally sound, economical and safe to use (Kilroy *et al.*, 2006). Research by Cefas (Stebbing *et al.*, 2011) demonstrated that the submersion of angling nets in hot water (45°C for 15 minutes), fulfilled these criteria and caused 100% mortality in the killer shrimp (*Dikerogammarus villosus*), suggesting that it could be an effective treatment to recommend to water users at the 'Clean' stage of *Check Clean Dry*. However, it is important to determine whether biosecurity treatments are effective against a wide range of INNS, as water users cannot be expected to know which species are present at any given site.

In *Chapter Four*, I evaluated the successes and challenges of a long term freshwater biosecurity programme running in New Zealand – a country widely recognised as a leading example of aquatic biosecurity best practice (Caffrey *et al.*, 2014; Chapple *et al.*, 2013; Meyerson and Reaser, 2002). The aim of the research was to identify the successes and challenges of New Zealand's freshwater biosecurity programme in order to develop best-practice guidelines for the delivery of similar campaigns in the UK and wider Europe. To do this, I conducted questionnaires with water users in New Zealand to compare their biosecurity awareness and compliance with those in the UK. I also conducted semi-structured interviews with key stakeholders to explore their perceptions of the successes and challenges of New Zealand's biosecurity programme.

In *Chapter Five*, I investigated the parasite profile of native and invasive non-native species to explore the role that sub-lethal infectious agents may play in freshwater invasions, focusing on the invasion of signal crayfish in the UK. I combined a long term histology dataset collected by the Centre for Ecology, Fisheries and Aquaculture Science (Cefas) with histology data collected through a primary field study. I used the data to look for evidence of parasite introduction, acquisition or enemy release in crayfish, processes which may be involved in determining the success of non-native signal crayfish, or the resilience of native white-clawed crayfish in the UK.

Tourism and recreational activities are widely regarded as pathways for the spread of non-native stowaways (Kolar and Lodge, 2001; Clout and De Poorter, 2005; Meyerson and Reaser, 2002). Having explored the potential for INNS to be transported via freshwater recreational activities (Chapters 2, 3 and 4), in *Chapter Six* I conducted a global review to investigate whether INNS establish in sites where recreational

activity takes place. Using a meta-analysis approach to quantitatively synthesize the results of multiple ecological studies (Stewart, 2010), I explored whether the diversity and abundance of non-native species were higher in sites disturbed by recreational activities than in undisturbed sites in terrestrial, freshwater and marine environments across a broad range of tourist-related vectors and INNS taxa.

In *Chapter Seven,* the findings of the research outlined above are summarised and their potential management implications discussed in the context of forthcoming invasive non-native species policy changes in Europe.

2 BIOSECURITY AND VECTOR BEHAVIOUR: EVALUATING THE POTENTIAL THREAT POSED BY ANGLERS AND CANOEISTS AS PATHWAYS FOR THE SPREAD OF INVASIVE NON-NATIVE SPECIES AND PATHOGENS

2.1 Summary

Invasive non-native species (INNS) endanger native biodiversity and are a major economic problem. The management of pathways to prevent their introduction and establishment is a key target in the Convention on Biological Diversity's Aichi biodiversity targets for 2020. Freshwater environments are particularly susceptible to invasions as they are exposed to multiple introduction pathways, including non-native fish stocking and the release of boat ballast water. Since many freshwater INNS and aquatic pathogens can survive for several days in damp environments, there is potential for transport between water catchments on the equipment used by recreational anglers and canoeists. To quantify this biosecurity risk, I conducted an online questionnaire with 960 anglers and 599 canoeists to investigate their locations of activity, equipment used, and how frequently equipment was cleaned and/or dried after use. Anglers were also asked about their use and disposal of live bait. My results indicate that 64% of anglers and 78.5% of canoeists use their equipment/boat in more than one catchment within a fortnight, the survival time of many of the INNS and pathogens considered in this study and that 12% of anglers and 50% of canoeists do

so without cleaning or drying their kit between uses. Furthermore, 8% of anglers and 28% of canoeists had used their equipment overseas without cleaning or drying it after each use which could facilitate both the introduction and secondary spread of INNS in the UK. My results provide a baseline against which to evaluate the effectiveness of future biosecurity awareness campaigns, and identify groups to target with biosecurity awareness information. My results also indicate that the biosecurity practices of these groups must improve to reduce the likelihood of inadvertently spreading INNS and pathogens through these activities.

2.2 Introduction

Invasive non-native species (INNS) are a primary driver of biodiversity loss and a major economic problem, with management and mitigation costing an estimated US$120 billion in the USA (Pimentel *et al.*, 2005), US$6.3 billion in Australia (CSIRO, 2009) and US$2.6 billion in the UK each year (Williams *et al.*, 2010). Their ecological impacts range from habitat degradation, to competition with native species, to the introduction of pathogens and disease (Prenter *et al.*, 2004b; Okamura and Feist, 2011b; Hatcher and Dunn, 2011). As the eradication of an established INNS is rarely possible (Mack *et al.*, 2000; Kolar and Lodge, 2001), preventative management is an important and cost effective control strategy (Caplat and Coutts, 2011). To this end, the management and prevention of INNS introductions is recognised as a global priority for biodiversity conservation and is listed as one of the Convention on Biological Diversity's (CBD) Aichi biodiversity key targets for 2020 (Secretariat of the Convention on Biological Diversity, 2011).

Freshwater ecosystems are particularly vulnerable to INNS (Strayer and Findlay, 2010). They are exposed to a wide range of transmission pathways including fish stocking, the redirection of water supplies, release of boat ballast and bilge water, release of exotic and ornamental plant and animal species, and the transfer of recreational angling and boating gear between sites (Rahel, 2007; Keller *et al.*, 2009; Strayer and Findlay, 2010; Havel and Shurin, 2004; Oidtmann *et al.*, 2011; Jacobs and MacIsaac, 2007). Recent research indicates that fishing, boating and leisure activities are collectively responsible for almost 40% of aquatic species introductions into Europe (Gallardo and Aldridge, 2013b). The management of vectors such as these is considered to be one of the most effective strategies to prevent introduction and spread of invaders since numerous INNS threats can be controlled simultaneously (Briski *et al.*, 2012; Chan *et al.*, 2012).

In the UK, freshwater ecosystems contain seven of the UK Environment Agency's 10 'most wanted' invasive non-native species (Environment Agency, 2011) and are thought to be threatened by a further 11 (Gallardo and Aldridge, 2013b).

TABLE 2.1 APPROXIMATE SURVIVAL TIMES OF SELECTED FRESHWATER PATHOGENS AND INVASIVE NON-NATIVE SPECIES

Species	Survival time outside host (pathogens) or in damp conditions (INNS)		Reference
NOTIFIABLE PATHOGENS			
Gyrodactylus salaris	2-5 days	R	(Olstad *et al.*, 2006)
Koi herpes virus	3 days	P	(Shimizu *et al.*, 2006)
White spot syndrome virus	3 -4 days	R	(Nakano *et al.*, 1998)
Aphanomyces astaci	16 days	P	(Oidtmann, 2000)
Batrachochytrium dendrobatidis	7 days	P	(Johnson and Speare, 2003)
Amphibian ranaviruses	1 month	P	(Nazir *et al.*, 2012)
Infectious haematopoietic necrosis	1 month	R	(Nazir *et al.*, 2012)
Spring viraemia of carp	5 weeks	R	(Ahne, 1976)
Viral haemorrhagic septicaemia	49 days	R	(Ahne, 1982)
INVASIVE NON-NATIVE SPECIES			
Topmouth gudgeon	Minutes (fish)	P	N/A
Zebra mussel	3-5 days	P	(Ricciardi *et al.*, 1995)
Signal crayfish	3-7 days	P	(GB Non Native Species Secretariat, 2011)
Killer shrimp	15 days	P	(Fielding, 2011)
Floating pennywort	16 days	P	see Chapter 3
Parrots Feather	16 days	P	see Chapter 3
Chinese mitten crab	No data available	P	
Ponto-caspian shrimp	6 days	R	(Martens and Grabow, 2008)
Quagga mussel	3-5 days	R	(Ricciardi *et al.*, 1995)
MEAN SURVIVAL TIME (where known)	15 days		

Source: Notifiable freshwater pathogens listed by the World Organisation for Animal Health (OIE) and freshwater INNS listed in the Environment Agency's 10 'most wanted' invasive non-native species or as one of the potential invaders threatening Great Britain and Ireland (Gallardo and Aldridge, 2013b).
Note: P = species or pathogen is already present, R = this species poses a significant threat to UK freshwaters

Despite the estimated 4 million recreational anglers (Environment Agency, 2004) and 404,000 canoe owners (British Canoe Union, 2011) in the UK, these groups have received little attention with regard their potential role in the introduction and secondary spread of aquatic invasive non-native species and pathogens.

Since many INNS can survive in damp environments for a number of days or even weeks (Table 2.1), the potential exists for their introduction and spread between catchments on wet equipment used by anglers and boaters (Taugbøl *et al.*, 1993; Rahel, 2007). Prominent examples include the zebra mussel (*Dreissena polymorpha*) introduced to Ireland on the hulls of boats (Minchin *et al.* 2003); the pathogen *Aphanomyces astaci* (causative agent of crayfish plague) vectored in mud and on damp angling gear (Reynolds, 1988; Taugbøl *et al.*, 1993); and the killer shrimp (*Dikerogammarus villosus*) which is able to survive for up to 15 days on damp angling equipment (Fielding, 2011).

2.3 Methods

2.3.1 Ethics Statement

The questionnaire satisfied the University of Leeds' guidelines on ethical conduct (Ethics reference BIOSCI 12-043). All data were collected, stored and analysed anonymously. Respondents were asked for two items of demographic data (age group and sex) but no data was collected that would enable any respondent to be identified.

2.3.2 Questionnaire

An online questionnaire survey was conducted using Bristol Online Surveys software (University of Bristol, 2013) (see Appendix A). The secretaries of 316 angling clubs and 241 canoeing clubs in England were contacted from listings in the UK Environment Agency's 'Where to Fish?' guides, online angling club databases and the British Canoe Union's list of canoe clubs, and asked to circulate the questionnaire to their members.

Anglers and canoeists were asked about the type and frequency of angling/canoeing carried out. In order to gain a representative overview of how far each respondent typically travels to take part in their sport, they were asked to list the three sites that they fished/canoed at most recently and most frequently. The six sites were geocoded into latitude and longitude coordinates with Python's Geopy toolbox, using the Google Maps API. The catchment that each site fell into was identified using the Extraction

tool in the ArcGIS™ Spatial Analyst extension within the ArcGIS™ 10.1 Geographic Information System software (Environmental Systems Research Institute (ESRI), 2012), according to the European Union Water Framework Directive catchment areas.

Respondents were asked about the equipment used during each trip, and how frequently it was i) dried and ii) cleaned after use; iii) which cleaning products were used, if any; and iv) whether equipment had been used overseas, and in which countries. Anglers were also asked about their use of live bait (invertebrates and fish). Canoeists were asked about the factors that influenced whether they cleaned and dried their equipment at the end of each trip. To do this, they were asked to score each of 6 factors on a likert scale from 1 (not important) to 5 (very important) depending on how these factors influenced their decision to clean equipment after use. The factors were: i) availability of a hose, ii) cost of cleaning products, iii) time taken to clean boat, iv) availability of information about how to clean boat and v) how dirty their boat appeared to be. Canoeists were also asked if they were aware of the '*Check Clean Dry*' biosecurity campaign launched by the UK Department for Environment, Food and Rural Affairs (Defra) in 2010 to see whether there were differences in the biosecurity practices of those who had and had not heard of the campaign. Respondents were asked about their awareness of the biosecurity campaign on the last page of the online questionnaire having already answered questions about their actions in order to minimise potential bias.

The answer options for closed-format questions were determined through consultation with Environment Agency Fisheries Officers, UK Rivers Trusts and biosecurity experts from the Centre for Environment, Fisheries and Aquaculture Science (Cefas). A prototype version of the questionnaire was also piloted with 15 anglers and 12 canoeists to prevent any misunderstandings; to check that the online questionnaire worked effectively; and to ensure that sufficient answer options had been provided in the closed questions.

2.3.3 Hazard scores

In order to explore the relative biosecurity hazard posed by different groups of anglers and canoeists, respondents were scored against a set of criteria from 1 (low hazard) to 5 (high hazard) by interrogating them against a set of criteria (Table 2.2). The criteria were: the number of catchments visited (N), the frequency of the activity (F), the frequency of equipment cleaning (C) and the frequency of equipment drying (D).

Individual Hazard Score = N * F * C * D

By multiplying hazards together, interactions between factors were incorporated into the overall hazard score. As there is insufficient data available with which to inform the relative importance of the different risk criteria, I gave them equal weighting when calculating the hazard scores. As I was primarily investigating the potential role of anglers and canoeists in the secondary spread of INNS between UK catchments, anglers and canoeists who only visited one catchment scored zero. Regardless of how frequently they cleaned or dried their equipment, or how frequently they travelled, their total score would remain zero as they posed no likelihood of moving invasive non-native species or aquatic pathogens to another catchment.

TABLE 2.2 SCORING SCHEME FOR THE CRITERIA AGAINST WHICH EACH ANGLER OR CANOEIST WAS ASSESSED IN THE HAZARD ANALYSIS

Risk Factor	Description	Hazard Score
Frequency of angling/canoeing	Once a month or less frequently	1
	Once every three weeks	2
	One a fortnight	3
	Once a week	4
	More than once a week	5
Number of catchments visited	1 catchment	0
	2 catchments	2
	3 catchments	3
	4 catchments	4
	5 or 6 catchments	5
Cleaning of equipment	After every trip	1
	Every 2-5 trips	2
	Every 6- 10 trips	3
	Every 11+ trips	4
	Never	5
Drying of equipment	After every trip	1
	Every 2-5 trips	2
	Every 6- 10 trips	3
	Every 11+ trips	4
	Never	5

Note: Scores from 1 – 5 correspond to a hazard gradient from 1 (very low) to 5 (very high). As I was considering secondary spread between river catchments, respondents scored 0 if they said that they only visited one catchment.

2.4 Results

Fifty two angling clubs and 70 canoeing clubs circulated the online questionnaire to their members. In total, 599 canoeists and 960 anglers completed the questionnaire (response rates 17% and 25%, respectively).

Following best practice advice of White *et al*. (2005), the sample was verified using demographic information to ensure that respondents were representative of the angling/canoeing communities. Of the 960 angler respondents, 98% were men with the highest proportion of respondents aged between 55 and 64 (31%) followed by 45 and 54 (22%) and 65+ (17%). To ascertain whether the sample was representative of the UK angling population, a Kolmogorov–Smirnov test was performed in 'R' (R Development Core Team, 2012) to compare the age distribution of angler respondents to the age distribution of holders of Environment Agency rod licences in 2011- a requirement of all UK freshwater anglers. No significant difference was detected between the ages of the two groups (K-S Test, D=0.24, p>0.05), nor was there a significant difference between the sex ratios of the two groups, with 2% of angler respondents and 5% of rod license holders being female (K-S Test, D = 0.3, p>0.05).

Seventy percent of the 599 canoeist respondents were men, a sex ratio which was not significantly different from British Canoe Union figures on the sex ratio of UK recreational boat users (British Canoe Union 2011) (D=0.7, p>0.05). Respondents were from a broad range of age groups, with 35-44 year old and 45-54 year old groups with the greatest number of respondents (16.4% and 26%, respectively). Unfortunately, data on the age profiles of UK canoeists were unavailable.

Respondents were broadly spread across different angling and canoeing categories. Forty four percent of anglers were coarse anglers (typically pleasure anglers using rods from the bank and catching any freshwater fish other than game fish), 25% were game anglers targeting trout, 13% were match anglers (typically angling in heavily stocked commercial fisheries and frequently travelling to different sites to attend competitions), 7% were barbel anglers, 6% were game anglers targeting salmon and 5% were pike anglers. Almost half of canoeist respondents (47%) were recreational canoeists using rivers while 21% canoed on lakes, 19% were competitive canoeists, 11.9% were sea kayakers who also took part in freshwater canoeing and 1.5% were long distance touring canoeists.

2.4.1 Potential for secondary spread

Anglers visited a mean of 2.25 different UK catchments (Figure 2.2). There was a significant difference in the number of catchments visited by categories of angler (ANOVA $F_{5,954} = 9.56$, $p < 0.001$). Posthoc (LSD) tests revealed that salmon anglers – who visited a mean of 2.79 catchments – travelled significantly further than any other group of angler ($p<0.05$). Canoeists visited a mean of 2.84 different catchments (Figure 2.2). There was a significant difference between the distances travelled by different categories of canoeist (ANOVA, $F_{4,594} = 6.17$, $p < 0.001$), with competitive canoeists visiting significantly fewer catchments than the other groups (mean of 2.53, posthoc tests $p<0.05$ when compared to each of the other groups).

Sixty four percent of anglers and 79% of canoeists used their equipment in more than one catchment within a fortnight (Table 2.3). Forty nine percent of canoeists and 12% of anglers visited more than one catchment within a fortnight and neither cleaned nor dried their kit between uses (Table 2.3). The geographic movements of these higher risk anglers and canoeists are displayed in Figure 2.1.
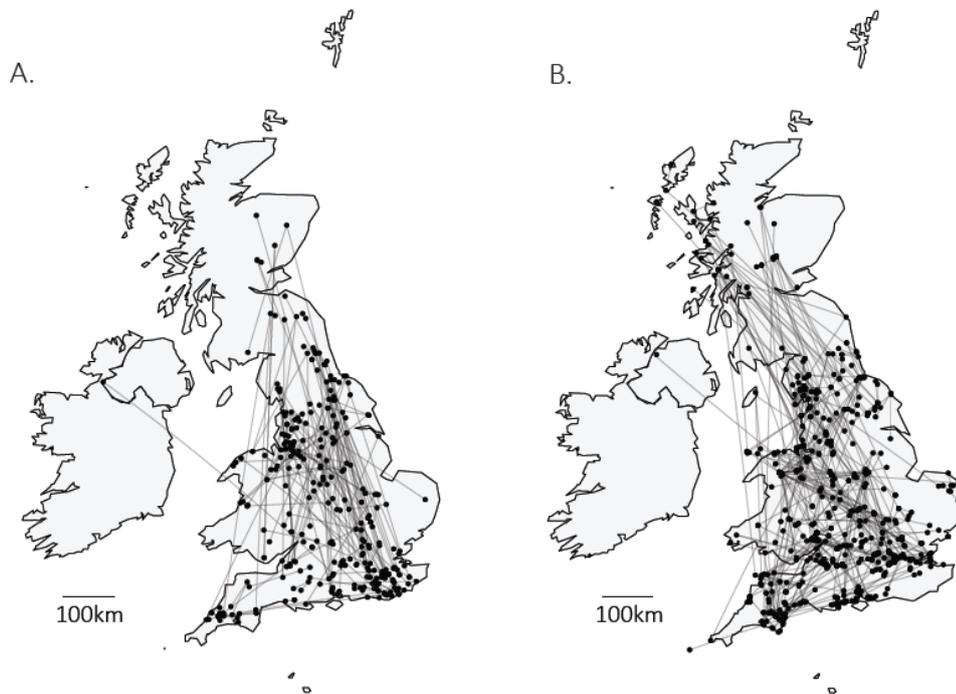
FIGURE 2.1 MAPS SHOWING THE LAST THREE UK SITES VISITED BY A) ANGLERS AND B) CANOEISTS WHO VISITED MORE THAN ONE CATCHMENT WITHIN A FORTNIGHT AND FAILED TO CLEAN OR DRY THEIR KIT BETWEEN USES.
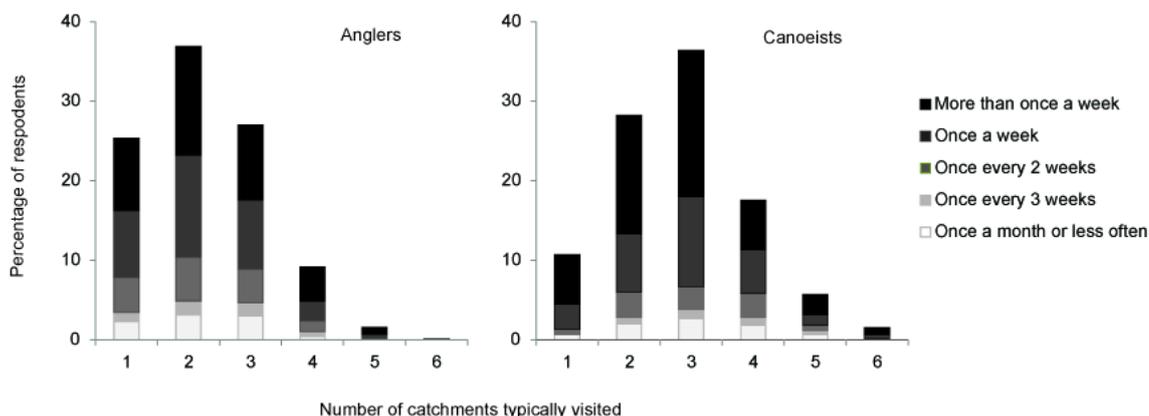
FIGURE 2.2 TYPICAL NUMBER OF UK CATCHMENTS VISITED BY CANOEISTS AND ANGLERS. SHADING SHOWS THE FREQUENCY WITH WHICH RESPONDENTS TRAVELLED BETWEEN THE CATCHMENTS THAT THEY VISITED.

Of the 614 anglers and 470 canoeists who used their equipment in more than one catchment within a fortnight, 22% of anglers and 10% of canoeists cleaned their kit after every use, 80% of anglers and 33% of canoeists dried their kit after every use and 21% of anglers and 6% of canoeists both cleaned and dried their kit after every use, the biosecurity advice recommended by Defra (Figure 2.3). Of the anglers who cleaned their kit after each use, 49% used tap water, 31% used disinfectant and 30% used detergent. Of the canoeists who cleaned their kit each time, 81% used tap water, 15% used detergent and 4% used disinfectant.
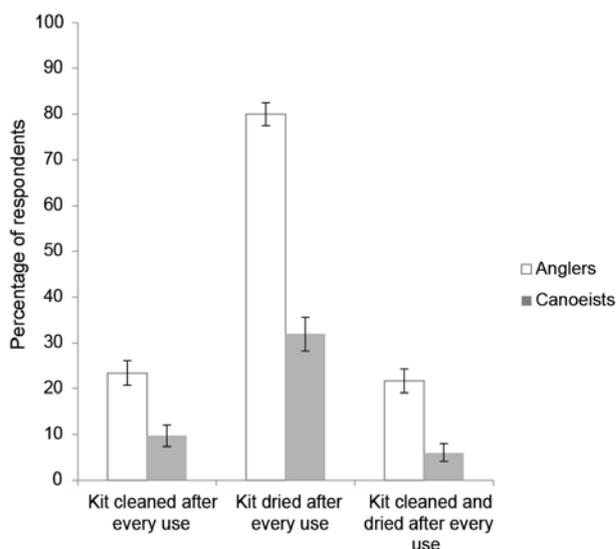
FIGURE 2.3 PERCENTAGE OF ANGLERS AND CANOEISTS WHO VISITED MORE THAN TWO CATCHMENTS WITHIN A FORTNIGHT AND WHO EITHER CLEANED, DRIED OR CLEANED AND DRIED THEIR EQUIPMENT AFTER EVERY USE. ERROR BARS SHOW 95% CONFIDENCE INTERVALS.

A large proportion of the anglers who travelled to more than one catchment within a fortnight without cleaning equipment after each trip used equipment associated with INNS/pathogen accumulation: rubber or felt soled waders (used by 36% and 4%, respectively) and keep nets (used by 25%).

2.4.2 Hazard scores

Overall, anglers had lower hazard scores than canoeists, due to the higher proportion drying equipment, and the lower number of catchments visited (Table 2.3). When different types of angler were compared, the median hazard scores of different groups were significantly different (Kruskal Wallis H = 29.80; 5 df; p<0.001). Salmon anglers had the highest average hazard score, followed by trout anglers while pike and barbel anglers had the lowest (Table 2.3). In contrast, there was no significant difference between the hazard scores of different categories of canoeist (Kruskal Wallis H= 2.086; 4df; p>0.05). However, competitive canoeists had the highest mean hazard score and touring canoeists had the lowest (Table 2.3).

Canoeists were asked about the factors affecting whether they cleaned equipment after use. The availability of a hose was the most important factor (mean score 3.1 out of 5) followed by time availability (mean score 2.86 out of 5). The least important factors were the cost of cleaning products and the availability of information about how to clean equipment (both scored a mean of 1.9 out of 5). The 22 percent (130) of canoeist respondents who had heard of the '*Check Clean Dry'* campaign exhibited biosecurity hazard scores that were 40% lower than those who had not (Kruskal-Wallis: H = 10.99; d.f. 1; p<0.001).

TABLE 2.3 SUMMARY OF THE RELATIVE HAZARD SCORES OF DIFFERENT CATEGORIES OF ANGLER AND CANOEIST BASED ON QUESTIONNAIRE RESULTS

Category	Median hazard score	% travelling to ≥2 catchments	% doing activity ≥ once per fortnight	% travelling to ≥2 catchments AND doing activity ≥ once per fortnight AND not cleaning equipment after every use	% travelling to ≥2 catchments AND doing activity ≥ once per fortnight AND not drying equipment after every use	% travelling to ≥2 catchments AND doing activity ≥ once per fortnight AND neither cleaning nor drying equipment after every use	% doing activity ≥ once per fortnight AND neither cleaning nor drying their equipment after every use AND using their equipment overseas
Game anglers (salmon)	40 (IQR 60)	88.5	80.3	63.9	19.7	19.7	8.2
Game anglers (trout)	30 (IQR 60)	82.6	68.6	56.8	14.0	13.6	8.1
Match anglers	20 (IQR 64)	74.2	72.6	52.4	15.3	12.9	8.9
Coarse anglers	16 (IQR 50)	69.6	56.8	45.1	11.9	11.2	9.0
Pike anglers	16 (IQR 52.5)	75.0	66.7	47.9	6.3	6.3	4.2
Barbel anglers	13.5 (IQR 60)	67.1	60.0	40.0	4.3	4.3	5.7
ALL ANGLERS	20 (IQR 50)	74.7	64.0	49.9	12.5	11.9	8.2
Competitive canoeists	100 (IQR 210)	85.6	83.8	55.0	79.3	52.3	36.0
River canoeists	96 (IQR 195)	89.4	74.6	54.1	68.6	50.9	31.1
Lake canoeists	80 (IQR 226)	87.2	77.6	50.4	68.6	48.8	19.2
Sea kayakers	75 (IQR 197.5)	97.2	85.9	47.9	67.6	40.8	18.3
Touring canoeists	60 (IQR 110)	100	88.9	44.4	77.8	44.4	11.1
ALL CANOEISTS	80 (IQR = 220)	89.3	78.5	52.6	70.6	49.5	27.7

Note: IQR = Interquartile range. Percentages show the co-occurrence of biosecurity hazards associated with potential transmission.

2.4.3 Potential for introduction into the UK

A large proportion of anglers (53%) and canoeists (46%) had used their equipment overseas ,trips of between 260km and 9500km from their last site in the UK (excluding Ireland). The majority visited other countries within Europe (84% of anglers and 96% of canoeists) although 20% of respondents had used their equipment in North America and 7% in Australasia.

Within Europe, France and Ireland were the most popular angling destinations (visited by 17% and 16% of anglers, respectively). France and Austria were the most popular canoeing destinations (visited by 69% and 20% of travelling canoeists, respectively). Three percent of anglers had used their equipment in Norway where the salmon fluke *Gyrodactylus salaris* poses a particular biosecurity threat to the UK (Peeler *et al.*, 2004).

Ninety nine percent of the 446 anglers who used their equipment overseas and went angling at least once a fortnight, failed to clean their equipment after every use, and 18% neither cleaned nor dried their equipment after every use. Moreover, 29% of the the anglers who travelled overseas, fished at least once a fortnight and neither cleaned nor dried their equipment between uses used rubber waders, 5% used felt-soled waders and 19% used keep nets. Of the 241 canoeists who used their equipment overseas and at least once a fortnight and used their equipment overseas, 94% failed to clean it after every use, 71% failed to dry it after every use and 69% neither cleaned nor dried it after use.

2.4.4 Use of live bait

Three hundred and seventy five of 960 angler respondents (39%) used live bait. Of those, 34% indictated using maggots, 34% indicated using bait fish, 23% indicated using earthworms and 18% indicated using bloodworms. The most commonly used bait fish were roach (*Rutilus rutilus*), rudd (*Scardinius erythrophthalmus*), perch (*Perca fluviatilis*), minnows (*Phoxinus phoxinus*) and gudgeon (*Gobio gobio*). The use of live bait was highest amongst pike anglers (47%) and barbel anglers (44%). Live bait use was lowest among trout anglers (36%).

Although the source varied between bait types (Table 2.4), the majority of bait users sourced bait from angling shops. Catching or collecting bait was the second most popular source of live bait. Of the 140 anglers who caught their own bait, fish and

earthworms were most commonly sourced (77% and 17%, respectively) (Table 2.4). Baitfish was most commonly caught at the same site that the angler intended to use it (84%). However, 16% of anglers collected their bait at a different site from where it was to be used and 7% released unused bait which had been from a different site into the river/lake after use (Figure 2.4).

TABLE 2.4 THE SOURCE AND METHOD OF DISPOSAL OF LIVE BAIT BY UK ANGLERS

		Bloodworms	Maggots	Earthworms	Fish
Source of bait (%)	Bait dealer	22.1	4.8	7.1	2.4
	Catch own	8.8	10.3	29.4	90.5
	Purchased at fishery	4.4	0.8	0.0	2.4
	Tackle shop	64.7	84.1	63.5	4.8
Disposal method (%)	Release into water	17.6	29.4	22.4	64.5
	Release onto land	7.4	7.9	21.2	0.8
	Freeze	27.9	18.3	18.8	27.4
	Throw in bin	7.4	4.0	5.9	3.2
	Take to next site	33.8	32.5	29.4	4.0
	Feed to garden birds	5.9	7.9	2.4	0.0

One hundred and forty bait users (37%) released unused bait into the water body at the end of their angling trip including bloodworms (9%) and baitfish (63%). Although the majority of anglers who released unused baitfish into the water had caught their fish at the same site, posing no biosecurity hazard, three had caught their baitfish at another site and one angler released bait fish sourced from a bait dealer. In addition, 9% of bait users released unused bait onto the land. This included 18 anglers who released unused earthworms on the river bank after use, all of which had been sourced from a different site to where they were released.

2.5 Discussion

This is the first study exploring the potential biosecurity risk posed by anglers and canoeists in the UK. My results highlight the fact that a high proportion of anglers and canoeists use their equipment frequently, and in multiple UK catchments, within the time that a range of INNS and aquatic pathogens can survive in damp conditions. This coupled with the low frequency with which anglers and canoeists clean and dry their equipment suggests that these groups have the potential to act as vectors for their spread.
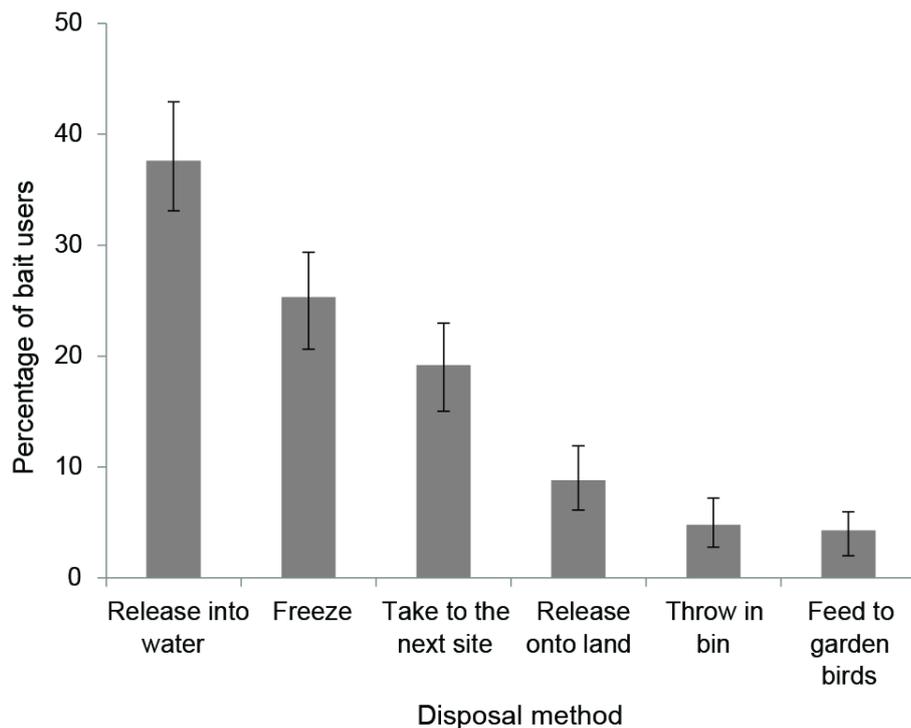
The results are in accord with studies in North America which showed that anglers and boaters were travelling hundreds of kilometres between sites (Gates *et al.*, 2009; Drake and Mandrak, 2010) and were frequently transporting muddy waders (Gates *et al.*, 2009), and the remnants of invasive aquatic plants on their boats (Johnson *et al.*, 2001; Stasko *et al.*, 2012). Within this research, I investigated the movement patterns of anglers and canoeists to evaluate the biosecurity risk that they would pose should they become contaminated with INNS or pathogens. I acknowledge that not all anglers and canoeists will use their equipment in a water body which has INNS present and of those who do, a low proportion of boats or equipment will become contaminated (Kelly *et al.*, 2012). However, the large number of anglers (4 million) and boat owners (404,000) in the UK and the frequency with which these groups appear to be using their equipment at different sites suggest that these groups pose an important pathway for the spread INNS should their equipment become contaminated.

2.5.1 Potential for Secondary Spread

Fewer canoeists cleaned or dried equipment than anglers and only a small proportion of both groups used disinfectant. The low proportion of canoeists cleaning their equipment reflected the behaviour of boat users at Lake Simcoe, Canada where 19%

rinsed their boats after use and only 3.2% allowed them to dry out for at least 5 days between uses (Kelly *et al.*, 2012). Despite the seemingly high proportion of anglers drying equipment after use, complete drying is required in order to kill INNS and pathogens through desiccation (Jerde *et al.*, 2012; Poznańska *et al.*, 2012). Considering the frequency with which anglers used their equipment it seems improbable that complete desiccation would have been achieved between trips.

The high frequency with which respondents took part in their activity may be an artefact of distributing the questionnaire to angling and canoeing clubs; themselves hubs of particular enthusiasts. Questionnaire surveys distributed in an 'opt in' manner can lend themselves to self-reporting bias (White *et al.*, 2005), however due to data protection restrictions preventing us from contacting individuals in a more structured manner, this was an effective way of obtaining a large sample size. While care was taken to design an un-biased survey, I recognise that when people are asked about their individual actions in an environmental context, some 'good behaviour' bias may exist.

2.5.2 Potential for introduction into the UK

A high proportion of anglers and canoeists used their equipment overseas, primarily elsewhere in Europe. Moreover, a low proportion of both groups cleaned their equipment between uses – actions which may risk the inadvertent introduction of new aquatic invaders and pathogens from overseas. A number of the species mentioned in Table 1 such as the salmon ecto-parasite *Gyrodactylus salaris* and invasive amphipod *Dikerogammarus villosus* pose a hazard to native species in the UK, yet exist in regions of Europe where anglers and canoeists had used their own equipment and failed to clean it after each use (Table 2.3) (Peeler *et al.*, 2004; MacNeil *et al.*, 2010).

Countries such as New Zealand communicate a strong biosecurity message to water users at the international border, insisting that anglers and boaters check and disinfect their equipment before entering the country, as well as banning the use of hazardous equipment such as felt soled waders (Ministry for Primary Industries, 2008). Considering the high proportion of UK water users who appear to use equipment overseas, there is likely to be a benefit from communicating a similar biosecurity message to these groups at UK ports and airports.

2.5.3 Use of live bait

Release of bait fish from another site or from a bait dealer, the release of unused aquatic bloodworms from an unknown source and the disposal of earthworms on banksides after use pose clear biosecurity hazards. In the USA, the release of live bait has been the third largest source of non-native fish introductions (Padilla and Williams, 2004) as well as being a major vector for the spread of invasive earthworms which have been associated with changing soil composition and local extinction of native plants (Keller *et al.*, 2007).

The number of anglers who released bait fish having caught them at another site or from a bait dealer was much lower in this study than a comparable study in Maryland, USA, where 65% of anglers released unused bait which was frequently (and illegally) non-native crayfish (Kilian *et al.*, 2012). Nonetheless, the release of unused bait fish by UK anglers is a recognised practice (Winfield *et al.*, 2011), and previous introductions of bait fish in the UK have been negatively correlated with the abundance of native fish species (Winfield *et al.*, 2011). The release of bait is still therefore a potential route by which invasive non-native species could be moved between UK catchments and one which needs controlling. Although more than a decade old, a study of anglers' attitudes to conservation in 2001 showed that only 19% of anglers saw the release of non-native bait fish as a conservation concern (Williams and Moss, 2001), despite the fact that failure to prevent the introduction of an invasive non-native species risks prosecution under the Wildlife & Countryside Act, 1981.

The control of bait movement is also important to control the indirect spread of associated INNS including *A.astaci* zoospores, zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) which can be transported via the gastrointestinal tract of fish (Oidtmann *et al.*, 2002; Gatlin *et al.*, 2012) and may be moved between sites by anglers using live bait fish (Peeler and Feist, 2011).

2.5.4 Biosecurity

My results indicate that in addition to the management of INNS vectors such as boat ballast water, and fish stocking, effective biosecurity practices are required to reduce the likelihood of INNS spread by recreational water users such as canoeists and anglers. In Europe, a large proportion (36%) of non-native species introductions are thought to have been due to fishing, boating and leisure activities (Gallardo *et al.*, 2011). My results suggest that angler and canoeist activities pose a potential pathway

for the spread of INNS or pathogens. As evidence indicates that pre-emptive management is effective at reducing the likelihood of aquatic INNS invasion via anthropogenic pathways (Dresser and Swanson, 2012), improving the biosecurity practices of these groups is important.

The lower proportion of canoeists cleaning/drying equipment after use as well as the higher proportion visiting three or more catchments was reflected in canoeists having higher biosecurity hazard scores than anglers. When canoeists were asked about factors affecting their cleaning/drying behaviours, the most important factor was the availability of a hose or cleaning station. The provision of more cleaning stations in 'hot spot' locations where boat and angling traffic is highest could therefore be fundamental to improving biosecurity practices.

Among anglers, I found game anglers, fishing salmonids, and match anglers to have the highest biosecurity hazard scores. Within the canoeing community, competitive canoeists and river canoeists had the highest hazard scores These groups were characterised by frequent canoeing/angling trips, often several times a week, visits to multiple catchments, and a low proportion of individuals cleaning and/or drying their equipment after every use. Biosecurity information should be targeted towards these groups of anglers, but I recognise that there was a lot of variation in biosecurity hazard scores within groups of canoeists so focusing biosecurity information towards specific types of canoeists may be less effective.

Defra launched a '*Check Clean Dry'* biosecurity awareness campaign in 2010 in response to the first reports of the killer shrimp *Dikerogammarus villosus* in the UK (Madgwick and Aldridge, 2011). The campaign was based on a biosecurity campaign in New Zealand designed to prevent the secondary spread of didymo (*Didymosphenia geminata*). In New Zealand, 80% of recreational water users were aware of the national 'Clean, Check, Dry' campaign and the spread of didymo appears to have slowed since the campaign was launched (Ministry for Primary Industries, 2008). My results indicate that the UK '*Check Clean Dry*' biosecurity campaign has only reached a small proportion of water users to date. Nonetheless, canoeists in this study who reported awareness of the campaign also exhibited lower biosecurity hazard scores.

Public engagement is vital to effectively manage INNS (Gozlan *et al.*, 2013). It is therefore important to engage with recreational water users to raise awareness and regularly evaluate the effectiveness of biosecurity campaigns, not only to ensure that they are having the desired effect, but to provide evidence to the public that their

actions make a difference (Baruch-Mordo *et al.*, 2011). My results highlight the need to increase biosecurity awareness among recreational water users; however it is important to engage with these groups so that they continue to enjoy their sport whilst taking biosecurity into account. I have provided an important baseline against which to monitor the effectiveness of future biosecurity awareness campaigns. My data also identify groups who pose a higher biosecurity hazard, and should therefore be targeted as a priority.

Finally, the data on equipment use can inform experiments that evaluate the effectiveness of different decontamination measures to prevent the survival of INNS and pathogens on angling and canoeing equipment and the spatial data can used to parameterise pathway risk assessment models to identify hotspot locations to target with biosecurity control measures.

3 INVADERS IN HOT WATER: A SIMPLE DECONTAMINATION METHOD TO PREVENT THE ACCIDENTAL SPREAD OF INVASIVE NON-NATIVE SPECIES

3.1 Summary

Watersports equipment can act as a vector for the introduction and spread of Invasive Non Native Species (INNS) in freshwater environments. To support advice given to recreational water users under the UK Government's *Check Clean Dry* biosecurity campaign and ensure its effectiveness at killing a range of aquatic INNS, I conducted a survival experiment on seven INNS which pose a high risk to UK freshwaters. The efficacy of exposure to hot water (45℃, 15 minutes) was tested as a method by which waters users could 'clean' their equipment and was compared to drying and a control group (no treatment). Hot water had caused 99% mortality across all species one hour after treatment and was more effective than drying at all time points (1 hour: $X^2 =$ 117.24 p<0.001; 1 day $X^2 = 95.68$, p<0.001; 8 days $X^2 = 12.16$, p<0.001 and 16 days $X^2 = 7.58$, p<0.001). Drying caused significantly higher mortality than the control (no action) from day 4 ($X^2 = 8.49$, p<0.01) onwards. In the absence of hot water or drying, 6/7 of these species survived for 16 days, highlighting the importance of good biosecurity practice to reduce the risk of accidental spread. In an additional experiment the minimum lethal temperature and exposure time in hot water to cause 100% mortality in American signal crayfish (*Pacifastacus leniusculus*), was determined to be 5 minutes at 40°C. Hot water provides a simple, rapid and effective method to

clean equipment. I recommend that it is advocated in future biosecurity awareness campaigns.

3.2 Introduction

Invasive Non Native Species *(INNS)* can have profound impacts on the marine, terrestrial and freshwater ecosystems they invade by replacing native species, altering community structure and introducing novel diseases (Mack *et al.*, 2000). Freshwater systems are particularly vulnerable to the introduction of INNS due to their exposure to multiple transport pathways along which new species can be either accidentally or intentionally introduced. Moreover, the ecological resilience of freshwater systems is already reduced by pollution, agricultural run-off and altered hydrology (Strayer, 2010), increasing the likelihood that non-native species will successfully invade (Dudgeon *et al.*, 2006).

Fishing, boating and leisure activities are collectively responsible for almost 40% of aquatic species introductions into Europe (Gallardo and Aldridge, 2013b). These pathways commonly include the release of boat ballast water and the stocking and subsequent escape of non-native fish or crustaceans introduced for aquaculture or sport. However, they also include the accidental transfer of invasive plants and invertebrate species "hitchhiking" on personal equipment such as angling nets, bait buckets, wet suits and waders used during recreational activities (Ludwig and Leitch, 1996; Buchan and Padilla, 1999; Johnson *et al.*, 2001b; Gates *et al.*, 2008; Stebbing *et al.*, 2011; Stasko *et al.*, 2012; Bacela-Spychalska *et al.*, 2013). Such accidental transfer is thought to have been responsible for new introductions, as well as facilitating the secondary spread of species once introduced (Johnson *et al.*, 2001; Bothwell *et al.*, 2009; Kilian *et al.*, 2012).

Freshwater ecosystems in the UK contain seven of the UK Environment Agency's 10 'most wanted' INNS (Environment Agency, 2011) and are thought to be threatened by a further 11 (Gallardo and Aldridge, 2013b). Many of these aquatic invasive non-native species can survive for several days in damp environments. For example, zebra mussels (*Dreissena polymorpha*) can survive outside water for at least 5 days (Ricciardi *et al.*, 1995) and killer shrimp (*Dikerogammarus villosus*) for at least 15 days (Fielding, 2011). As 64% of anglers visit more than one catchment within a fortnight (Anderson *et al.*, 2014a, Chapter 2), it is likely that many aquatic INNS could survive

the journey from an invaded catchment to an uninvaded catchment on damp equipment if effective biosecurity measures are not in place.

Once established, the eradication of these species is virtually impossible (Mack *et al.*, 2000; Kolar and Lodge, 2001; Briski *et al.*, 2012) and control measures costly (Oreska and Aldridge, 2010). Preventing their initial introduction and spread through effective biosecurity is therefore considered a far more effective management strategy (Vander Zanden *et al.*, 2010; Caplat and Coutts, 2011; Briski *et al.*, 2012). The *Check Clean Dry* campaign was launched in the UK by the Government's Department of Environment, Food and Rural Affairs (Defra) in 2010. The objective of the campaign is to raise awareness of good biosecurity practices among recreational water users to prevent the introduction and spread of aquatic INNS. The campaign provides broad guidance for best-practice:

"***Check*** *your equipment and clothing for live organisms – particularly in areas that are damp or hard to inspect.* ***Clean*** *and wash all equipment thoroughly. If you do come across any organisms, leave them at the water body where you found them.* ***Dry*** *all equipment and clothing – some species can survive for many days in damp conditions. Make sure you don't transfer water elsewhere."* (Defra, 2013)

Specific advice about the most effective method by which to clean equipment is required.

Thermal control is considered to be one of the most efficient, environmentally sound and cost effective methods by which to prevent the accidental spread of aquatic INNS (Beyer *et al.*, 2010; Perepelizin and Boltovskoy, 2011; O'Neill and MacNeill, 1991; Stebbing *et al.*, 2011). Preliminary research conducted by the Centre for Environment, Fisheries and Aquaculture Science (Cefas) indicated that submersion in hot water at 45°C was sufficient to cause 100% mortality in *D.villosus* within 15 minutes (Stebbing *et al.*, 2011). This advice has since been incorporated within local biosecurity awareness programmes (e.g. (Broads Authority, 2013).

Hot water at this temperature meets the essential criteria for an effective cleaning treatment: it is accessible, economical, requires no specific training or protective equipment to use and has no impact on the environment when disposed (potentially in large volumes) (Kilroy *et al.*, 2006). However, the recommended cleaning treatment needs to be effective at killing a wide range of aquatic INNS in addition to *D. villosus* as it is unrealistic to expect water users to use multiple treatments for different species, or to know which invasive non-native species are present in different waterways.

Previous studies indicate that hot water can also cause 100% mortality in zebra mussels (*Dreissena polymorpha*), quagga mussels (*Dreissena rostriformis bugensis*) and the planktonic lifestage of spiny water fleas (*Bythotrephes longimanus*) (Beyer *et al.*, 2010) as well as the invasive diatom didymo (*Didymo germinata*) (Kilroy *et al.*, 2006) suggesting potential efficacy of this treatment across a range of taxonomic groups. Whether the 45℃/15 minute protocol is effective across multiple INNS, including plants, remains to be tested however.

The study had three aims: i) to test whether the cleaning and drying components of the *Check Clean Dry* protocol were effective at killing a range of aquatic INNS should they become tangled in anglers keep nets; ii) to evaluate whether hot water at 45℃ is an effective method for killing a range of high impact aquatic INNS; and iii) to conduct a pilot experiment to test whether hot water could be a feasible biosecurity treatment for larger INNS such as American signal crayfish.

3.3 Methods

Survival experiments were conducted during October/November 2013 and February/March 2014 to evaluate the effectiveness of drying and hot water as treatments for decontaminating angling nets. Seven aquatic INNS representing a range of taxa and all currently present in the UK were used: zebra mussels (*Dreissena polymorpha*), killer shrimp (*Dikerogammarus villosus)*, bloody-red mysid (*Hemimysis anomala*), floating pennywort *(Hydrocotyle ranunculoides)*, curly water-thyme (*Lagarosiphon major)*, New Zealand Pigmyweed (*Crassula helmsii*), and parrot's feather (*Myriophyllum aquaticum*). Species were selected due to their classification as high impact invaders by the UK Technical Advisory Group for the EU Water Framework Directive.

A second experiment to test the effect of hot water temperature and duration of exposure on the survival of signal crayfish (*Pacifastacus leniusculus*) was conducted during March 2014. Adult crayfish were used as a proxy for juvenile crayfish (which may be difficult for anglers to detect) because juveniles were not accessible at the time of year when the experiment was undertaken. It was also reasoned that a treatment that is effective in killing adults is likely to also be effective for juveniles due to limited ontogenetic changes in body morphology between juvenile and adult crayfish life stages (Holdich 2001).

The animals and plants were collected from various sites across the UK using hand searching (*D. polymorpha*, *D. villosus*, *H. anomala*, *P. leniusculus*) or from UK retailers of aquatic pond plants where it was unfeasible to collect wild specimens (*L. major, M. aquaticum, H. ranunculoides, C. helmsii*). Once collected, plants/animals were stored in separate tanks of dechlorinated, aerated tap water at constant temperature (14 ± 1°C, light: dark cycle 12h: 12h) for at least 48 hours before the experiment to enable acclimation to laboratory conditions and recovery from collection or transport-induced stress. The temperature conditions were chosen to reflect the conditions in a garage or shed, the conditions in which most anglers store their equipment (Anderson *et al.,* 2014a, Chapter 2).

3.3.1 *Check Clean Dry* experiment

At the start of the experiment, plants were removed from the tank and cut into fragments of approximately 60 mm to simulate a fragment of plant that may become broken off and tangled up in an angling net. As the plant species were all vegetative reproducers, care was taken to include the reproductive part of the plant in each fragment. A *FluorPen* (FP 100 , Photon Systems Instruments, Czech Republic) was used to determine the equivalent variable fluorescence: maximal fluorescence (F_V:F_M) ratio in the aquatic plants. This ratio is commonly used as an index of plant stress (Willits and Peet, 2001). Only those with scores of at least 0.7 were deemed healthy and included in the experiment (Dan *et al.*, 2000).

D. polymorpha, *D. villosus* and *H. anomala* were randomly selected from the tank to prevent bias towards particular sizes. Only those swimming normally (*D. villosus, H. anomala*) or siphoning water and responding to stimuli (*D. polymorpha*) were used in the experiment (Beyer *et al.*, 2010). *D. polymorpha* ranged in total length from 8.0mm to 22.0mm (median 16.0 mm), *D. villosus* ranged from 8.7 to 20.9 mm (median 11.2 mm) and *H. anomala* ranged from 10.5 to 13.8 mm (median 12.5mm). There was no significant difference in the sizes of *D. polymorpha* (Kruskal Wallis, H = 2.1, df = 3, p= 0.55), *D. villosus* (H = 3.17, df = 3, p=0.36) or *H. anomala* (H = 7.39, df = 3, p = 0.06) assigned to different treatments.

To mimic the conditions of an angler's keep net, each animal or plant fragment (n = 240 per species) was placed in a bag (50 mm x 50 mm) constructed from the mesh (2 mm spacing) of a typical polyester coarse angling keep net (Keepnets Direct, UK). The bags were sealed with staples and submerged in dechlorinated tap water at 14± 1°C for one hour to simulate an angling trip. Once damp, the nets were subjected to one of

three treatments: 1) hot water (45°C); 2) hot water (45°C) and drying, and 3) drying only; or a no-treatment control (See Table 1). For the hot water treatments, a 15 minute exposure period was selected as this duration has been previously shown to be effective at causing 100% mortality in *D. villosus* (Stebbing *et al.*, 2011) and because this is the maximum period of time that a treatment could realistically be applied in the field. For the drying treatments, net bags were laid on plastic trays in a temperature controlled room (14 ± 1°C, light: dark cycle 12h: 12h, gently circulating air 1.23m/s). In the control, net bags were placed in thin, transparent unsealed plastic bags to hinder drying and stored in the same way as the drying treatments. The relative rates at which the net bags dried in each treatment are supplied in Appendix B.

Animals/plants were observed and recorded as alive/dead at six time points after the initial treatment: 1 hour, 1 day, 2 days, 4 days, 8 days, and 16 days (Table 3.1). My previous research (Chapter 2) indicated that 86% of anglers use their equipment at least once a fortnight so the time units were chosen to represent time intervals during which angling equipment might be stored for between uses. Because the plants and animals had to be handled and/or exposed to water to test for survival, separate batches of 10 animals were tested at each time point. Having been tested, individuals were not returned to the experiment.

3.3.2 Testing survival

D. polymorpha were assumed dead if their shells gaped and they did not respond to stimuli either immediately after the experiment or after 1 hour recovery in a container of dechlorinated water at at 14± 1°C (Ricciardi *et al.*, 1995; Beyer *et al.*, 2010; Comeau *et al.*, 2011). *D. villosus* and *H. anomala* were considered dead if they were discoloured (or had begun to decompose) and neither responded to stimuli nor swam after being put in a container of dechlorinated water for 1 hour. For the plants, a FluorPen was used at the end of the experiments to measure the variable to maximal fluorescence of leaves ($F_v:F_m$). This measurement is widely used as an indication of plant stress (Willits and Peet, 2001), and plants with $F_v:F_m$ values of 0.3 or below were considered to be dead (Dan *et al.*, 2000).

TABLE 3.1 SUMMARY OF EXPERIMENTAL SET UP. THE DESCRIPTION OUTLINES THE TREATMENT THAT EACH POLYESTER NET (CONTAINING AN INDIVIDUAL ANIMAL OR PLANT FRAGMENT, N = 240 PER SPECIES) WAS EXPOSED TO AFTER HAVING BEEN SUBMERGED IN DECHLORINATED WATER AT AN AMBIENT TEMPERATURE FOR ONE HOUR TO SIMULATE THE MINIMUM LENGTH OF AN ANGLING TRIP.

Treatment	Description	Number of individuals checked at each time point					
		1h	1 day	2 days	4 days	8 days	16 days
Hot water only	60 x individual mesh nets submerged in a waterbath at 45°C for 15 minutes in a randomly assigned order. Immediately afterwards, nets put inside individual (unsealed) plastic bags and stored on a tray in climate controlled room at 14± 1 °C.	10	10	10	10	10	10
Hot water and drying	60 individual mesh nets submerged in water bath at 45°C for 15 minutes in a randomly assigned order. Immediately afterwards, nets laid out on tray in climate controlled room at 14± 1 °C.	10	10	10	10	10	10
Drying only	60 mesh nets laid out on trays in climate controlled room at 14 ± 1°C.	10	10	10	10	10	10
Control	60 mesh nets put inside individual (unsealed) plastic bags and stored on a tray in climate controlled room at 14 ± 1 °C.	10	10	10	10	10	10

3.3.3 Crayfish experiment

At the beginning of the experiment a single animal was removed from the holding tank, sexed, measured from the tip of the rostrum to the end of the cephalothorax (mm) and placed into a water bath at 30, 40, 50 or 60°C (±1°C) for either five minutes, one minute or five seconds for one of the temperatures (five animals per treatment x twelve treatments). Where all individuals survived a treatment after five minutes, or one minute, the treatment was not repeated for the shorter time period(s). Once the animal had been submerged for the required duration, it was removed and placed into dechlorinated water at 14± 1°C for a recovery period of 30 minutes. Behavioural observations to determine morbidity were made one and 30 minutes into the recovery period. Animals were considered dead if they would not right themselves if placed on

their back and were not responsive to stimulus. No animal was used more than once. The carapace length of animals used ranged from 30 mm to 70 mm (median 45 mm) with no significant difference between treatments (Kruskal Wallis H = 5.52, df=8, p=0.70).

3.3.4 Data analysis

Generalised linear models (GLMs) with binomial errors were used to identify whether species or treatment were significant predictors of survival (proportion alive) at each time point (1 hour, 1 day, 2 days, 4 days, 8 days, 16 days). To test the relative effectiveness of two different treatments at a particular time point, paired X^2 tests (R package: prop.test) were used to compare differences in the proportion of individuals alive. Dose response curves were plotted to illustrate changes in mortality over time and to estimate LT_{50} and LT_{90} for the treatments which did not cause 100% mortality. All statistical analyses were carried out using 'R' (R Development Core Team, 2012).

3.4 Results

3.4.1 *Check Clean Dry* experiment

Mortality differed between treatments and increased over time for all treatments. The hot water treatment and hot water and drying treatment resulted in 99% and 97% mortality within one hour, respectively, whereas it took 7.52 days to reach LT_{90} with the drying treatment and a projected 17.16 days to reach LT_{90} for the control group (Table 3.2, Figure 3.1).

More specifically, the hot water treatment resulted in 100% mortality in six of the seven species and 90% mortality in the seventh species (*C. helmsii*) within 1 hour, regardless of whether the nets were allowed to dry or remained damp afterwards. The hot water and dry treatment showed similar results, with 100% mortality across 6 of the 7 species and 80% mortality in *C. helmsii* after 1 hour. A much longer time period was required for the drying treatment to cause mortality, with 19% of individuals subjected to the drying treatment still alive after 8 days and 10% still alive after 16 days. In the control group, mortality was low with 70% of individuals alive after 7 days and 30% still alive after the full 16 days, among all species except *H. anomala*. *H. anomala* showed high mortality (100% within 1 day across all treatments except drying (Fig.1.).

TABLE 3.2 MEAN NUMBER OF DAYS TAKEN FOR EACH SPECIES TO REACH 50% MORTALITY (LT50) AND 90% MORTALITY (LT90) IN THE CONTROL AND DRYING TREATMENTS. RESULTS WERE CALCULATED FROM DOSE-RESPONSE CURVES.

Species	LT 50 (days)		LT90 (days)	
	Drying treatment	Control	Drying treatment	Control
C. helmsii	15.42	>100*	22.53	>100*
H. ranunculoides	4.13	13.35	4.34	19.04
L. major	2.25	16.31	3.21	17.14
M. aquaticum	6.19	18.52	8.73	27.65
H. anomala	0.15	0.10	0.95	0.10
D. polymorpha	4.81	16.93	6.62	23.46
D .villosus	3.43	6.45	8.54	15.59
MEAN	6.93	11.94*	7.52	17.16*

*As none of the *C.helmsii* died during in the control experiment, it was not possible to accurately calculate its projected survival under the control treatment. The species from was therefore excluded the mean calculation and T-tests.

Treatment was a significant predictor of mortality after 1 hour (GLM, Estimate = 1.28, SE = 0.15, Z = 8.4, p<0.001), 1 day (GLM, Estimate=2.36, SE = 0.26, Z = 9.02, p<0.001), 8 days (Estimate=0.698, SE = 0.14, Z = 4.75, p<0.001), and 16 days (Estimate=0.624, SE = 0.17, Z = 3.59, p<0.001), (Figure 3.1). Species was not a significant predictor of mortality at any of the four time points (binomial GLM, p >0.05).

There were no significant differences in survivorship between the hot water and dry treatment and hot water only treatment at any of the time points, indicating that drying equipment after submersion in hot water has no additional benefit (Table 3.3). The hot water only treatment killed a significantly higher proportion of individuals than the drying treatment or control at every time point (Figure 3.1, Table 3.3 Results of paired X2 tests to compare the level of mortality (proportion) between treatments after 1 hour, 1 day, 8 days and 16 days. and Figure 3.1).

TABLE 3.3 RESULTS OF PAIRED X^2 TESTS TO COMPARE THE LEVEL OF MORTALITY (PROPORTION) BETWEEN TREATMENTS AFTER 1 HOUR, 1 DAY, 8 DAYS AND 16 DAYS.

Treatment comparison	1 hour	1 day	8 days	16 days
CLEAN (HOT WATER) ONLY vs. CLEAN (HOT WATER) AND DRY	NA	2.31	NA	NA
CLEAN (HOT WATER) ONLY vs. DRY ONLY	117.24***	95.68***	12.16***	7.58**
CLEAN (HOT WATER) ONLY vs. CONTROL	113.77***	101.37***	70.77***	43.44***
DRY ONLY vs. CONTROL	NA	0.05	34.34***	25.20***
CLEAN (HOT WATER) AND DRY vs. CONTROL	110.03***	86.96***	70.77***	43.44***

Note: Figures show X^2 value. NA = result was the same for both treatments so X^2 tests could not be performed.
*p<0.05, **p<0.01, ***p<0.001

Although hot water is clearly the most effective treatment, it may not always be available to recreational water users. Drying, despite not being as effective at causing mortality as hot water (Figure 3.1), caused significantly higher mortality than the control from day 4 (X^2 = 8.49, p<0.01) onwards (Table 3.3), at which point the nets had dried out completely (Appendix B) Over half of species exposed to the drying treatment reached LT90 in one week (7.52 days), while aquatic plants such as *L. major* and *H. ranunculoides* survived only 3-4 days under the drying treatment (Table 3.2). In contrast, *C. helmsii* could survive over 23 days of drying (Table 3.2). Overall, drying took significantly less time to cause 50% mortality (Independent samples T test: t = -2.76, df = 10, p < 0.05) and 90% mortality (t = -2.89, df = 10, p < 0.05) compared to the control treatment.
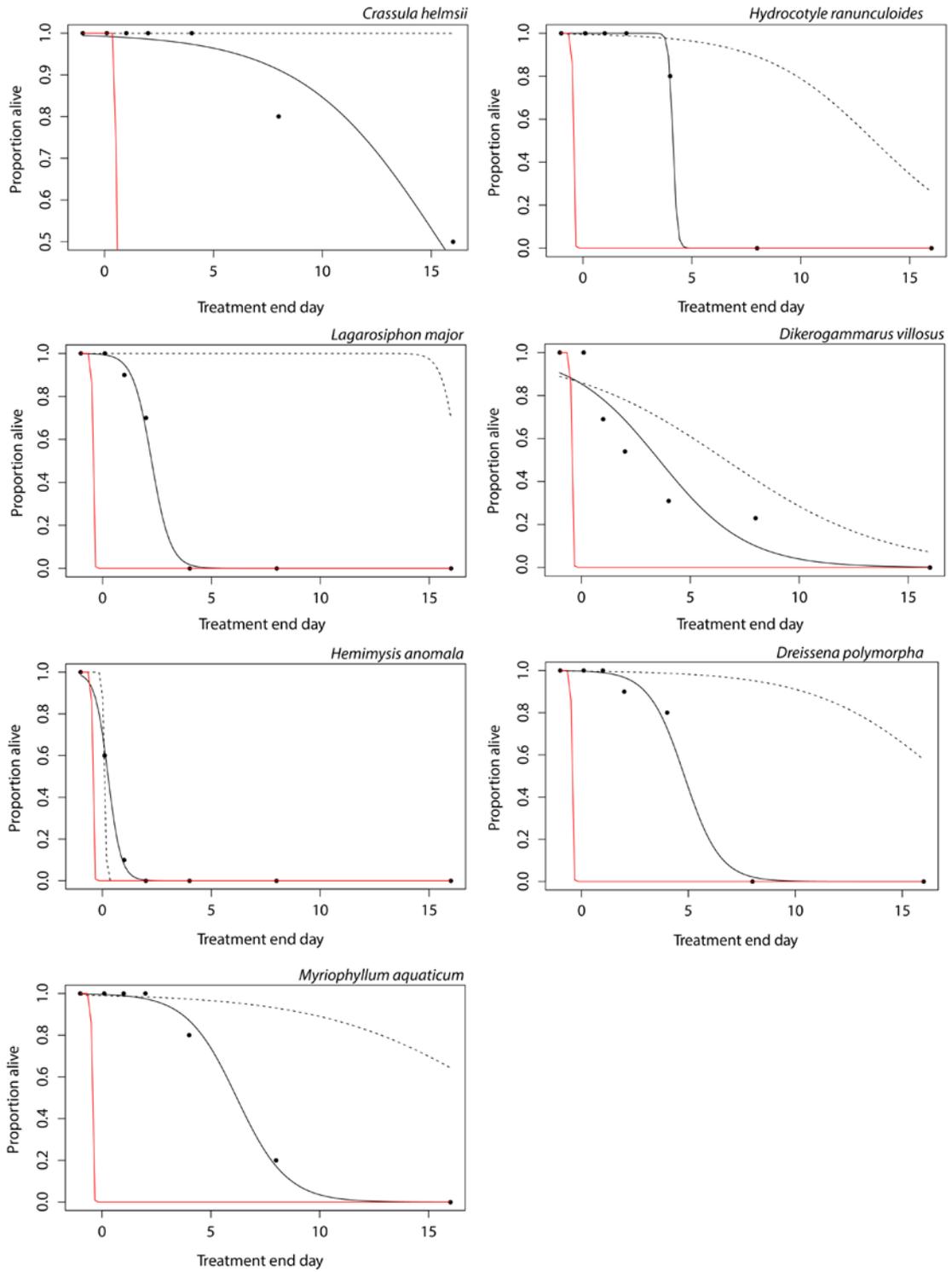
FIGURE 3.1 DOSE RESPONSE CURVES SHOWING PROJECTED SURVIVAL OVER TIME FOR HOT WATER ONLY (RED LINE), DRYING (BLACK LINE AND DATA POINTS) AND CONTROL (DASHED LINE) TREATMENTS.

3.4.2 Crayfish experiment

No mortalities were observed when *P. leniusculus* were exposed to either 50°C or 60°C for five seconds (Table 3.4). After exposure to 50°C for five seconds, animals were initially unable to right themselves and inactive, however, all animals appeared to recover fully after 30 minutes. After exposure to 60°C for five seconds, chronic effects were observed and behaviour deteriorated during the recovery period. Exposure to 60°C for one minute caused 100% mortality while exposure to 50°C for one minute caused 75% mortality. However, no mortalities were observed when animals were exposed to 40°C for one minute. When animals were exposed to 40°C, 50°C or 60°C for five minutes, 100% mortalities were observed however animals exposed to 30°C for five minutes recovered.

TABLE 3.4 RESULTS OF THE PERCENTAGE MORTALITIES OBSERVED IN THE HEAT EXPOSURE EXPERIMENT WITH CRAYFISH. FIGURES EXPRESSED AS PERCENTAGE OF CRAYFISH IN EACH TREATMENT GROUP. RECOVERY WAS MEASURED ONE MINUTE AND 30 MINUTES AFTER TREATMENT ENDED FOR EACH TEMPERATURE.

Exposure	5 minutes (n= 20)		1 minute (n = 15)		5 seconds (n = 10)	
Recovery	1m	30m	1m	30m	1m	30m
60°C	100	100	65	100	0	0
50°C	100	100	75	75	0	0
40°C	100	100	0	0		
30°C	0	0				

3.5 Discussion

Hot water (45°C for 15mins) caused 99% mortality across the seven aquatic INNS used in the primary experiment one hour after treatment. These results demonstrate that submerging water sports equipment in 45°C water for 15 minutes is an extremely effective method for killing a range of invasive non-native animals and plants in a short time frame. Moreover, hot water was effective regardless of whether or not the net which the invader was in was subsequently dried, or remained damp. *C. helmsii* and *M. aquaticum*, were the only two species to survive submersion in hot water one hour after treatment, although all individuals were dead one day after treatment. Particular caution should be taken when using recreational equipment in areas where these plants are known to be present.

The results of this hot water experiment were similar to those of previous studies which reported 100% mortality in *D. polymorpha* and *D. rostriformis bugensis* after five minutes at 43°C; adult spiny waterfleas (*Bythotrephes longimanus*) after 10 minutes at 43°C (Beyer et al., 2010); and *B. longimanus* eggs at 50 °C for ≥ 10minutes (Branstrator *et al.*, 2013). Although some of these INNS reached 100% mortality in cooler temperatures or a shorter time period, I believe it is important to recommend a consistent treatment which is effective against a wide range of species, without the need for waterusers to know which INNS are present. As 45 °C for 15mins was identified as the most efficient time/temperature combination to cause 100% mortality in *D. villosus* (Stebbing et al., 2011), I recommend that this longer time period is used as a consistent treatment.

Adult *P. leniusculus* are unlikely to remain attached to equipment without being noticed, but were used in this study as a proxy for juvenile *P. leniusculus*. Although 100% mortalities were observed when *P. leniusculus* were exposed to 60°C for 1 minute, this water temperature could degrade watersports equipment and has the potential to cause burns in children (Feldman et al., 1998). With 100% mortality observed with 5 minutes exposure at 40°C, the recommendation of exposing water sport equipment in 45°C water for 15 minutes is considered more than sufficient to cause mortality in juvenile *P. leniusculus*.

In the absence of hot water, drying was still found to be a significantly more effective treatment than doing nothing (control) and caused 90% mortality in a mean of 7.52 days in all species except *C. helmsii*, suggesting that it would be suitable as a biosecurity treatment for anglers who go fishing once a fortnight or less frequently in areas where *C. helmsii* is not present. In this experiment, the desiccation treatment took longer to cause mortality in plants than previous studies. For example, drying Eurasian water milfoil (*Myriophyllum spicatum*), resulted in 71% mortality within 1 hour and 100% mortality within 1 day (Jerde et al., 2012). In an animal experiment, air exposure of ≥ 6 hours prevented the dormant egg stages of *B. longimanus* from hatching (Branstrator *et al.*, 2013). In contrast, the plants in this study took at least of 2.25 days to reach LT_{50} and 3.21 to reach LT_{90} and the animals took at least 22 hours to reach LT_{90} (Table 3.2). The longer time-to-mortality in this desiccation treatment is likely to be due to the plant fragments and animals remaining enclosed in damp nets which retained water for a number of days after initial submersion (Appendix B) whereas the plants/animals were not enclosed in the aforementioned studies. My results demonstrate that drying can take many days, particularly for INNS entrapped

in large equipment and in cool or damp conditions and is a more subjective biosecurity treatment (i.e. people's perceptions of what 'dry' is may vary). These results support previous studies which show that complete desiccation is required for it to be effective (Jerde et al., 2012; Poznańska et al., 2012), making drying an unsuitable decontamination method for use by anglers who go fishing frequently.

Despite some mortality, six of the seven species (all except *H. anomala*) in the control group were able to survive for at least 16 days in damp conditions. As recent research suggests that 64% of anglers visit multiple catchments within a fortnight (Chapter 2, Anderson *et al.*, 2014a), this demonstrates the potential for invaders to survive in damp equipment in the absence of biosecurity. Several of the species in this experiment were not previously thought to be able to survive for this long out of water: *D. villosus* has only been reported to survive for 15 days out of water (Fielding, 2011) and *D. polymorpha* for 3-5 days (Ricciardi et al., 1995). My results also demonstrate that aquatic plants including *H. ranunculoides* and *M. aquaticum* can survive out of water for at least 16 days which, to the best of my knowledge, has not been previously reported. Unlike the other species tested, *H. anomala* showed high mortality (100% within 1 day in all treatments except drying). This species appeared particularly fragile, so it is probable that handling in the laboratory or physical damage by the nets resulted in mortality. Based on my results, it seems unlikely that *H. anomala* would survive transport in an angling net, therefore water-based transfer methods (such as the ballast water of boats) may be more important vectors for this species; as presumed for its introduction into the Great Lakes (Brooking *et al.*, 2010).

Hot water provides a rapid and easy method to clean equipment as part of the *Check Clean Dry* protocol and I believe it is a simple and effective method to recommend to the anglers (e.g. 78% of those in the UK) who do not currently clean their kit after use (Anderson *et al.*, 2014a). While I have demonstrated the effectiveness of this method at killing a range of INNS, I stress that further research must be conducted to test the effectiveness of hot water as a treatment to kill aquatic pathogens, such as *Gyrodactlylus salaris*, a salmon ectoparasite which is considered to be the most important exotic fish-disease threat to the UK (Peeler et al., 2004); *Aphanomyces astaci*, the causal agent of crayfish plague (Oidtmann et al., 2002) and *Batrachochytrium dendrobatidis*, the causal agent of chytrid disease in amphibians (Johnson and Speare, 2003). I acknowledge that aquatic parasites such as these pose a similar economic and ecological risk to INNS and that anglers using equipment in

multiple countries pose a risk of parasite dispersal (Anderson *et al.*, 2014a). I advocate the continued use of Virkon Aquatic® (DuPont, 2014) as a biosecurity agent for anglers travelling between countries or using equipment in areas where aquatic parasites may be present. Further work into the effectiveness of hot water as a control measure for parasites would therefore be of significant use in demonstrating hot water as a single 'catch all' biosecurity message for both invasive non-native species and aquatic pathogens.

3.6 Conclusion

Hot water fulfils the criteria for an effective biosecurity treatment. Not only does it cause 99% mortality within an hour, it is environmentally sound and cost effective (Beyer *et al.*, 2010; Perepelizin and Boltovskoy, 2011; O'Neill and MacNeill, 1991; Stebbing *et al.*, 2011) and the recommended temperature of 45℃, is below the temperature at which hot water is thought to be able to cause burns in children (52 ℃) making it safe to use by children as well as adults (Feldman *et al.*, 1998). However, I still recommend that water is disposed of on land and away from a water source after use.

These results provided evidence that hot water is effective at killing a range of high impact invasive non-native species in a short time frame. The use of hot water (45℃ for 15 minutes) for the 'Clean' stage of the UKs national *Check Clean Dry* biosecurity awareness campaign would greatly enhance biosecurity efforts. In addition to anglers, this method could be used by water sports participants with wetsuits or equipment that can easily be submerged, as well as ecologists, environmental scientists and field centre staff and volunteers who use nets, waders and other equipment to undertake freshwater fieldwork in the UK.

4 AQUATIC BIOSECURITY BEST PRACTICE: LESSONS LEARNED FROM NEW ZEALAND

4.1 Summary

Managing the pathways and vectors by which invasive non-native species (INNS) are introduced is the most effective way of preventing non-native species invasions. It is a fundamental component of new EU invasive non-native species legislation and one of the Convention on Biological Diversity's Aichi biodiversity key targets for 2020. Recreational water users are a high-risk vector for the accidental spread of INNS in the absence of biosecurity (taking steps to reduce the risk of spread). I examined the successes and challenges of a long-running aquatic biosecurity awareness campaign in New Zealand, a leader in aquatic biosecurity best practice. I combined self-completion questionnaires with 230 recreational water users and semi-structured interviews with 15 key stakeholders in New Zealand's Bay of Plenty region to evaluate the effectiveness of the campaign. My results demonstrated that compliance with biosecurity was high among New Zealand water users and that awareness of the campaign was a significant predictor of biosecurity action. The development of regional partnerships and the support of national legislation appeared to be key components of the country's streamlined approach to INNS prevention and management. Based on New Zealand's experience, I make recommendations for delivering effective aquatic biosecurity awareness campaigns in Europe.

4.2 Introduction

Invasive non-native species (INNS) are one of the biggest drivers of global biodiversity loss (Mack *et al.,* 2000; WWF, 2014). They have both ecological and social impacts, replacing native species, altering nutrient cycling and introducing novel diseases, as well as reducing natural hazard prevention and eco-tourism, and increasing threats to human health (Hatcher *et al.,* 2012; Pimentel *et al.,* 2005; Vilà *et al.,* 2010b; Williams *et*

al., 2010). In the EU, INNS cost an estimated €12 billion per year to manage (Shine *et al.*, 2009).

Complete eradication of an established INNS is rarely possible (Mack *et al.*, 2000; Kolar and Lodge, 2001), therefore prevention is recognised as the most effective management approach (Caffrey *et al.*, 2014; Caplat and Coutts, 2011). To this end, the Convention on Biological Diversity (CBD) Aichi Biodiversity Targets for 2020 and new EU legislation on the prevention and management of INNS both focus on identifying and managing the pathways and vectors by which INNS can be introduced and spread (Secretariat of the Convention on Biological Diversity, 2011; European Commission, 2013).

Anglers and pleasure boaters (herein "recreational water users") are one such vector, having been responsible for much of the spread of freshwater INNS in Europe (Gallardo and Aldridge, 2013b), the USA (Rothlisberger *et al.*, 2010) and New Zealand (deWinton *et al.*, 2009). This is because many INNS can survive for days in the anchor wells, bilges or hulls of boats, on trailers, or on damp equipment used by water users, allowing them to "hitchhike" between sites (Anderson *et al.*, 2014a; Bothwell *et al.*, 2009; Keller *et al.*, 2009; Keller *et al.*, 2007; Rothlisberger *et al.*, 2010). However, the likelihood of this accidental spread can be reduced through biosecurity measures: taking steps to ensure that INNS are removed from boats and equipment before moving to another site. Educational campaigns to raise awareness of biosecurity among high risk groups are an important component of the new EU INNS legislation (European Commission, 2013), but are currently in their infancy in Europe.

In contrast, New Zealand is widely recognised as a leading example of aquatic biosecurity best practice (Caffrey *et al.*, 2014; Chapple *et al.*, 2013; Meyerson and Reaser, 2002). The country's comprehensive biosecurity programmes are coordinated by a dedicated team in government (Biosecurity New Zealand, within the Ministry for Primary Industries (MPI)) and supported by unified legislation: the Biosecurity Act 1993 (Ministry for Primary Industries, 1993). Current opinion advocates building evidence-based biosecurity programmes in Europe based on New Zealand's experience (Caffrey *et al.*, 2014).

In 2004, the detection and rapid spread of the invasive diatom *Didymo germinata* in New Zealand's South Island catalysed biosecurity behaviour change efforts. MPI's centrepiece response, the '*Check Clean Dry*' campaign, recognised that human activities posed the biggest threat to the containment of didymo (Kilroy *et al.*, 2006; Bothwell *et*

al., 2009), and that improving the biosecurity practices of recreational water users was likely the most cost-effective management approach (Colmar Brunton, 2013). Since its inception, the campaign has been broadened to include other threats to New Zealand's waterways e.g. invasive aquatic plants (deWinton *et al.*, 2009).

Campaign funding is split between national promotional activities and support for regionally implemented biosecurity awareness initiatives. The Bay of Plenty is one such region. Home to some of the New Zealand's biggest freshwater lakes, the Bay of Plenty is one of the country's most popular destinations for water-based activities (Miller *et al.*, 2006; Edgar, 2008). It is therefore well suited as a case study with which to evaluate the effectiveness of New Zealand's biosecurity awareness initiatives.

The primary aim of this study is to evaluate the effectiveness of New Zealand's aquatic biosecurity awareness initiatives to help catalyse the delivery of similar initiatives in Europe. The research has two components:

i.   A questionnaire study with which to: i) quantify biosecurity awareness and actions taken by water users; ii) identify the factors which motivate or deter water users from taking biosecurity actions; and iii) understand the most cost effective communication channels for biosecurity awareness information.

ii.   Semi-structured interviews with key stakeholders to triangulate the results of the questionnaire and to explore perceptions of invasive non-native species and biosecurity initiatives in greater depth (Creswell and Miller, 2000; Olsen, 2004).

4.3 Methods

4.3.1 Ethics statement

The self-completion questionnaire and semi-structured interview protocols satisfied the University of Leeds' guidelines on ethical conduct (Ethics reference BIOSCI 12-016).

4.3.2 Field site

The Bay of Plenty region is home to the Rotorua Lakes: a set of 12 freshwater lakes with a mean area of $18.76 \pm 7.1km^2$ (Figure 4.1). The region sees approximately 500,000 domestic and over 800,000 international tourists each year, many of whom take part in activities linked to the lakes (Edgar, 2008; Miller *et al.*, 2006). The lakes'

popularity and close proximity to one another makes them particularly susceptible to the introduction and spread of aquatic invasive non-native species on recreational boats and equipment (Clayton *et al.*, 1981; Miller *et al.*, 2006; de Winton *et al.*, 2009; Lass and Eldershaw, 2012).
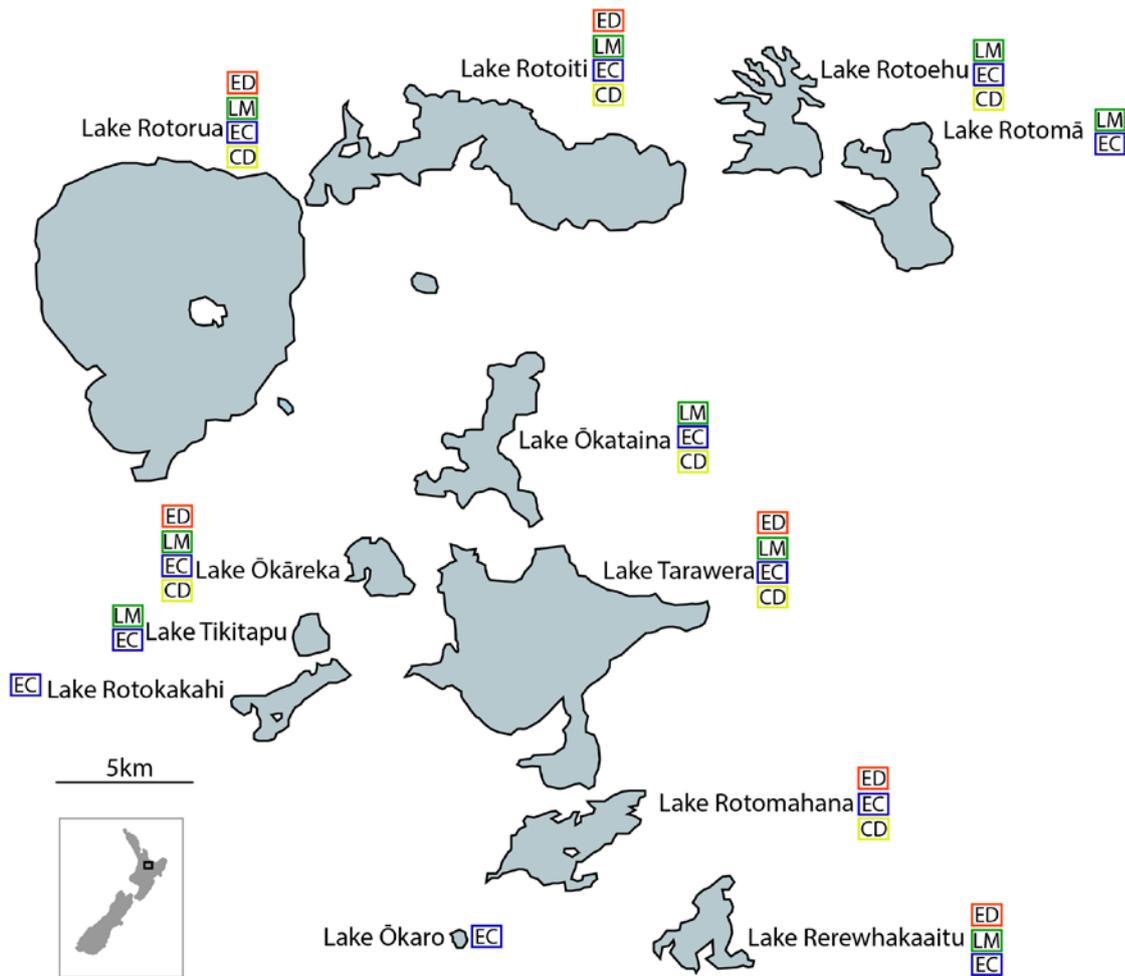
FIGURE 4.1 MAP SHOWING THE BAY OF PLENTY LAKES AND THE INVASIVE NON-NATIVE PLANT SPECIES CURRENTLY PRESENT IN EACH LAKE. ED = *ELODEA DENSA*, LM = *LAGAROSIPHON MAJOR*, EC = *EGERIA CANADENSIS*, CD = *CERATOPHYLLUM DEMERSUM*.

Several species have already been introduced, including curly water thyme (*Lagarosiphon major*), leafy elodea (*Elodea densa*), Canadian waterweed (*Egeria canadensis*) and hornwort (*Ceratophyllum demersum*) (Figure 4.1). These invasive macrophytes are also thought to be vectors for the movement of the eggs of invasive fish including rudd (*Scardinius erythrophthalmus*), koi carp (*Cyprinus carpio*) and brown bullhead catfish (*Ameiurus nebulosus*), present in the lakes and rivers of neighbouring regions (Miller *et al.*, 2006). The aim of the region's biosecurity strategy is therefore to stop the further spread of the existing invasive plants, as well as preventing new INNS introductions.

4.3.3 Biosecurity awareness activities in the Bay of Plenty

The Bay of Plenty's *Stop the Spread* campaign was established in 2004. As didymo has not yet been reported in New Zealand's North Island, the regional campaign focuses on encouraging water users simply to *check* their equipment, vessels and trailers after use and remove any vegetation to prevent the introduction and spread of invasive aquatic plants and associated fish eggs. Each year, the regional council employs two biosecurity advocacy staff during peak visitor season (November-February). The staff engage with water users at boat ramps and visit water sports operators, campsites and hotels to distribute biosecurity awareness materials (including leaflets, cleaning guides, spray bottles and posters). They also man decontamination stations, brief competitors at water sports events, send press releases to local media outlets and maintain biosecurity awareness signs at all boat ramps.

4.3.4 Questionnaire survey

Respondents were asked a number of questions that addressed the following topics:

i. **The biosecurity hazard that their actions posed:**
  - The type of watersports they did and how frequently.
  - Whether they used their boat/equipment at other sides and (if so) where.
  - Whether they took actions to clean/check/dry their boat/equipment after use and (if so) how frequently.

ii. **Their motivations for taking biosecurity actions:**

  How important (from "extremely important" to "not at all important") they
  considered a series of factors to be when deciding whether to

clean/check/dry their boat/equipment at the end of their trip. The factors were:

- the availability of a hose or cleaning station;
- the expense of buying cleaning products;
- the time available to clean everything;
- the availability of information about how to clean their equipment;
- whether there are signs up reminding them to clean it;
- how dirty their boat or equipment looks

iii. **How likely they would be to clean their boat/equipment after use if a series of potential initiatives were put into place (likert scale from 1= highly likely to 5= highly unlikely). The initiatives were:**
- free cleaning stations with hoses/jet washers;
- an information board showing them how to clean their boat or equipment;
- a $500 spot fine if they were found arriving at or leaving a lake without having cleaned their equipment;
- signs up reminding them to clean their boat or equipment before leaving; and more information about how they would be helping the local environment by cleaning their boat/equipment.

iv. **Awareness and understanding of the region's biosecurity campaign:**
- If/how they had heard of the regional biosecurity campaign
- Whether they could select what it was about from a list of options.

v. **Knowledge about invasive non-native species:**
- Whether they could correctly identify i) an invasive non-native plant in the region (hornwort) and ii) an invasive fish in the neighbouring region (rudd) from three possible images.
- Whether they could correctly select which invasive non-native species were in the lakes from a list of options.

vi. **Demographic information**
- Respondents were asked for their gender, age group, nationality and current place of residence (in order to differentiate between locals and tourists). They were also asked if they visited the lake for competition or leisure.

The full questionnaire is provided as Appendix C.

4.3.5 Questionnaire sampling strategy

Questionnaires were conducted between December 2013 and February 2014 at nine of the twelve Rotorua Lakes (Lake Rotorua, Lake Rotomā, Lake Ōkāreka, Lake Ōkataina, Lake Rotoehu, Lake Rotoiti, Lake Tarawera, Lake Tikitapu and Lake Rerewhakaaitu) (Figure 4.1). The remaining three lakes did not permit watersports (Lake Rotomahana and Lake Rotokakahi) or were too small for watersports (Lake Ōkaro, 0.31km^2).

I sampled my target population of watersports participants using the Bay of Plenty lakes using intercept surveys with a skip interval. Every third person (over the age of 18) leaving the lake following a water-based activity was asked if they were willing to complete a two-sided questionnaire taking approximately five minutes. To control for potential biases, the order in which the lakes were visited was randomly stratified by time of day (morning or afternoon) and by day of the week.

The questionnaire was refined using guidelines in Dillman, Smyth & Christian (Dillman *et al.*, 2009) and the answer options for closed-format questions were determined through consultation with biosecurity experts (n = 5) in the UK and New Zealand. Following a pilot of the questionnaire with 10 water users in the region, additional answer options were added to the closed questions (e.g. type of watersport carried out) and one question was reworded to improve comprehension.

A total of 230 recreational water users completed the questionnaire (response rate: 98%). Following best practice advice of White *et al.* (2005), I compared the gender ratio of my sample population to a parallel survey of New Zealand pleasure boaters (Marine Safety Authority of New Zealand, 1999; Maritime New Zealand, 2008). A Kolmogorov–Smirnov test did not reveal any significant differences between the ratios of the two groups (D=0.05, p>0.05), suggesting that my sample was representative of the broader watersports community in New Zealand, in terms of gender. Unfortunately, further demographic data on recreational water users in New Zealand were unavailable.

4.3.6 Stakeholder interviews

Interviews with key stakeholders were conducted on a one to one basis using a semi-structured format. One interviewer (LGA) conducted all interviews to mitigate inter-observer bias. Stakeholders (n=15), who were sampled purposively, included representatives from local and national government, tourist operators (e.g. boat tour

companies), fisheries officers, watersports clubs and environmental management organisations. Topics explored included engaging the public and organisations with biosecurity, opinions on existing and potential biosecurity initiatives in the region, as well as perceived successes and challenges of the aquatic biosecurity programme.

4.3.7 Data analysis

I analysed the questionnaire data using Generalised Linear Models (GLMs) with binomial errors to assess relationships between the response variables (proportion aware of the campaign, proportion of people taking biosecurity actions after every trip) and multiple predictor variables. Predictor variables were checked for inter-correlation before being entered into a model. Residual diagnostics and goodness of fit (via the dispersion parameter) were used to assess model suitability. At each step, non-significant predictor variables were dropped (X^2 test) and the model was re-run, until all remaining covariates were significant. Simplified models were assessed for influential data points and to ensure they met model assumptions (Crawley, 2007). Paired X^2 tests (R package: prop.test) were used to compare proportional data between groups. All quantitative data analysis was performed in 'R' version 3.1.1 (R Development Core Team, 2012).

Interview notes and audio files were imported into qualitative data analysis software NVivo 10 (QSR International Pty Ltd., 2012) for transcription and analysis. Analysis involved coding transcripts into common themes derived from the topics covered and additional topics generated by respondent's answers. Quotations were identified and were used to illustrate particular points or alternative responses.

4.4 Results

4.4.1 Respondents

Questionnaire respondents spanned a range of water sports and included locals (n = 143, mean distance travelled 46.31 ± 7.0 km to reach the lakes) and visitors (n = 87, mean distance travelled 143.48 ±15.54 km). Eighty five percent of respondents had used their vessel or equipment at other water bodies aside from the one that they were using when they were surveyed. (For full demographics see Appendix D).

Overall, semi-structured interview respondents were engaged with biosecurity and resporte having experienced ecological (e.g. reduced abundance and diversity of native plants and invertebrates); social (e.g. inability to continue watersports on

affected lakes, more frequent capsize due to oar/rudder entanglement); and economic impacts (e.g. profit affected by lake closures, vessels damaged) from INNS.

4.4.2 Awareness of biosecurity campaign

Overall, 71% of questionnaire respondents were aware of the region's *Stop the Spread* campaign. Type of water user (Binomial GLM; estimate= -0.41 ± 0.10, p<0.001) and whether people were leisure users or competitors (estimate = 1.46 ± 0.72, p< 0.05; model R^2 =0.98) were both significant predictors of awareness. Aside from sail boat users (who had 100% awareness but of whom three were only 3 respondents), awareness was highest among motorboat users (79.7%) and anglers (76.1%).

The majority of those who had heard of the campaign could correctly recall what it was about. Eighty five percent thought it was about checking their vessel before visiting another site, 75% thought it was to stop the spread of didymo and 60% thought that it was to stop the spread of aquatic INNS (other than didymo).

The majority of water users had heard about the campaign via signs at boat ramps (53.6%), or had been approached by biosecurity advocacy staff at a boat ramp (19%). Boat ramp signs were the most cost-effective communication channel (aside from word of mouth), while face to face conversations with biosecurity advocacy staff were the least cost-effective (Table 4.1).

Environmental management, fisheries and tourism stakeholders agreed that signs at boat ramps were an effective communication channel, but regarded them as the first step of many steps to achieving effective biosecurity.

TABLE 4.1 COST-EFFECTIVENESS OF BIOSECURITY COMMUNICATION CHANNELS

Initiative	Direct annual cost (NZD)	Heard through channel (%)	Reach	Cost per person (NZD)
Signs at boat ramps	1000	53.9	17,351	0.06
Employing advocacy staff	20,000	19.0	6112	3.27
Production of merchandise with awareness messages	25,000	19.0	6112	4.09
Briefing at competitions (roughly 12 events/yr)	240 per event	12.7	4092	0.70
Leaflet	1000	7.9	2535	0.39
Newspaper	1000	15.2	4873	0.20

Note: Reach = estimated number of water users in the region who heard of the Stop the Spread campaign through each channel. I conservatively estimated the total number of recreational water users visiting the area to be 45,307 (see Appendix E) and if I estimate that 71% (32,168) are aware of the campaign, the reach is the percentage of the 32,168 who would have heard about the campaign through each channel.

"It's one thing to get the message in front of them [recreational water users], but it's another thing to get them to do something about it. We need to keep the messages fresh, change them here and there, move the signs around so that people notice them again." (Fisheries officer)

4.4.3 Biosecurity compliance

Awareness of the *Stop the Spread* campaign was a significant predictor of whether people checked their boat/equipment for INNS after every use (estimate = 0.93 ± 0.31, $p<0.01$; type of water user: estimate = -2.09 ± 1.03, $p<0.05$; model $R^2 = 0.96$). Motor boat users were most likely to check their equipment after every use (Appendix D). Models did not reveal any significant predictors of whether people cleaned or dried their equipment after every use. However, the *Stop the Spread* campaign advocates checking for (and removing) weeds on vessels and trailers – a method which can remove $88 \pm 5\%$ of invasive macrophtyes (Rothlisberger *et al.*, 2010).

4.4.4 Motivating individuals to take biosecurity actions

The most important factor determining whether water users cleaned their equipment at the end of their activity was the availability of a hose or cleaning station, with 66% of questionnaire respondents selecting it as either a 'very important' or 'extremely important' factor in their decision. The least important factors were the availability of information about how to clean their equipment and the cost of cleaning products, with 76% and 82% of respondents indicating that these were 'not very important', or 'not at all important', respectively.

Water users were almost equally motivated to clean their equipment every time in response to a 'carrot' approach (the provision of cleaning stations, mean likert score 1.9) as a 'stick' approach (the prospect of a \$500 fine should they be found leaving a site with invasive plants on their vessels, trailers or equipment (mean likert score 2.13). They were least likely to be motivated by an information board explaining how to clean equipment (mean likert score 3.7).

In the interviews, water sports clubs and tourism operators said they would use cleaning stations if they were provided, reflecting the results of the questionnaire respondents. However, environmental management stakeholders expressed reservations about their feasibility, due to the high number of lake entry points; misuse (for car-washing) in other regions; and a belief that they took the responsibility away from individuals.

Tourist operators, water sports clubs, fisheries officers and environmental managers believed that spot-fines would encourage people to check for weeds before visiting another site, though managers were concerned that inspections would strain resources. One tourist operator established an organisational biosecurity policy after being threatened with a fine.

4.4.5 Motivating organisations to take biosecurity actions

Tourism operators and water sports clubs had good awareness of biosecurity overall and were motivated to clean their boats/equipment once they understood the implications of not doing so. Exchange visits were suggested by several stakeholders as a useful way of demonstrating what could happen if organisations failed to take biosecurity seriously.

> *"People react most strongly to what is in front of them. Taking people from the North Island to see the didymo incursion in the South Island really shocked them into taking action and spreading the message. They saw for themselves what could happen if they didn't"* (National government stakeholder)

Eco-certification schemes were also suggested by local and national government stakeholders as a tool to encourage water-based companies to become "environmental guardians".

Events were seen by environmental managers as a particular biosecurity threat, with competitors descending on the lakes from across New Zealand, if not internationally. Stakeholders from water sports clubs admitted not always taking biosecurity action before events but said they always decontaminated their boats at events if facilities were provided. In contrast, environmental managers said events (often at weekends) were difficult to staff, that they took biosecurity responsibilities away from individuals, and that the use of the facilities was difficult to enforce.

> *"We provided disinfectant dips for one event with 600 entrants, but only 40 entrants came to use the facilities."* (Environmental manager)

4.4.6 Barriers to effective biosecurity

Interviewees expressed concern at the inconsistency of biosecurity messages and the cleaning products recommended in different parts of New Zealand, which caused confusion for people travelling with their boats/equipment.

"There seem to be mixed messages: Stop the Spread, Check Clean Dry, lots of different detergents suggested. It's all a bit confusing. It would be good to have one simple message, not seven different ways to clean stuff." (Water sports club stakeholder)

4.4.7 Awareness of INNS in the region

The questionnaire revealed that few water users were able to correctly identify either hornwort (*Cetarophyllum demersum*) or rudd (*Scardinius erythrophthalmus*) from photographs. Twenty three percent were able to identify one of the two species and only 8% able to identify both species. Similarly, only 4% of water users knew that elodea (*Elodea canadensis*) was present in the lakes, or that brown bullhead catfish (*Ameiurus nebulous*) were not present (31%).

Environmental managers differed in their views on identification of INNS by water users: some were concerned, whilst others felt that taking action to prevent their spread was more important. Interviewees agreed that regular water users could provide valuable help with surveillance if they were able to notice and report new INNS.

"It would be good to have big pictures of the non-native weeds and fish that we're on the lookout for at the boat ramps so that people can easily recognise them, even if they don't know their specific names. Something simple and visual." (Environmental manager)

Tourism operators have already provided help with surveillance having been the first to report hornwort (*Cetarophyllum demersum*) in Lake Ōkāreka to the regional council. However, two of the tourism operators who were interviewed were unable to identify hornwort from the images used in the questionnaire, suggesting that more training is needed.

4.4.8 Effectiveness of the regional partnership approach

In the interviews, partner organisations agreed that the formation of an INNS management strategy (Bay of Plenty Regional Council, New Zealand, 2011) had increased collaboration between environmental managers in the area and commented that it had been used as a best-practice model elsewhere in New Zealand. The partners meet twice a year to discuss progress and plans.

"The concept of setting up a formal agreement between the parties about who has responsibility for what and what the goals are has really improved things. Funding applications have become much easier because an existing network of organisations is already in place to carry out the work."
(Environmental manager)

4.5 Discussion

New EU invasive non-native species legislation calls for member states to take a preventative approach to INNS management (European Commission, 2013). The legislation specifically advocates the development of awareness campaigns to improve biosecurity practices among high-risk groups, such as recreational water sports enthusiasts.

Engaging with the public and stakeholders is vital for the success of environmental education initiatives (Bremner and Park, 2007; García-Llorente *et al.*, 2008; Baruch-Mordo *et al.*, 2011). Yet social research into the effectiveness of INNS management has often been overlooked (Bremner and Park, 2007; García-Llorente *et al.*, 2008; García-Llorente *et al.*, 2011; Schüttler *et al.*, 2011; Sharp *et al.*, 2011). Using a social research approach, I have gained a novel insight into the successes and challenges of a preventative approach to INNS management with which to develop recommendations for future campaigns in European countries.

4.5.1 Key findings from New Zealand

4.5.1.1 Biosecurity Awareness
Public awareness of aquatic biosecurity appears to be high in New Zealand. In the Bay of Plenty case study region, 71% of water users had heard of the *Stop the Spread* biosecurity campaign, a significantly higher proportion of water users than had heard of a similar biosecurity campaign in the UK (22%; X^2 = 120.7, d.f. =1, p<0.001) (Anderson *et al.*, 2014a, Chapter 2).

Importantly, high awareness of the biosecurity campaign in the Bay of Plenty converted into action with 61% of respondents checking their boat/equipment after use, 57% cleaning and 50% drying their boat/equipment after every use. Moreover, the significant link between awareness of the campaign and the proportion of people checking boat/equipment for INNS indicates that the campaign is positively influencing water users' biosecurity actions. The proportion of water users taking

measures to clean and dry their equipment in New Zealand were higher than those reported in similar studies both in the UK (Anderson *et al.*, 2014a) and United States (Kelly *et al.*, 2013b; Rothlisberger *et al.*, 2010a).

4.5.1.2 Communications Channels

The most cost-effective communication channel for sharing biosecurity information was signage at boat ramps. I recommend that aquatic biosecurity information is disseminated at the access points of high risk water bodies in Europe, such as popular angling or water sports lakes. Although signs were the most cost effective, the use of multiple communication channels appeared to reinforce water users' awareness of biosecurity messages with many water users indicating that they had heard about the campaign from several sources. Previous research also indicates that using a range of communications types is more likely to increase INNS awareness and pro-environmental behaviour change (Teillac-Deschamps *et al.*, 2009).

It was also apparent from my results that there is a large biosecurity risk inherent at water sports events and angling tournaments. Feedback from my interview respondents highlighted the importance of targeting event organisers with biosecurity messaging, providing briefings and decontamination stations at angling and water sports events, and enforcing their use.

4.5.1.3 Motivating water-users

When water users were asked what would motivate them to clean their boats/equipment after every use, the prospect of a fine for not doing so, and the incentive of having a cleaning station available were the most popular initiatives. In New Zealand, The Biosecurity Act 1993 includes provisions for spot fines for water users, however the feasibility of a similar system in Europe would require further research. Although cleaning stations proved popular among water users, there were logistical difficulties inherent in placing them on busy boat ramps in the Bay of Plenty (Miller *et al.*, 2006). Moreover, several environmental managers felt the provision of cleaning stations took the responsibility away from individuals. However, there may be scope to install cleaning stations at high risk sites in Europe. A permanent cleaning station has already been established at a popular water sports reservoir in the UK (Royal Yachting Association, 2010), while temporary facilities are being offered by some rivers trusts (South Cumbria Rivers Trust, 2014).

Organisations in the USA have overcome non-compliance with biosecurity by stipulating that boats are decontaminated before entering or leaving the site as part of a mooring agreement, or enforcing a "no wash, no fish" policy for entrants of angling tournaments (Hickey, 2010). Similarly, angling tournament organisers in Ireland require anglers to disinfect their equipment before entry (Lakelands & Inland Waterways, 2014). Initiatives such as these could help increase biosecurity compliance at events.

4.5.1.4 Motivating organisations

My interview results suggested that businesses were motivated to take biosecurity actions once they understood the potential threat that invasive non-native species posed to their livelihoods. This reflects previous research which indicated that an increased understanding of the impacts of INNS among stakeholders improves levels of advocacy with INNS management interventions (Bremner and Park, 2007; García-Llorente *et al.*, 2011). The prospect of a fine was considered to be a particularly good incentive to motivate businesses having previously compelled water sports organisations in the region to change their practices and implement company biosecurity policies. Exchange visits to areas affected by INNS were considered an effective way to demonstrate the impacts of INNS to water sports operators in unaffected areas.

Eco-certification schemes were also proposed as a potential tool through which companies could improve the sustainability of their practices (including biosecurity practices) while simultaneously improving their reputation to environmentally-conscious customers. Eco-certification schemes are recommended as effective tools for promoting good environmental practice among tourism operators (Christ *et al.*, 2003; Secretariat of the Convention on Biological Diversity, 2004). For example, an eco-certification scheme is offered to water sports operators working in the vicinity of the Great Barrier Reef who demonstrate sustainable practices and reduced carbon emissions (Zeppel, 2012). However, there is some debate around whether sufficient consumer demand exists for eco-certification schemes, particularly for tours and attractions (as opposed to accommodation) (Esparon *et al.*, 2014). Tourists must buy into the goals of an eco-certification scheme in order for it to be successful (Esparon *et al.*, 2014). Further research into the purchasing motivations of watersports customers would be a practical first step towards any of these options.

4.5.1.5 Regional approach with overarching national support

New Zealand's nationally coordinated but regionally implemented approach to biosecurity appeared to be a particular strength. Research suggests that community level approaches to environmental management can enhance communication between parties, encourage participatory decision making, and promote conflict resolution between stakeholders, fostering a collaborative approach to INNS management (Sharp *et al.*, 2011). In the Bay of Plenty, regional biosecurity activities are bolstered by government funding and knowledge exchange, as well as being supported by national legislation. The effective communication network between coordinators in the national biosecurity team and biosecurity representatives in each region ensures each region remains motivated and that neighbouring regions are managing INNS in an integrated manner. The network – which gathers for a weekly teleconference – also acts as a valuable channel through which reports of new and potentially invasive species can be rapidly disseminated.

The Bay of Plenty region's five-year INNS management strategy supports a holistic approach to INNS control incorporating awareness raising activities with monitoring and eradication work. The strategy, which outlines the roles and responsibilities of the different partners in the region, was found to be a key component of the region's overall INNS management programme. Several stakeholders commented that they had had a much more cohesive approach to managing INNS since the regional strategy was implemented.

4.5.2 Potential challenges to effective biosecurity

4.5.2.1 Inconsistent biosecurity messages

Due to New Zealand's regional approach to biosecurity, the decontamination treatments recommended to water users are tailored to tackle the problematic species in each region. Stakeholders expressed concerns about message consistency and confusion among the public over what to use to decontaminate vessels and equipment when travelling between regions. It is unrealistic to expect water users to know which INNS are present in a region. I therefore recommend that biosecurity campaigns both in New Zealand as well as in Europe use consistent biosecurity messaging (Caffrey *et al.*, 2014) and advocate one cleaning product or protocol that is easy and economical for people to source, that is effective at killing a wide range of INNS, and use and has no impact on the environment when disposed of (potentially in large volumes) (Kilroy

et al., 2006). Recent research indicates that submersion hot water at (45°C for 15 minutes) kills 99% of invasive plants and animals on angling equipment (Chapter 3).

4.5.2.2 Evaluative Research

In the absence of a control site, it proved difficult to tease apart which aspects of the Bay of Plenty's INNS strategy (Bay of Plenty Regional Council, New Zealand, 2011) have been the most effective (and most cost effective) at preventing the introduction and spread of aquatic INNS. In New Zealand, the selection of a valid control site (a water body or region of waterbodies where no biosecurity campaign is currently taking place) is almost impossible due to widespread biosecurity messaging at waterways across the country. In Europe, with biosecurity programmes in their infancy, we are afforded an unparalleled opportunity to use Before After Control Impact (BACI) studies to test the effectiveness of (and subsequently adapt) different aspects of an INNS management strategy.

4.5.2.3 Awareness of local INNS

Previous studies suggest that increased awareness and recognition of INNS among the public have led to increased acceptance of management interventions (Bremner and Park, 2007; Somaweera *et al.*, 2010). Despite their high levels of biosecurity awareness, a low proportion of water users in the Bay of Plenty recognised local INNS. Only 23% of water users could correctly identify one of the INNS threatening lakes in the Bay of Plenty and only 8% of people could identify both species. Some water sports companies were also unable to accurately identify INNS in the lakes that they use.

The value of basic biosecurity is that it can be embedded into people's activities without requiring specialist knowledge of harmful organisms. Nonetheless, the value of informal monitoring or "passive surveillance" by the public in the detection of new INNS is widely recognised (Whittle *et al.*, 2013). Water users have the potential to report sightings of invasive plants or fish in the water bodies that they use. However the public need to be able to accurately identify INNS for this to be effective (Somaweera *et al.*, 2010).

A recent report recommends that citizen science – the collection and analysis of environmental data by members of the public – should play a much stronger role in the surveillance of INNS in New Zealand (The Royal Society of New Zealand, 2014) and its use is also recommended in Europe (Caffrey *et al.*, 2014). This is largely because the public are able to monitor a much broader area for INNS than a small team of

environmental scientists would be able to (Crall *et al.*, 2010). As signs at boat ramps were the most effective way of disseminating biosecurity information, I recommend that biosecurity awareness information is accompanied by images detailing which aquatic INNS to look out for and how to report them. Public monitoring efforts could complement biosecurity activities both in New Zealand and in Europe, where monitoring and reporting new non-native species are key components of new legislation (European Commission, 2013).

4.6 Conclusion

The development of educational campaigns to raise awareness of biosecurity among high risk groups is an integral component of the new EU invasive non-native species legislation (European Commission, 2013). Based on my findings in New Zealand as a best practice example, I present a number of good practice guidelines for the design and implementation of aquatic biosecurity awareness campaigns in Europe.

I recommend that European biosecurity awareness campaigns should include the following elements:

- The formation of regional biosecurity partnerships with overarching national support.
- The development of integrated regional invasive non-native species management strategies to improve cohesion between partner organisations.
- Simple and consistent biosecurity advice.
- The use of multiple communications channels to disseminate biosecurity advice, with a focus on signs at the entry/exit points of waterbodies.
- The provision of basic information about which species to look out for and how to report them to promote "passive monitoring".
- The provision of decontamination stations at high risk sites and watersports events.
- The promotion of stakeholder exchange visits and the development of eco-certification schemes to encourage best practice amongst water sports and tourism operators.
- The use of Before-After-Control-Impact (BACI) studies to evaluate and adapt invasive non-native species management initiatives.

5 Does enemy release or environmental quality explain patterns of infection in UK crayfish?

5.1 Summary

The enemy release hypothesis (ERH) is frequently proposed to explain the success of INNS in their introduced ranges, postulating that in the absence of regulation by infectious agents, INNS should reach larger body sizes, and higher population densities giving them a competitive advantage. However, the ERH is often explored in isolation without considering the influence that environmental variables can have on host-parasite dynamics. In this study, I explore whether crayfish species or environmental quality are predictors of the presence or prevalence of infectious agents in native white-clawed crayfish (*Austropotamobius pallipes*) and non-native invasive signal crayfish (*Pacifastacus leniusculus*), using i) a 7 year dataset of histology records collected by Cefas and ii) a community study comparing the presence and prevalence of infectious agents in 3 x isolated *A. pallipes* populations; 3 x isolated *P. leniusculus* populations and 3 populations where the two species had overlapped in the past. My results support the ERH, demonstrating that non-native *P. leniusculus* are hosts to a significantly lower diversity of infectious agents (mean Simpson's = 0, n = 1 infectious agent) than native *A. pallipes* (mean Simpson's = 0.17, n = 4 infectious agents) in the UK (GLMM Log likelihood X^2 = 7.5, p <0.05, Model R^2 = 0.98). The only infectious agent present in both species was an intranuclear bacilliform virus which may have been acquired by *P. leniusculus* from *A. pallipes* in the past. My results revealed a significant link between the chemical status of the waterbody, and the presence of the lethal agent *A. astaci* in *A. pallipes* populations. This demonstrates the need for regular epidemiological monitoring to examine interactions between environmental quality and the presence of INNS and patterns of aquatic animal disease.

5.2 Introduction

Conservationists have long sought to understand the traits that make invasive non-native species (INNS) so successful in their introduced ranges (van Kleunen *et al.*, 2010; Parker *et al.*, 2013). Understanding these attributes can help in assessing comparative risk and formulating preventative management measures.

Infectious agents can play an important role in biological invasions, influencing both the success of an introduced species, and the resilience of native species in the introduced range; ultimately determining the outcome of the invasion (Hatcher *et al.*, 2012; Dunn *et al.*, 2012; Dunn and Hatcher, 2015). These agents can be involved in the invasion process in three main ways.

First, non-native species can introduce infectious agents into native populations. Such introduced agents may '*spill over*' into native populations, potentially resulting in an emerging disease (Tompkins *et al.*, 2011; Okamura and Feist, 2011b; Hatcher *et al.*, 2012). The likelihood of the native population becoming infected with an introduced agent will depend on the factors which include the number of infected individuals introduced into the native population; the host-specificity of the infectious agent; the immunity of native species; and whether the introduced habitat has the necessary environmental conditions (e.g. water chemistry, presence of secondary hosts) for the infectious agent to survive (Okamura and Feist, 2011b; Hatcher *et al.*, 2012).

Secondly, non-native species can acquire infectious agents in their introduced range (Kelly *et al.*, 2009c, p.2), resulting in one of two outcomes. If the non-native species is a competent host, it may act as a reservoir for the agent, amplifying its prevalence and allowing infection to '*spill back'* into the native population (Kelly *et al.*, 2009c; Poulin *et al.*, 2011; Strauss *et al.*, 2012). In contrast, if the INNS is a less competent host than the native species i.e. the agent cannot develop in the INNS, but can infect it anyway, it may act as a sink for the agent, reducing infection prevalence in native species through a dilution effect (Poulin *et al.*, 2011).

Finally, due to stochastic and selective pressures during the invasion process, non-native species may lose their infectious agents (Dunn, 2009), a concept termed '*enemy release'* (Keane and Crawley, 2002). There are a number of mechanisms to explain the loss of infectious agents, for example: i) only a small number of infected individuals surviving the invasion process; ii) selective pressures in the introduced habitat favouring fitter (i.e. uninfected/resistant) individuals; iii) reduced transmission opportunities in the introduced range due to low (founder) population density, or

absence of an intermediate host; iv) the founder population could be an uninfected life history stage (e.g. marine larvae (Torchin et al., 2002)) (Dunn, 2009).

As infectious agents commonly regulate host population density (Keane and Crawley, 2002; Shea and Chesson, 2002) and limit host body size (Torchin *et al.*, 2001; Torchin *et al.*, 2003), the Enemy Release Hypothesis (ERH) posits that invasive non-native species may have a competitive advantage in their introduced range if they are free from such agents of control (MacLeod *et al.*, 2010).

The ERH explains the success of INNS through three predictions: 1) the specialist enemies of the INNS will be absent from the new region; 2) host switching of native enemies to INNS will be rare; and 3) generalist enemies will have a greater impact on native competitors (Keane and Crawley, 2002).

Biogeographical studies (those comparing the diversity of infectious agents in the native and invasive range of a species) have reported that introduced animals and plants may escape up to 75% of the infectious agents in their native range (Torchin and Mitchell, 2004). However such studies may over-represent the effects of enemy release if they do not compare the invasive population with the specific source population from which it was founded (Colautti *et al.*, 2004; Colautti *et al.*, 2005) as there may be genetic heterogeneity in different native populations which could influence their resistance to infectious agents as well as spatial heterogeneity in prevalence (MacLeod *et al.*, 2010). Community studies (those comparing the diversity of infectious agents in native and invasive conspecifics in the introduced range) have often contradicted the ERH, showing similar levels of infectious agents in both species (Colautti *et al.*, 2004).

Under natural conditions, host-parasite interactions are likely to be affected by external factors in the host population's abiotic environment (Sures, 2008; Johnson and Paull, 2011). For example, freshwater habitats are affected by multiple environmental stressors including pollution, habitat degradation, agricultural run-off and flow modification, as well as INNS (Dudgeon *et al.*, 2006; WWF, 2014). Environmental factors such as these can affect the competitive ability of native and non-native hosts and their resistance to disease causing agents as well as affecting the survival of free living stages of these agents, and their potential virulence (Keane and Crawley, 2002; Prenter *et al.*, 2004a; Sures, 2008; Poulin *et al.*, 2011). It is therefore considered best practice to consider enemy release in the context of other environmental factors (Torchin *et al.*, 2001; Roy *et al.*, 2011).

UK freshwater environments are increasingly imperiled by aquatic INNS (Dudgeon *et al.*, 2006; Jackson and Grey, 2012; Gallardo and Aldridge, 2014). One of the worst freshwater INNS is the non-native American signal crayfish *Pacifastacus leniusculus* (UK Technical Advisory Group on the Water Framework Directive, 2014). *P. leniusculus* was first introduced into the UK during the 1970s for the purposes of aquaculture but subsequently escaped and dispersed forming widespread wild populations (Holdich *et al.*, 2014). Not only has it outcompeted native *A. pallipes* for food and habitat (Vorburger and Ribi, 1999; Dunn *et al.*, 2008), it is an asymptomatic carrier of the oomycete *Aphanomyces astaci*, the causative agent of crayfish plague (Alderman *et al.*, 1984). Crayfish plague has been associated with sudden and acute mortality events (Alderman *et al.*, 1984; Longshaw, 2011) and subsequent local extinction in *A. pallipes*, and is a major contributor to its listing as an endangered species on the IUCN Red List (Füreder *et al.*, 2010).

Although widespread mortalities are an obvious impact of this and other invasions, the role that sub-lethal infectious agents play in invasion dynamics have received less attention in UK crayfish (Longshaw, 2011). A recent study of infectious agents in *A. pallipes* showed a high diversity and prevalence of infection while a similar study of non-native crayfish imports showed that 66.4% of non-native crayfish were apparently free from infection (Longshaw *et al.*, 2012a; Longshaw *et al.*, 2012c). Although these studies indicate that enemy release may play a role in invasion success, other studies suggest that the fitness of native crayfish, as well as the prevalence of some infections in native crayfish, may also be affected by the quality of the local environment (Haddaway, 2010; Imhoff, 2010). Here, I test whether enemy release or environmental factors are better predictors of infectious agent diversity and prevalence in UK crayfish populations.

In the first part of the study, I analysed a 7 year dataset relating to crayfish disease surveillance compiled by the Centre for Environment, Fisheries and Aquaculture Science (Cefas) in Weymouth, UK. Cefas is an Executive Agency of the Department for Environment, Food and Rural Affairs (Defra) and the lead advisor on aquatic animal health in the UK. It also houses the EU Reference Laboratory for Crustacean Diseases (http://www.crustaceancrl.eu/). My aim was to determine whether the species of crayfish present, or the habitat quality (the ecological and chemical classification of each sub-catchment according to the Water Framework Directive) were predictors of the presence and prevalence of infectious agents in *A. pallipes* and *P. leniusculus* crayfish populations in the UK.

Secondly, I performed a community study (comparing native and invasive non-native species of crayfish in the introduced range (Colautti *et al.*, 2004)) to test whether Keane & Crawley's (2002) predictions for ERH were supported in isolated *A. pallipes* populations (n=3 populations), isolated *P. leniusculus* populations (n=3 populations) and mixed species crayfish populations (n=3 populations). I hypothesized that i) few infectious agents would be shared between native and non-native crayfish and ii) generalist infectious agents would have a greater prevalence in native than non-native crayfish in mixed species populations.

5.3 Methods

5.3.1 Cefas 7 year dataset

All histological surveys of *A. pallipes* (25 populations; 210 individuals) and *P. leniusculus* (44 populations; 818 individuals) conducted by Cefas between 2007 and 2014 were combined into a single 7 year dataset. *A. pallipes* is listed as an endangered species (IUCN 2014) and a UK protected species, hence smaller sample sizes were collected. Only crayfish populations which were subjected to full histological examinations were included in the dataset in order to investigate the influence of co-infections. In addition to the histological screening, a separate set of *A. pallipes* populations (25 populations; 123 individuals) were analysed separately to investigate predictors of *Aphanomyces astaci*, the causative agent for crayfish plague, which is detected using molecular screening instead of histology.

5.3.2 2012 Field sampling to investigate enemy release

For the second part of this study, crayfish were collected from three *A. pallipes* populations and six *P. leniusculus* populations under license from Natural England (Licence number: 20122156, granted to LA) and the Environment Agency (FR2 licenses), respectively, between June and October 2012. Efforts to locate overlapping mixed-species populations of crayfish were unsuccessful, in part due to a lack of up to date documentation on the distributions of the two species. Therefore, as a proxy for mixed populations, I used 3 single-species *P. leniusculus* populations (Bookill Gill Beck, Cawthorne Dike and the River Ure, see Table 5.2) where the co-occurrence of *A. pallipes* had been recorded within the previous two years (Dunn et al 2008; Haddaway et al 2012b; Haddaway et al 2012a, pers comm. S Peay 2012). The other six populations were selected because they had been isolated from introductions of other

crayfish species in the past, according to the rivers trusts, local records centres, National Biodiversity Network Gateway and ecological consultants consulted.

5.3.3 Histological screening

Samples were prepared and histological analyses conducted in accordance with a standard crustacean disease screening protocol (see www.crustaceancrl.eu) and as applied in previous studies on crayfish disease (Longshaw 2011; Longshaw et al 2012a; Longshaw et al 2012b). All crayfish were examined for external abnormalities (e.g. missing claws, damage to carapace), sexed and measured (carapace length) before being exposed to an overdose of chloroform vapours to humanely euthanize them prior to sampling for histology.

Juvenile crayfish (≤ 10mm carapace length) were euthanized and fixed whole by direct injection of Davidson's Freshwater Fixative (Hopwood, 1996). Larger animals (>10mm carapace length) were dissected and samples of the carapace, gill, gonad, intestine, hepatopancreas and tail muscle were collected immediately and preserved in Davidson's freshwater fixative for 24h before being transferred to 70% industrial methylated spirits (IMS). If required the tissue samples were decalcified in a rapid decalcification solution.

The tissues were processed to wax blocks using an automatic vacuum infiltration tissue processor (Vision Biosystems Peloris). Sections were cut at 3 to 5 μm and routinely stained with haematoxylin and eosin (H&E) in an automatic tissue stainer. The tissues were examined on a light microscope using brightfield illumination. A record was made of any pathologies or infectious agents in organs and tissues and, where appropriate, an indication of the level of infection severity. Images were captured using a LUCIA™ (Nikon, UK) screen measurement system.

5.3.4 Molecular screening for *Aphanomyces astaci*

Samples were collected from the cuticle and sub-cutis of all adult crayfish and individually placed in 100% ethanol and stored at -20℃. The full methods used to extract DNA from tissues have been previously described in (Oidtmann, 2004). Briefly, DNA was extracted using the DNeasy tissue kit (Qiagen) following the manufacturer's instructions. Animals were screened for *A. astaci* using the PCR protocol described in (Oidtmann *et al.*, 2006).

5.3.5 Environmental parameters

The Water Framework Directive requires all European Union (EU) Member States to assess and classify the status of their river catchments according to a unified set of ecological and chemical standards (Water Framework Directive, 2012). The ecological classification ('high' to 'bad' on a five point scale), scores each water body against a set of biological (abundance of fish and rooted plants), physico-chemical (temperature and nutrient levels) and hydro-morphological (water flow and physical habitat) criteria (Water Framework Directive, 2012). The chemical classification ('good' or 'fail') examines the presence of polluting substances that could adversely affect the ecology of the catchment by checking whether the water meets Environmental Quality Standards (EQSs) for substances listed in Annex IX (Dangerous Substances Directive and associated daughter Directives) and Annex X (WFD Priority List Substances) (Water Framework Directive, 2012). To ensure that the environmental parameters were policy-relevant as well as being biologically robust, I identified the chemical and ecological status of the sub-catchment each crayfish population in the Cefas dataset was sourced from using the Environment Agency's Catchment Data Explorer website (Environment Agency, 2014).

5.3.6 Statistical analysis

Generalised linear mixed-effects models (GLMMs) were used to determine which variables were predictors of the presence/absence, prevalence and diversity (Simpson's index) of pathogens in *A. pallipes* and *P. leniusculus* populations in the UK based on the Cefas dataset. Explanatory variables included crayfish species; presence of other infectious agents in the population; prevalence of other infectious agents in the population; chemical status of the sub-catchment; and ecological status of the sub-catchment. For single-species *P. leniusculus* sites I added an additional explanatory variable in the model (former presence of *A. pallipes* crayfish in the catchment). Due to the wide temporal and geographical range of the data, site and year were included as random factors in each model. All models were fitted with a binomial error distribution and a logit link function due to the fact that the response variables consisted of binary or proportion data (Crawley, 2007).

I checked that the models met the assumptions of homogeneity of variance by visually assessing plots of residuals vs. fitted values and checked normality of residuals with quantile-quantile plots and histograms. Log-likelihood tests were used to compare simplified models to null models (random effects only) and conditional R^2 values were

calculated to describe the proportion of variance explained by both the fixed and random factors (Johnson, 2014).

5.4 Results

5.4.1 Predictors of infection: Cefas 7 year dataset

Overall, *A. pallipes* had a higher diversity of infectious agents than *P. leniusculus*. Histology results revealed that *A. pallipes* populations were infected with four agents: the microsporidian parasite *Thelohania contejeani*, a hepatopancreatic bacilliform virus, *Psorospermium haeckeli* and the clitellate annelid *Branchiobdella astaci* (mean Simpson's index = 0.17) while *P. leniusculus* were only infected with a hepatopancreatic bacilliform virus (Figure 5.1). In line with this, crayfish species was the only significant predictor of infectious agent diversity in the minimum adequate model (Simpson's index) (Table 5.1).

The only infectious agent in both crayfish species was a bacilliform virus (BV). It is not yet known whether this BV is the same taxon in both host species. Although none of the variables (ecological status, chemical status or crayfish species) were significant predictors of the presence/absence of BV in crayfish populations, crayfish species was a significant predictor of the prevalence of BV infection (Table 5.1), with a higher prevalence observed in *A. pallipes* populations (13/24 populations infected, mean prevalence = 0.39) than *P. leniusculus* populations (11/44 populations infected, mean prevalence = 0.13).

5.4.2 Predictors of infection in *A. pallipes*

The presence and prevalence of the three other infectious agents in *A. pallipes* (*T. contejeani, P. haeckeli* and *B. astaci*) were explored in the *A. pallipes* dataset in isolation. *T. contejeani* was present in 11/24 *A. pallipes* populations in the long term dataset and the mean prevalence among infected populations was 0.27 (range 0.09 - 0.37). However, *B.astaci* and *P.haeckeli* were only present in 2/24 populations each (note: not the same two populations) and infected populations had a mean prevalence of 0.19 and 0.60, respectively. Perhaps surprisingly, neither the ecological nor chemical status of the catchment were significant predictors of the presence or prevalence of the three parasites (model P values >0.05).

TABLE 5.1 RESULTS OF MIXED EFFECTS MODELS SHOWING SIGNIFICANT PREDICTORS OF INFECTIOUS AND PREVALENCE OF INFECTIOUS AGENTS IN CRAYFISH POPULATIONS USING THE 7 YEAR CEFAS DATASET.

Response variable	Significant predictor(s)	Non-significant predictors	Model R^2	X^2	P value
Model 1: Parasite diversity (Simpson's Index)	Crayfish species	Ecostat Chemstat	0.98	7.50	<0.05
Model 2: Prevalence of bacilliform virus (both species)	Crayfish species	Ecostat Chemstat Presence of Ph, Ba, Tc Prevalence of Ph, Ba, Tc	0.24	11.30	<0.001
Model 3: Response: Prevalence of bacilliform virus (*A.pallipes* only).	Presence of *Tc*	Ecostat Chemstat Presence of Ph, Ba Prevalence of Ph, Ba, Tc	0.27	0.01	<0.001
Model 4: Presence of crayfish plague (*Aphanomyces astaci*) in *A.pallipes*.	Chemstat	Ecostat	0.21	4.20	<0.05

Note: Predictors = the variables which remained in the minimum adequate model. Non-significant predictors = variables removed to reach the minimum adequate model. X^2 = result of log likelihood test comparing minimum adequate model to null model. Ecostat = Ecological status of the sub-catchment according to the Water Framework Directive. Chemstat = Chemical status of the sub-catchment according to the Water Framework Directive. Ba = *Branchiobdella astaci*, Ph = *Psorospermium haeckeli*, Tc = *Thelohania contejeani*, BV = bacilliform virus

However, the presence of *Thelohania contejeani* in the population was a significant predictor of BV prevalence (Table 5.1). The mean prevalence of BV was 0.58 in *A.pallipes* populations with *T. contejeani* but 0.23 in populations with no *T. contejeani* infection present.

The populations of *A. pallipes* which tested positive for *Aphanomyces astaci,* the causative agent of crayfish plague, were widely distributed across the UK. Infection prevalence of the sampled animals ranged from 10% to 100%. My results revealed a significant link between the chemical status of the water body and the presence of crayfish plague: waterbodies with a "fail" for chemical status were more likely to test positive for *A. astaci*. However none of the variables were significant predictors of the prevalence of *A. astaci*.

5.4.3 2012 Field sampling to investigate enemy release

To further explore whether there was evidence to support enemy release, I analysed the subset of nine sites with three different population compositions: i) *A. pallipes* in isolation; ii) *P. leniusculus* in isolation; iii) *P. leniusculus* with recent *A. pallipes* overlap. In accordance with the results of the 7 year dataset, *A. pallipes* populations were

infected with three types of infectious agent (*T. contejeani*, BV, *Branchiobdella astaci*) while signal crayfish populations were only infected with BV (Figure 5.2). Population composition was the only significant predictor of BV prevalence (Estimate =2.62±0.89, t = 2.96, p<0.05; Model R^2= 0.21), with higher prevalence associated with single species *A. pallipes* populations (0.72) and *P .leniusculus* populations which had recently overlapped with *A. pallipes* (0.2) and low prevalence among *P. leniusculus* only populations (<0.01) (Table 5.1, Figure 5.2).

TABLE 5.2 LOCATION AND COMPOSITION OF CRAYFISH POPULATIONS SAMPLED AS PART OF THE 2012 COMMUNITY STUDY OF ENEMY RELEASE. RESULTS SHOW THE PREVALENCE OF THE THREE PARASITES RECORDED DURING THE STUDY.

Species	Site (coordinates)	n	*T. contejeani*	Bacilliform virus	*B. astaci*
A. pallipes	Wyke Beck, Leeds, West Yorkshire (53.8225, -1.4819)	24	0.37	0.58	0.25
A. pallipes	Clapham Beck, Clapham, North Yorkshire (54.118116, -2.391811)	33	0.09	0.80	0.13
A. pallipes	River Kent, Kendal, Cumbria (54.341219, -2.747489)	11	0.09	0.80	0
P. leniusculus (formerly mixed species)	Bookill Gill Beck, Long Preston, North Yorkshire (54.022255 -2.242651)	28	0	0	0
P. leniusculus (formerly mixed species)	Cawthorne Dike, Cawthorne, South Yorkshire (53.575938, -1.555192)	33	0	0.6	0
P. leniusculus (formerly mixed species)	River Ure, West Tanfield, North Yorkshire (54.203132, -1.589163)	9	0	0	0
P. leniusculus	River Clyde, Elvanfoot, Scotland (55.433032, -3.649609)	22	0	0.2	0
P. leniusculus	Aske Estate, Richmond, North Yorkshire (54.424541, -1.724253)	24	0	0	0
P. leniusculus	Loch Ken, Dumfries and Galloway, Scotland. (55.0116161,-4.0593604)	32	0	0	0

Note: results show the presence and prevalence of the three parasites recorded during the study
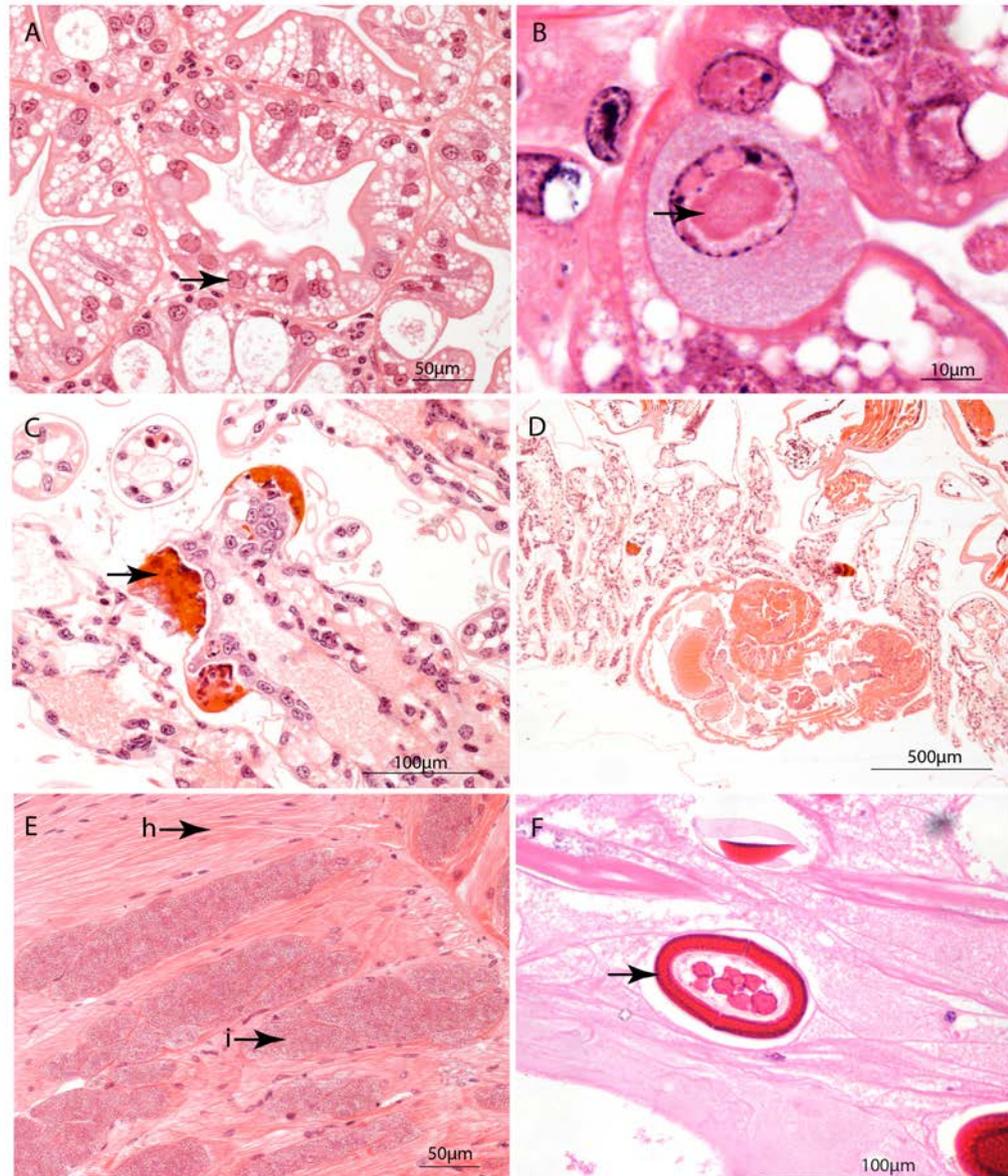
FIGURE 5.1 MICROSCOPE IMAGES SHOWING INFECTIOUS AGENTS OF CRAYFISH BASED ON H&E STAINING. A) LOW POWER VIEW OF *P. LENIUSCULUS* HEPATOPANCREATIC TUBULE EPITHELIAL CELL NUCLEI INFECTED WITH BV (SOLID PINK VIROPLASM IN CENTRE SURROUNDED BY CELL ORGANELLES). B) HIGH POWER VIEW OF HEPATOPANCREATIC TUBULE WITH BV-INFECTED NUCLEUS MARKED WITH ARROW. C) MELANISED GILL TISSUE OF AN *A. PALLIPES* INFECTED WITH *B. ASTACI*. D) CROSS-SECTION THROUGH AN INDIVIDUAL *B. ASTACI* PARASITE INFECTING THE GILL OF *A. PALLIPES*. E) TAIL MUSCLE TISSUE OF *A.PALLIPES* HEAVILY INFECTED WITH *T. CONTEJEANI*. ARROW MARKED 'H' SHOWS HEALTHY, STRIATED MUSCLE TISSUE. ARROW MARKED 'I' SHOWS INFECTED MUSCLE TISSUE WHICH HAS BEEN REPLACED WITH SPORES. F) LONGITUDINAL SECTION THROUGH *PSOROSPERMIUM HAECKELI* SPOROCYST IN THE CONNECTIVE TISSUE OF AN *A. PALLIPES* HOST
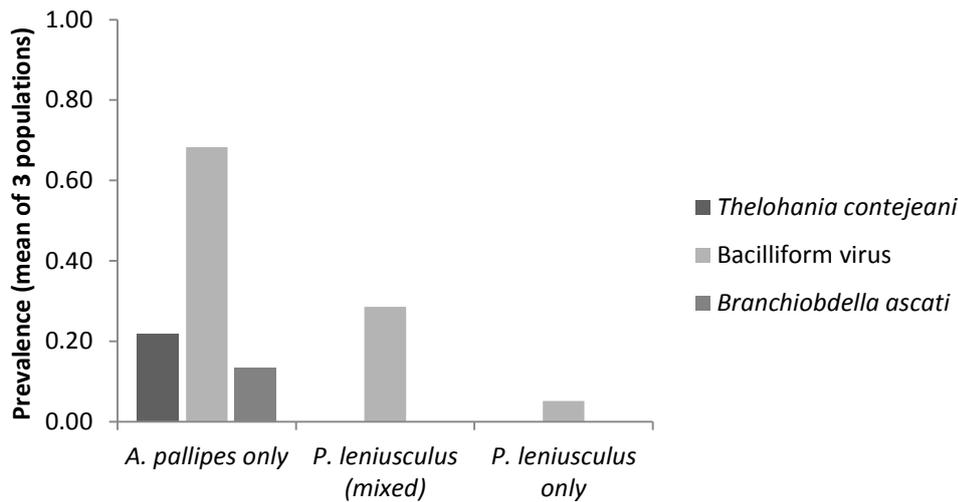
FIGURE 5.2 PRESENCE AND MEAN PREVALENCE OF PARASITES ACROSS THE THREE POPULATION COMPOSITIONS: ISOLATED *A. PALLIPES* POPULATIONS (N=3 POPULATIONS), ISOLATED *P. LENIUSCULUS* POPULATIONS (N=3 POPULATIONS) AND *P. LENIUSCULUS* POPULATIONS WITH RECENT *A. PALLIPES* OVERLAP

5.5 Discussion

The enemy release hypothesis posits that INNS will display greater fitness in their introduced ranges because they are freed from their limiting infectious agents (Keane and Crawley, 2002; Mitchell and Power, 2003). The results of this study provide support for enemy release by demonstrating that non-native *P. leniusculus* are hosts to a significantly lower diversity of infectious agents than native *A. pallipes* in the UK (Table 5.2)*.* In particular, the results of my 2012 field study supported the predictions made by Keane and Crawley (2002): the switching of infectious agents from native to non-native hosts was rare, and the one shared parasite (BV) had a greater prevalence in native, than non-native crayfish. I also found a significant link between the chemical status of the waterbody, and the presence of the lethal parasite *A.astaci* in *A. pallipes* populations. In contrast, I found little evidence to suggest that the presence and prevalence of sub-lethal crayfish infections are affected by the chemical or environmental status of their habitat.

5.5.1 Intranuclear bacilliform virus

Hepatopancreatic bacilliform virus (BV) infections were detected in both *P. leniusculus* and *A. pallipes* populations. Morphologically similar viruses have been found in a number of other marine and freshwater decapod species including crabs and penaeid shrimps (Stentiford *et al.*, 2004; Stentiford and Feist, 2005; Bateman and Stentiford,

2008), suggesting that they are generalist pathogens of Crustacea. The fact that the highest prevalence of BV was detected in single-species *A. pallipes* populations; high prevalence was detected in *P. leniusculus* populations which had previously overlapped with *A. pallipes;* and low prevalence (or absence) was detected in isolated *P. leniusculus* populations may suggest that *P. leniusculus* acquired bacilliform virus from *A. pallipes*. However, the presence of bacilliform virus in an isolated *P. leniusculus* population does not fit this hypothesis and may suggest that (perhaps a different strain of) bacilliform virus accompanied *P. leniusculus* from the its native range, something that is considered to be more common with asymptomatic parasites (Mitchell and Power, 2003; Longshaw *et al.*, 2012a). A third alternative is that the infected Scottish population of *P. leniusculus* had indeed had prior contact with *A. pallipes* in the past, despite this not having been recorded. The development of a molecular test to isolate and sequence BV from both species is required to further explore whether the virus is distinct in both species with strict host specificity, or whether it has been acquired by *P. leniusculus* in its introduced range (Longshaw, 2011).

In previous studies, no gross pathological changes were observed in crayfish infected with BV, suggesting that this pathogen has minimal impact on the host and is not a major driver of crayfish mortality, nor a determinant of competitive success (Stentiford *et al.*, 2004; Longshaw, 2011; Longshaw *et al.*, 2012a; Longshaw *et al.*, 2012c). However, I did observe a higher prevalence of *T. contejeani* in *A. pallipes* populations which were infected with bacilliform virus. The potential for the virus to compromise the immunity of native hosts and increase their susceptibility to other parasites merits further exploration.

5.5.2 *Thelohania contejeani*

Thelohania contejeani is a microsporidian parasite which causes a chronic infection in crayfish, infecting muscle fibres and replacing them with parasite life stages, thus restricting movement and causing the eventual death of the infected host (Alderman and Polglase, 1988; Oidtmann *et al.*, 1996). In addition to causing mortality the parasite affects hosts predatory abilities; it has been associated with a 30% reduction in food intake, reduced prey attack rate and increased prey handling time: sub-lethal impacts which facilitate competitive exclusion and are thought to play an important role in competitive interactions between *A. pallipes* and *P. leniusculus* (Haddaway *et al.*, 2012).

The presence of *T. contejeani* was common among *A. pallipes* populations (present in 46% of populations from the long term dataset) with a maximum prevalence of 37%, consistent with other European studies (Cossins and Bowler, 1974; ROGERS *et al.*, 2003; Dunn *et al.*, 2008; Longshaw *et al.*, 2012c). Despite examining 50 populations (966 individuals) of *P. leniusculus* in the field study and Cefas datasets combined, I did not detect any microsporidian infection in non-native crayfish. The lack of detection was perhaps surprising given that *P. leniusculus* populations which had previously tested positive for microsporidian infection were re-sampled as part of this study (Dunn *et al.*, 2008)*. This finding may suggest that although *T. contejeani* can infect *P. leniusculus* (as detected using PCR (Dunn *et al.*, 2008; Imhoff *et al.*, 2012)), it is a less competent host, so the parasite burden may be lower, preventing an infection from being detected using histopathology. An experiment which indicated that *P. leniusculus* could become infected with *T. contejeani* by consuming the infected tissue of *A. pallipes* (Imhoff *et al.*, 2012) was not reversed (uninfected crayfish were not fed the muscle tissue of infected *P. leniusculus*) which may have determined whether *P. leniusculus* muscle tissue was infected (Imhoff *et al.*, 2012)*. Further experimental studies are required to determine the competence of *P. leniusculus* as a host for this agent.

5.5.3 *Branchiobdella astaci*

Branchiobdellids, ectoparasites which are generally considered to be symbionts, were found in two *A. pallipes* populations. In line with previous suggestions, histology images showed an association between the presence of *B.astaci* and gill melanisation, a localised immune response which may have impaired the functioning of the crayfish gill tissue (Alderman and Polglase, 1988; Rosewarne *et al.*, 2012). However, *B.astaci* has not been associated with crayfish mortality (Longshaw, 2011), nor co-infection, and did not appear to cause any gross pathological signs in *A. pallipes*, suggesting that it would have minimal impact on competitive interactions between native species and INNS.

5.5.4 Environmental parameters

I found little evidence to support a link between environmental quality and the presence, or prevalence of sub-lethal infectious agents. Given the narrow environmental thresholds associated with *A. pallipes* populations – for example, their presence is associated with higher levels of dissolved oxygen and lower levels of ammonia and phosphate (Haddaway *et al.*, 2015) and the fact that they are regarded

as an indicator of good environmental quality, I may have expected crayfish in inferior habitats to be more susceptible to disease.

The environment of the host is considered to be an important determinant of disease dynamics (Strayer, 2010; Johnson and Paull, 2011). A relatively small-scale study of *A. pallipes* in the River Wharfe catchment in Yorkshire, reported a positive correlation between the presence of *T. contejeani* and the level of zinc, lead, and dissolved oxygen in the waterbody (Imhoff, 2010). Similarly, a laboratory experiment with penaeid shrimp revealed that the prevalence of a shrimp-specific bacilliform virus increased from 23% to 75% after 35 days when shrimp were exposed to aquatic pollutants (polychlorinated biphenyls) but only increased from 23% to 46% in the (non-exposed) control group (Couch and Courtney, 1977). In contrast to these previous studies, my results did not reveal a relationship between the presence or prevalence of either *T. contejeani* or bacilliform virus, and the chemical status of the catchment.

I did, however, find a significant relationship between the chemical status of the water body and the prevalence of *A. astaci*, the causative agent of crayfish plague. The prevalence of *A.astaci* was higher in catchments which received 'fail' status for their chemical classification. This may be because the immunity of *A. pallipes* is reduced in more polluted river catchments, or because these catchments provide the optimal conditions for oomycete spores. The motility of *A. astaci* spores is dependent on water temperature, while high magnesium levels and low calcium levels are considered less favourable for spores (Oidtmann, 2000). Alternatively, as external factors such as pollution are considered to reduce the resilience of freshwater ecosystems to invasion (Dudgeon *et al.*, 2006; Strayer, 2010), this pattern may have been the result of polluted catchments being more susceptible to invasion by *P. leniusculus*, the asymptomatic carrier of *A. astaci*, rather than being the result of host immunity, as to the best of my knowledge, *A. pallipes* are not known to have overcome mortality from *A. astaci* (Longshaw, 2011).

The lack of a significant relationship between the quality of the environment and the presence and prevalence of sub-lethal infections may have been the result of a lack of specificity in measure of chemical and environmental quality that I used in the study. The Water Framework Directive catchment classification takes multiple environmental parameters into account to produce an overall "status" which may omit the impacts of particularly important stressors. Moreover, localised pollution events – perhaps only affecting a 100m strength of river where crayfish are present – may have

been missed at the broad spatial scale at which the catchments are assessed. I therefore recommend that more localised studies are conducted in future to explore these findings in higher resolution.

The objectives of this study were to determine whether host species or environment were predictors of infection in UK crayfish. My results have provided evidence in support of enemy release, revealing that in the UK, the invasive non-native *P. leniusculus* is a host to significantly lower diversity and prevalence of infectious agents than native *A. pallipes*. Further work is required to identify the diversity and impact of agents infecting *P. leniusculus* in the original source population, however, the apparent lack of infectious agents in introduced *P. leniusculus* populations could go a long way to explaining their success at replacing *A. pallipes* in the UK. Although I did not find a relationship between environmental quality and the presence or prevalence of sub-lethal infections in crayfish, my results revealed a link between water quality and the presence of *A. astaci*: the causative agent of crayfish plague. As this agent has contributed to widespread mortalities and the local extinction of *A. pallipes* populations in Europe, understanding the environmental factors which might increase its virulence, or trigger a disease outbreak, are crucial to conservation and merit further exploration. In the UK, Cefas collect data on aquatic animal disease outbreaks (i.e. mortality events) however, no regular epidemiological monitoring takes place to examine interactions between environmental quality, the presence of INNS and patterns of aquatic animal disease. As anthropogenic factors continue to degrade freshwater environments, increasing their susceptibility to INNS (Strayer, 2010) and emerging wildlife diseases (Okamura and Feist, 2011b), I believe the establishment of a baseline monitoring programme to better understand interactions between these factors and provide an early warning before outbreaks occur warrants further exploration.

6 THE ROLE OF TOURISM AND RECREATION IN THE INTRODUCTION OF NON-NATIVE SPECIES: A GLOBAL META-ANALYSIS

6.1 Summary

Managing the pathways by which non-native species are introduced and spread is considered the most effective way of controlling species invasions. Tourism and outdoor recreation involve the frequent congregation of people, vehicles and vessels from geographically diverse areas. They are therefore perceived to be major pathways for the movement of non-native species, and ones which will become increasingly important with the continued growth of these sectors. However, a global assessment of the relationship between tourism activities and the introduction of non-native species – particularly in freshwater and marine environments – is lacking. I conducted a global meta-analysis to determine the impact of tourism and outdoor recreation on non-native species in terrestrial, marine and freshwater environments. My results provide quantitative evidence that the abundance (mean effect size (*Hedges g*) = 0.88, p<0.001) and richness of non-native species (mean effect size (*Hedges g*) = 0.95, p<0.001) are significantly higher in sites disturbed by tourist activities than undisturbed sites. The pattern was consistent across terrestrial, freshwater and marine environments; across a variety of vectors (e.g. horses, hikers, yachts); and across a range of taxonomic groups. These results highlight the need for widespread biosecurity interventions to prevent the inadvertent introduction of INNS as the tourism and outdoor recreation sectors grow.

6.2 Introduction

Understanding and managing the pathways by which non-native species are introduced into new regions is considered the most effective way to prevent future biological invasions (Belz *et al.*, 2012; Briski *et al.*, 2012; Caffrey *et al.*, 2014; Chapple *et al.*, 2013; Hulme, 2009; Mack *et al.*, 2000). As such, effective pathway management forms one of the Convention on Biological Diversity's Aichi Biodiversity Targets for 2020 and is a key element of the new EU regulation on the prevention and management of invasive non-native species (European Commission, 2013; Secretariat of the Convention on Biological Diversity, 2004). However, the development of pathway management plans and biosecurity measures must be grounded in evidence about the vectors and mechanisms by which non-native species can be transported (Chapple *et al.*, 2013; Hulme, 2009; Hulme *et al.*, 2008).

Tourism is considered to be a major pathway for the spread of non-native species (Clout and De Poorter, 2005; Convention on Biological Diversity, 2014; Kolar and Lodge, 2001; Meyerson and Reaser, 2002). Not only can the congregation of large numbers of people, vehicles and vessels from geographically diverse areas provide a regular supply of non-native propagules (Lockwood *et al.*, 2005; Tobin *et al.*, 2010), common recreational activities such as hiking, mountain biking and off-road driving can act as forms of habitat disturbance, potentially facilitating species invasion (Jauni *et al.*, 2014; Pickering *et al.*, 2010; Pickering *et al.*, 2007; Tobin *et al.*, 2010). Disturbance occurs when an activity either partially or totally destroys the plant/animal biomass in an area, changing niche opportunities for the species within the habitat (Byers, 2002; Jauni *et al.*, 2014). Non-native species are often particularly successful in disturbed habitats as their superior rates of growth and reproduction enable them to quickly colonise disturbed areas (Barros and Pickering, 2014; Britton-Simmons and Abbott, 2008; Jauni *et al.*, 2014).

Existing research has focused on the role of tourism and outdoor recreation as vectors for non-native species in terrestrial environments, notably protected areas and national parks (for example (Allen *et al.*, 2008; Barros and Pickering, 2014; Cowie and Werner, 1993; Newsome *et al.*, 2008; Pickering *et al.*, 2007). There, as transport vectors are often restricted, recreational activities form one of the few pathways by which non-native species can be introduced (Pickering *et al.*, 2007). Previous studies have revealed that activities such as hiking and horse-riding can act as vectors for the dispersal of non-native seeds as well as pathogens such as *Phytophthora ramorum,* the

causative agent of sudden oak death (Allen *et al.*, 2008; Cushman and Meentemeyer, 2008; Pickering, 2008).

Despite the terrestrial focus in the literature to date, recreational activities can also act as vectors for the introduction of non-native species in aquatic environments (Bax *et al.*, 2003; Davenport and Davenport, 2006; Thurstan *et al.*, 2012). For example, recreational boats have been a major vector for the spread of the zebra mussel *Dreissena polymorpha* and invasive macrophtyes between lakes and rivers within Europe, the USA and New Zealand (Minchin *et al.*, 2003; Rothlisberger *et al.*, 2010). In marine environments, yachts have been responsible for the introducing non-native bivalves, algae, ascidians and bryozoan into ports in Australasia and the Caribbean (Floerl and Inglis, 2003; Thresher, 1999; Willette *et al.*, 2014). Yet to date, there has been no quantitative global review of the impacts of tourism and recreation on the abundance and diversity of non-native species in aquatic systems.

Internationally, tourist arrivals are expected to grow from 1 billion in 2013, to 1.8 billion by 2030 (World Tourism Organization, 2014) and nature-based tourism (i.e. wildlife viewing and outdoor recreation, often centred around protected areas and national parks) is a key growth area (Balmford *et al.*, 2009; Christ *et al.*, 2003; Davenport *et al.*, 2002; Pickering *et al.*, 2007). As nature-based tourism and outdoor recreation (hereafter grouped under 'recreation' for simplicity) often take place in relatively pristine habitats, biodiversity hotspots and in developing countries which rely upon tourist income (Christ *et al.*, 2003), it is vital to better understand the invasion pathway created by tourist activities, so that it can be effectively managed.

Meta-analysis provides a valuable tool with which to quantitatively synthesize the results of multiple studies to identify large scale patterns and facilitate evidence-based conservation management (Stewart, 2010, Haddaway 2015). The aim of this study was to conduct a global meta-analysis to quantitatively determine whether the diversity and abundance of non-native species were higher in sites disturbed by recreation than undisturbed sites in terrestrial, freshwater and marine environments.

6.3 Methods

6.3.1 Search strategy

I performed the literature review following recognised protocols for systematic reviews and meta-analyses (Cooper, 2009; Collaboration for Environmental Evidence, 2013).

In March 2014 I searched for relevant studies using three literature databases: Scopus, ISI Web of Science and Science Direct. The first 100 hits of an internet search performed using Google.com (filetype:pdf) were also checked for relevance. The search was restricted to English language articles but included all publication years. A list of tourist and recreational activities was collated from previous studies of tourism in terrestrial, freshwater and marine environments (McCrone and New Zealand. Department of Conservation, 2001; Pickering and Mount, 2010; Thurstan *et al.*, 2012). Due to inconsistencies in terminology in the literature (Blackburn *et al.*, 2011), non-native species and invasive non-native species were grouped together. The specific search terms were: *("horse riding" OR "mountain biking" OR "bicycle" OR "cyclist" OR "off-road vehicle" OR "4x4 vehicle" OR "all-terrain vehicle" OR "rock climbing" OR mountaineer* OR "scuba div*" OR surf* OR angl* OR boat* OR vessel OR anchor OR canoe OR kayak* OR sail* OR yacht* OR "leisure craft" OR "personal water craft" OR "cruise ship" OR "passenger ship" OR ferry OR camp* OR hik* OR trails OR "walking tracks" OR paths OR safari OR ski* OR snowboard* OR wintersport OR "wildlife watch*" OR "bird watch*" OR visitor OR touris* OR ecotour* OR eco-tour* OR passenger OR travel* OR leisure OR sightsee* OR footwear OR luggage OR clothing OR "tourist transport" OR "tourist vehicle" OR train OR railway OR car OR vehicle OR coach OR bus OR recreation OR aeroplane OR "air transport" OR airport OR plane OR "human vector" OR "human activity" OR (("protected areas" OR "nature reserve" OR "national park" OR "marine reserve" OR "marine park" OR "marine protected area") **AND** (visitor OR user OR tourist))) AND ("invasive species" OR "introduced species" OR "non-native species" OR "alien species" OR "non-indigenous species" OR "exotic species") **AND** ("species richness" OR diversity OR cover OR abundance OR density OR biomass).*

6.3.2 Screening and data-extraction

My original search returned 3088 studies after duplicates were removed. Titles and abstracts were vetted by two reviewers (Lucy Anderson and Steve Rocliffe) and the Kappa statistic was used to evaluate inter-reviewer agreement (Kappa = 0.84: near perfect level of agreement) (Figure 6.1).

I retrieved and reviewed 290 studies in full against inclusion criteria (Table 6.1). Studies could be observational or experimental in nature but had to have the primary goal of quantifying the impact of a tourist or outdoor recreation activity, tourist-specific transport vector or visitors to a tourist destination (such as a national park or island). After screening, 32 studies were included in the meta-analysis, representing

37 effect size data points (species richness n = 18, abundance n = 19, Figure 6.1). Full details of the included studies are provided in Appendix F.

TABLE 6.1 META-ANALYSIS INCLUSION CRITERIA AGAINST WHICH THE SUITABILITY OF 290 STUDIES WAS ASSESSED

Inclusion criteria	Exclusion criteria
1. Primary study including a quantitative comparison of abundance (e.g. biomass, density, percentage cover, total abundance) and/or species richness (total number of species, mean number of species, proportion of species or Simpson's diversity index) of non-native species in a site affected by a tourist activity, and a comparable control site.	1. Experiment not replicated (only one treatment and one control site).
	2. No control site, or insufficient information provided about the characteristics of the control site to assess its suitability.
	3. Study does not report confidence intervals or sample sizes.
2. Study provides exact P value or a statistical result (Z, F, t, r, r^2 or X^2) accompanied by the sample size or degrees of freedom. Alternatively, study can provide raw data on the mean abundance/species richness in the treatment and control sites with associated sample sizes.	4. Treatment and control sites spatially confounded.
	5. Study includes evidence of intentional non-native species introduction which may confound results. For example through seeding (ski-resorts) or stocking (angling lakes).
	6. Study of road/vehicles, railways or boats where it is unclear whether the primary vehicles/vessels are industrial (e.g. cargo ships, goods trains, works vehicles) or strictly tourist related (yachts, recreational boats, tourist cruise ships etc.)

I coded each study according to sample size; sample selection (purposive, randomised, blocked, not stated, other); spatial scale (<2 ha, 2-10ha, >10ha); temporal scale (<2 year since tourist activity began; 2-5 years or >5 years); the activity/vector in focus; habitat type (freshwater/marine/terrestrial); study taxa; study design (Before-After (BA), Control-Impact (CI), Before-After-Control-Impact (BACI), other); and whether the study was observational or experimental. I also collected abundance data (biomass, density, percentage cover, total abundance) and species richness data (total number of species, mean number of species, proportion of species or Simpson's diversity index). For studies where abundance/species richness data were separated across spatial or temporal scales, I took the mean value, weighted by the sample size at each spatial/temporal scale (Shackelford *et al.*, 2013). Where results were presented graphically, I extracted the mean and variation (e.g. Standard error or 95% confidence intervals) from the figure using *ImageJ* (Rasband, 2014). To avoid pseudoreplication,

each study could only contribute one abundance and/or one diversity effect size to the meta-analysis.
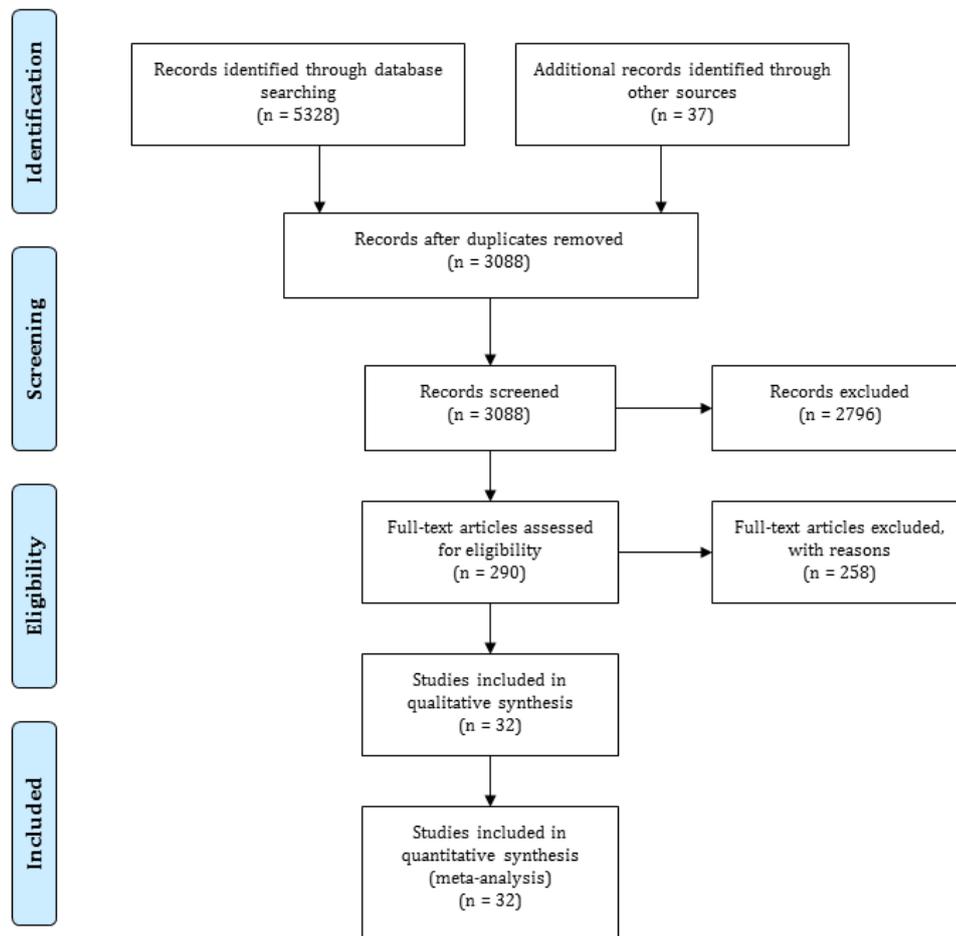
FIGURE 6.1 FLOW DIAGRAM DEPICTING STAGES OF THE LITERATURE SEARCH.

6.3.3 Calculating effect sizes

For each study, I calculated the effect size between the abundance/species richness of non-native species in control sites and sites experiencing recreation using the R package *compute.es* (Del Re, 2013). A value of 0.001 was added to raw abundance and species richness figures in order to calculate the effect size of studies where non-native species were not found in the control site (Molloy *et al.*, 2009). I used *Hedges g* as a weighted and standardised effect size metric (Hedges and Olkin, 1985). Positive *g* values indicate that non-native species richness or abundance was higher in sites with recreational activity than in undisturbed sites. A value of *g* greater than or equal to 0.8

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can be interpreted as a large effect size; 0.5 a moderate effect size; and 0.2 is a small effect size (Cohen, 1988).

6.3.4 Statistical models

Using the *metafor* package in R (R Development Core Team, 2012; Viechtbauer, 2010), I created random effects models to calculate the grand-mean effect size across all non-native species abundance studies, and all non-native species richness studies. Random effects models are considered appropriate for ecological studies because they allow effect size estimates to vary both due to sampling error and as a result of real ecological differences between studies (Bancroft *et al.*, 2007). Due to the small sample size, I calculated bias-corrected 95% confidence intervals around the two mean effect sizes by bootstrapping 10,000 iterations using the *boot* package in R (Canty and Ripley, 2014) . The grand mean effect size was considered to be significantly different from zero if the confidence intervals did not overlap zero.

6.3.5 Factors explaining the heterogeneity in effect size

The total heterogeneity statistic (Q) was used to determine whether the heterogeneity in grand mean effect sizes was significantly greater than what would be expected from sampling error alone (Cooper, 2009; Hedges and Olkin, 1985). Where the Q statistic was significant, sub-group analyses were conducted using mixed effects models (study ID included as a random factor) to determine whether ecosystem (terrestrial/aquatic), taxa, study type (observational/experimental) or vector type could explain the variation in effect sizes. Parametric 95% confidence intervals (suitable for sample sizes of n<10 (Bancroft *et al.*, 2007)) were calculated around each subgroup mean to determine whether the mean effect size had a significant effect on non-native species richness/abundance.

6.3.6 Assessment of publication bias

I used a number of standard methods to check for publication bias. A visual assessment of effect size plotted against the standard normal distribution (normal quantile plot) revealed that all data points fell within 95 % confidence intervals (Appendix E). Failsafe tests revealed that it would take an additional 1016 abundance studies and additional 1887 species richness studies with effect sizes of zero to change the result of the meta-analyses from significant to non-significant (Rosenberg, 2005). Finally, rank correlation tests were non-significant for abundance (Kendall's tau =

0.05, p = 0.75) and species richness (Kendall's tau = 0.07, p=0.67) indicating that there were no significant correlations between effect size and variance. I am therefore confident that my meta-analyses were not affected by publication bias.

6.4 Results

The studies included in the meta-analysis had a broad geographic distribution. The majority were from North America (n=13), Australasia (n = 6) and Europe (n=4) however studies from Africa, Asia, South America and Antarctica were also included (Appendix F). They comprised 22 terrestrial studies, eight marine studies and two freshwater studies. Due to the small sample size of freshwater studies, I combined freshwater and marine studies into an "aquatic" category. The activities covered in the meta-analysis included visits to national parks, hiking, horse-riding, recreational boating, yachting and the recreational harvesting of shellfish.

The large positive effect sizes obtained from the meta-analyses indicate that both the abundance (mean effect size (g) = 0.88, p<0.001) and species richness (mean effect size (g) = 0.95, p<0.001) of non-native species were significantly higher in sites that were disturbed by recreational activities than in undisturbed sites (Figure 6.2). The pattern was repeated in both terrestrial and aquatic environments, across multiple non-native taxa (including terrestrial and aquatic plants, invertebrates and fungal pathogens) and a suite of different vectors (Figure 6.2).

In both the non-native species richness and abundance meta-analyses, the Q statistic was significant (Table 6.2), indicating that the heterogeneity in effect sizes between studies was higher than would be expected by sampling error alone (Bancroft *et al.*, 2007; Cooper, 2009)*. However,* none of the subgroups (vector type, habitat type, study type (observational/experimental) or aquatic vs. terrestrial) were significant predictors of between-group heterogeneity (Table 6.2), suggesting that the impact of recreation was similar across all vectors/habitats. Due to high levels of correlation between three subgroup categories: study type (observational/experimental); spatial scale; and temporal scale, I only included the subgroup *study type* in my analyses.

TABLE 6.2 TOTAL HETEROGENEITY (QT) AND BETWEEN-GROUP HETEROGENEITY (QB) OF EFFECT SIZES IN STUDIES COMPARING THE ABUNDANCE AND DIVERSITY OF NON-NATIVE SPECIES BETWEEN SITES DISTURBED BY RECREATIONAL ACTIVITIES AND UNDISTURBED CONTROL SITES.

Explanatory variable	Non-native abundance				Non-native species richness			
	QT	QB	df	p	QT	QB	df	p
	99.26		18	<0.0001	104.33		18	<0.0001
Vector type		1.37	3	0.71		3.97	2	0.13
Aquatic vs. terrestrial		1.07	1	0.30		0.88	2	0.35
Ecoregion (Ter/Mar/Fw)		2.85	2	0.24		1.37	2	0.50
Study type		0.23	1	0.62	NA (only observational studies)			

Note: As there was a significant correlation between study type, study area and duration of study, only study type was analysed in the subgroup analysis.

Forest plots of the mean effect size and bootstrapped confidence intervals for each subgroup suggested that recreational activities in aquatic systems had moderately larger positive effect on the abundance of non-native species than recreational activities in terrestrial systems (Figure 6.2a), however these differences were not significant (Table 6.2), nor were the differences in effect size between different vector types (Figure 6.2a and Table 6.2).

In contrast, recreational activities in terrestrial systems appeared to have a moderately larger positive effect on the richness of non-native species than recreational activities in aquatic systems (Figure 6.2b). Both boating and hiking had significant positive effects on non-native species richness (Figure 6.2b). Horse-riding did not have a significant effect on non-native species richness (Figure 6.2b), however only one study was included due to a paucity of suitable horse-riding studies so this result should be treated with caution.

A) NON NATIVE SPECIES RICHNESS

Subgroup	Hedges g (95% CI)	N
Full model	0.951 (0.639 to 1.263)	18
Aquatic	0.557 (0.152 to 1.599)	3
Terrestrial	0.769 (0.225 to 1.105)	15
Horses	-0.17 (-0.99 to 0.64)	1
Boats	0.557 (0.152 to 1.599)	3
Visitors/trails	0.810 (0.837 to 1.130)	14
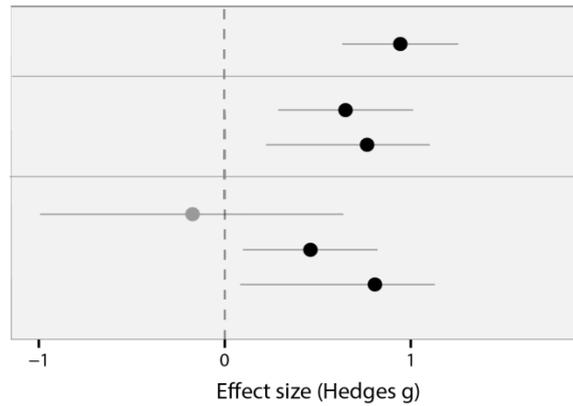
B) NON NATIVE SPECIES ABUNDANCE

Subgroup	Hedges g (95% CI)	N
Full model	0.879 (0.139 to 1.324)	19
Aquatic	1.103 (0.152 to 1.599)	8
Terrestrial	0.769 (0.090 to 0.974)	11
Horses	0.556 (0.929 to 0.813)	2
Visitors/trails	0.810 (0.837 to 1.130)	9
Boats	1.107 (0.152 to 1.599)	7
Observational	0.839 (0.191 to 1.997)	15
Experimental	0.961 (0.697 to 1.225)	4
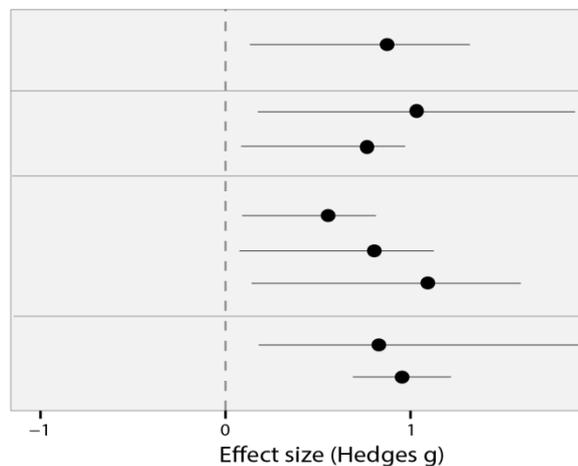
FIGURE 6.2 FOREST PLOTS SHOWING THE EFFECT OF RECREATIONAL ACTIVITIES ON A) NON-NATIVE SPECIES RICHNESS AND B) NON-NATIVE SPECIES ABUNDANCE. EFFECT SIZE VALUES >0 SHOW THAT THE SPECIES RICHNESS OR ABUNDANCE OF NON-NATIVE SPECIES WAS GREATER IN SITES WHERE RECREATIONAL ACTIVITIES TOOK PLACE. THE MEAN EFFECT SIZE AND 95% CONFIDENCE INTERVAL IS SHOWN FOR THE OVERALL RESULT AND EACH SUB-GROUP ANALYSIS. BIAS-CORRECTED CONFIDENCE INTERVALS WERE BOOTSTRAPPED FOR GROUPS N<10 AND PARAMETRIC CONFIDENCE INTERVALS FOR GROUPS N≥10. CONFIDENCE INTERVALS THAT OVERLAP THE DASHED LINE AT ZERO ARE NOT SIGNIFICANTLY DIFFERENT FROM ZERO.

6.5 Discussion

Effective INNS management requires an understanding of the relative importance of different pathways of spread (Hulme, 2009). My results provide quantitative evidence in support of the hypothesis that tourism and recreation are pathways for the spread

of non-native species across the globe (Clout and De Poorter, 2005; Kolar and Lodge, 2001; Meyerson and Reaser, 2002). The results of the meta-analysis demonstrate that the abundance and richness of non-native species are significantly higher in sites disturbed by recreational activities than in undisturbed sites, and that this pattern is consistent across multiple non-native taxa, in both terrestrial and aquatic habitats, and across a suite of different vectors.

6.5.1 Terrestrial environments

The literature search revealed that the majority of empirical studies conducted to investigate the impacts of recreational activities on INNS are terrestrial in focus (15/18 studies of non-native species richness and 11/19 studies of non-native species abundance). The meta-analysis revealed that there was a significantly higher abundance and diversity of non-native species in terrestrial sites with recreational activities than undisturbed terrestrial sites (Figure 6.2). These results were in accord with previous studies. For example, a review of 18 vegetation surveys in Kosciuszko National Park, Australia, revealed that 48 non-native species had been reported in the park's natural vegetation compared to 152 in areas disturbed by tourist activities (Pickering *et al.*, 2007). In addition, a long term study of visitors to US National Parks showed that there were significantly higher numbers of non-native species in parks with higher visitor numbers (Allen *et al.*, 2008), a pattern that was reflected in forests with/without visitor access in Poland (Sikorski *et al.*, 2013). However, unlike many previous studies this meta-analysis incorporated tropical and temperate habitats and both continental and island studies.

I did not find sufficient evidence that horse-riding has a significant effect on the diversity of non-native species (Figure 6.2), however previous studies have reported that over 100 species of invasive non-native plant can germinate from horse dung, and that disturbance from trampling facilitates germination (Ansong and Pickering, 2013) so the lack of a positive result may have been due to a small sample size. Although only two studies could be included, horse-riding did appear to have a significant effect on the abundance of non-native species, with significantly more reported in sites where horse-riding took place, than in undisturbed sites. I believe further control-impact studies are required to fully understand the impact of this vector.

Unlike the aquatic studies included in the meta-analysis which incorporated a wide range of taxa, the majority of terrestrial studies (21/22) focused on non-native plants. The only non-plant study (Cushman and Meentemeyer, 2008) showed that the

prevalence of the fungal pathogen *Phytophthora ramorum* was higher on trails than in undisturbed vegetation in a Californian National Park. The impact that terrestrial recreational activities are having on other types of non-native taxa (such as other pathogens and invertebrates) demands further attention.

6.5.2 Marine and freshwater (aquatic) environments

My study is the first quantitative global analysis of the relationship between recreational activities and non-native species in marine and freshwater environments. In accord with findings on terrestrial activities, the meta-analysis revealed that the abundance and diversity of aquatic non-native species – including seagrasses, seaweeds, macrophtyes, molluscs, amphipods and bryozoans – were significantly higher in aquatic environments where recreational boating or yachting took place, than in undisturbed sites. No significant differences in effect size were found between terrestrial and aquatic environments, suggesting that the impacts of recreational activities are equally important, and require management interventions of a similar magnitude.

Recreational boating and angling are receiving growing recognition as vectors for non-native species (Anderson *et al.*, 2014a; Bacela-Spychalska *et al.*, 2013; Kilian *et al.*, 2012; Rothlisberger *et al.*, 2010) and are thought to have been responsible for over a third of non-native species introductions into Europe (Gallardo and Aldridge, 2013b). Examples include the introduction of the zebra mussel *Dreissena polymorpha* from England to Ireland via the hulls of recreational boats (Minchin *et al.*, 2003) with subsequent impacts on the fisheries, water treatment works and aquatic transport industries (Kelly *et al.*, 2013a); and the introduction of the Ponto-Caspian gammarid shrimp *Dikerogammarus villosus* into watersports lakes in the UK and European Alps (Bacela-Spychalska *et al.*, 2013; Gallardo *et al.*, 2012). In New Zealand, the distribution of the invasive diatom didymo (*Didymosphenia geminata*) is consistent with angler-mediated introduction and dispersal (Kilroy and Unwin, 2011). The invasion was first reported in 2004 and had cost the New Zealand government over NZD $127.8 million to manage by 2011 (Deloitte, 2011).

In marine environments, the introduction of non-native species through aquatic activities could potentially compromise the conservation value of marine reserves. Much like their terrestrial counterparts, marine reserves can attract high rates of visitation by tourists, leading to a congregation of potential transport vectors including boat anchors, SCUBA equipment, boat ballast and bilge water and fouled

hulls (Burfeind *et al.*, 2013). The reduced levels of harvesting within marine reserves may also ironically allow non-native species that are inadvertently introduced to become more abundant (Byers, 2005), however others argue that marine reserves are associated with greater native species richness (Lester *et al.*, 2009)and are therefore more resilient to biological invasions (Stachowicz *et al.*, 2002)

In addition to marine reserves, long distance yachts could be one of few vectors capable of introducing INNS to the marine environment surrounding oceanic islands (Floerl and Inglis, 2003), ecosystems in which species invasions are considered the most acute threat to biodiversity loss (Mack, 2000; Wilcove *et al.*, 1998). Yachts are thought to be responsible for the introduction of five non-native species including sponges, a macroalga, a bryozoan and a hydroid to Palmyra, an unoccupied North Pacific atoll (Knapp *et al.*, 2011) as well as introducing *Halophila stipulacea*, a non-native seagrass, to many islands across the Caribbean (Willette *et al.*, 2014).

6.5.3 The relationship between study design and effect size

Forest plots revealed that the mean effect of recreation on non-native species abundance was higher in experimental studies than observational studies, although both were significant. Experimental studies typically occurred at smaller spatial and temporal scales than observational studies (< 1ha in area and <1 year in duration). The larger effect size of experimental studies may be explained by experimental simulations being more intense or happening more suddenly than would take place in natural conditions (Britton-Simmons and Abbott, 2008), whereas effects of non-native species in observational studies may have been diluted by space and time.

6.5.4 Additional impacts of tourism

I focused this study on tourist and recreational activities associated with unintentional non-native species spread. However, tourist infrastructure – for example the building of footpaths and lodges, and the planting of exotic species in hotel gardens and ski resorts – have also been associated with the intentional introduction of non-native species. For example, tourist development was identified as the main determinant of non-native plant abundance and diversity in study of 37 Mediterranean Islands (Pretto *et al.*, 2012). Similarly, 152 of the 156 non-native plants recorded in Kosciusko National Park in Australia were associated with tourist infrastructure including ski resorts and hotel gardens (Pickering *et al.*, 2007), and the seeding of ski runs in the conversion of alpine habitats to ski resorts is also a source of non-native species

spread to neighbouring areas (Titus *et al.*, 2003; Titus and Tsuyuzaki, 1999). A review of the links between tourist infrastructure and the abundance/diversity of non-native species would add valuable further insight into the link between tourism and non-native species.

6.5.5 Issues relating to study design

A major obstacle encountered during this study was the paucity of studies which provided sufficient information to calculate an effect size. For example, 24% of the studies which reached the final stage of assessment (n=69) failed to meet the inclusion criteria based on the lack of a control site in their experimental design. Not only did this lead to an unintentional dominance of plant-based studies in the meta-analyses, it revealed a wider issue in ecological study design. As research techniques in applied ecology begin to follow the rigorous systematic methods which have been adopted in healthcare science, I implore ecologists to adopt balanced study designs from which effect sizes can be calculated (Pullin *et al.*, 2004; Pullin and Knight, 2001; Stewart, 2010; Sutherland *et al.*, 2004). This will facilitate the use of meta-analysis in ecology as an evidence-based conservation management tool (Stewart, 2010; Haddaway, 2015).

6.5.6 Management implications

As nature-based tourism and outdoor recreation continue to grow in popularity (Balmford *et al.*, 2009), the transport of non-native species to remote habitats such as oceanic islands, polar regions (previously considered so remote they were 'immune' to invasions (Mack, 2000)) and biodiversity hotspots, could have catastrophic consequences. This is because the endemic flora and fauna living in these environments have often evolved in isolation and are therefore less resilient to novel threats, such as non-native species and the pathogens that may accompany them (Bataille *et al.*, 2009; Cox and Lima, 2006). Moreover, more than half of the world's poorest countries fall within biodiversity hotspots and rely on nature-tourism income (Christ *et al.*, 2003). The introduction and subsequent impacts of non-native species into these areas could therefore have serious economic, as well as ecological, ramifications (Balmford *et al.*, 2009; Christ *et al.*, 2003). However, the tourist income generated in these areas could also provide a source of revenue to fund management initiatives to prevent and mitigate the impacts of INNS (Steven *et al.*, 2013). For example, tourism funds up to 64% of global conservation measures for some bird species, measures which include the removal of INNS from critical habitat (Steven *et*

al., 2013). Such investment can in turn increase potential for eco-tourism in the long term (Glen *et al.*, 2013).

Reducing unintentional introductions through the tourism pathway will require effective prediction, surveillance, awareness-raising and control (Tatem, 2009), and will rely on international cooperation (Clout and De Poorter, 2005). Awareness raising initiatives have already been developed to improve the biosecurity practices of recreational water users (Anderson *et al.*, 2014a), hikers (Kauri Dieback Programme Partners, 2014) and airline passengers (Wittenberg and Cock, 2001) and have resulted in compliance by 71% of water users in New Zealand (Chapter 4). Minimum impact codes of conduct for visitors to national parks have been proposed as a way of reducing non-native plant introduction by hikers and horse riders (Ansong and Pickering, 2013; Newsome *et al.*, 2008) as well as visitors taking part in recreational activities (e.g. motor boating, diving, snorkelling) in marine reserves (Thurstan *et al.*, 2012). Similarly, inspections of tourists' footwear and luggage on arrival to pristine sites such as Antarctica have been suggested as a way to substantially reduced propagule loads (Lee and Chown, 2009). However, many of these initiatives will need to be reinforced by legislation in order to be adopted (Hulme *et al.*, 2008). On a larger scale, disinfection protocols have been implemented for inter-island aeroplanes and boats in the Galapagos Islands where the mosquito *Culex quinquefasciatus* had frequently been transported in aircraft (Bataille *et al.*, 2009; Causton and Peck, 2005).

Invasive non-native species are a major threat to global biodiversity. In order to meet international conservation commitments, countries are obliged to identify and manage pathways for the spread of invasive non-native species (Secretariat of the Convention on Biological Diversity, 2011). This meta-analysis has demonstrated that tourism and recreation can be significant pathways for the introduction of non-native species across all ecosystems. As the nature-tourism and outdoor recreation sector continue to grow (Balmford *et al.*, 2009), so too will the need for effective and long-lasting biosecurity interventions.

7 GENERAL DISCUSSION

In January 2015, a new EU regulation on the Prevention and Management of the Introduction and Spread of Invasive Alien Species (European Commission, 2013) came into force. The regulation – which is expected to profoundly improve conservation initiatives within the EU (Beninde *et al.*, 2014) – states that "*prevention is more environmentally desirable and cost-effective than reacting* [to introduced INNS] *and should be prioritised*" (European Commission, 2013). Managing the pathways by which INNS may be introduced and spread will be fundamental to preventing their introduction as part of the new regulation (Beninde *et al.*, 2014). Such pathway management requires an understanding of the movement patterns of potential vectors, the effectiveness of INNS control measures (i.e. biosecurity), and compliance with such control measures (Hulme *et al.*, 2008). Within this PhD, I have combined social and ecological research to identify human-mediated pathways for the spread of INNS in freshwater environments (Chapter 2, 4 and 6), and to a lesser extent in marine and terrestrial environments (Chapter 6). I have also identified effective control measures (Chapter 3), and gained an understanding of the most effective ways to disseminate biosecurity awareness information and increase biosecurity compliance among high-risk groups, using New Zealand as a best-practice example (Chapter 4). My findings provide an evidence base from which to develop a freshwater biosecurity strategy to reduce the accidental introduction and spread of freshwater INNS in the UK, and could also be applied to wider Europe.

The discussion which follows will summarise the findings of each data chapter and discuss them in the context of the new EU legislation, providing specific recommendations from which to develop – and improve – freshwater biosecurity in the UK.

7.1 Recreational water users as a pathway for the introduction and spread of INNS

In Chapter two I conducted the first UK study to explore the potential biosecurity risk posed by recreational water users as vectors for the introduction and spread of

freshwater INNS. Using a questionnaire approach, I gained quantitative information about the movement patterns of UK anglers and canoeists as well as their awareness of, and compliance with, biosecurity; elements which are fundamental to the development of pathway management plans (Hulme *et al.*, 2008; Hulme, 2009). The results revealed that 64% of UK anglers and 78.5% of UK canoeists use their equipment/boat in more than one catchment within a fortnight, and 12% of anglers and 50% of canoeists do so without taking any actions to clean or dry their equipment between uses. Moreover, 8% of anglers and 28% of canoeists used their equipment overseas – including parts of Europe where high-risk pathogens, such as the invasive non-native salmon ecto-parasite *G. salaris* are present (Sviland *et al.*, 2012) – without cleaning or drying it upon their return.

The survival experiment I conducted in Chapter 3 demonstrated that seven out of eight high-impact INNS threatening the UK (including floating pennywort, parrot's feather, New Zealand stonecrop, curly water thyme, zebra mussels and killer shrimp) can survive in damp conditions (e.g. the fold of an angler's keep net or wader) for at least 16 days. This is considerably longer than many of these species were previously thought able to survive for (Table 2.1), and is a period of time within which UK anglers and canoeists visit multiple catchments (Chapter 2). Although I recognise that not all anglers and canoeists will come into contact with INNS during their activities, the combined results of Chapters 2 and 3 indicate that in the absence of biosecurity, recreational water users have the potential to act as vectors for the overland dispersal of INNS should they use their equipment where INNS are present. The fact that the killer shrimp has been reported in a number of watersports lakes in the UK and Europe (Madgwick and Aldridge, 2011; Bacela-Spychalska *et al.*, 2012), and the quagga mussel – considered the most high-risk freshwater INNS threat to the UK (Roy *et al.*, 2014) – was recently discovered in the UK for the first time in Wraysbury Reservoir, London, a popular watersports venue (GB Non Native Species Secretariat, 2014b) demonstrate the potential significance of this vector.

Having explored the potential for INNS to be transported via freshwater recreational activities in Chapters 2 and 3, I investigated whether INNS were establishing in sites where recreational activity takes place in Chapter 6. To do this, I conducted a global meta-analysis to test whether the diversity and abundance of non-native species were higher in sites where tourism and recreation took place than in undisturbed sites in terrestrial, freshwater and marine environments.

The meta-analysis revealed that the abundance (mean effect size (*Hedges g*) = 0.88, p<0.001) and richness (mean effect size (*Hedges g*) = 0.95, p<0.001) of non-native species were significantly higher in sites disturbed by tourism/recreational activities than in undisturbed sites, and that this pattern was consistent across multiple non-native taxa, in both terrestrial and aquatic habitats, and across a suite of tourist-related vectors. Although this PhD has focused primarily on the impacts of recreational activities in freshwater environments, the results of Chapter 6 suggest that recreational activities are also important pathways for INNS in terrestrial and marine environments, and require management interventions of a similar magnitude. These results highlight the need to develop biosecurity awareness initiatives in all habitat types to reduce the likelihood of non-native stowaways being transported via recreational activity (e.g. horse-riding, hiking, nature-tourism and yachting). However, for the rest of this discussion, I will focus on freshwater environments in particular.

Key recommendation:

- The development of a *Check Clean Dry* communications strategy to raise the level of biosecurity awareness among recreational water users in the UK.

7.2 Identifying effective biosecurity control measures

In Chapter 3, I conducted a survival experiment to examine the effectiveness of different biosecurity treatments (submersion in hot water; drying; submersion in hot water AND drying; control) at killing freshwater INNS on damp angling nets. The aim of the work was to identify a biosecurity treatment that was safe, effective and environmentally sound that could be recommended to UK water users as part of the national *Check Clean Dry* campaign. As well as revealing how long un-treated INNS can survive for (see 7.1), the results revealed that hot water (45°C for 15 minutes) is a very effective biosecurity treatment. Exposure to hot water (45°C for 15 minutes) had caused 99% mortality among the invasive non-native animals and plants used in the experiment one hour after treatment. This not only demonstrates the immediacy of its action, but its efficacy across a range of taxa. In contrast to hot water, drying took a mean of 7.52 days to cause 90% mortality. This is a particular concern because 80% of UK anglers and 32% of UK canoeists rely on drying as a means of decontamination (Chapter 2). Not only is drying a highly subjective biosecurity treatment (what people consider to be 'dry' may vary), the survival experiment demonstrated that drying may

be an ineffective biosecurity treatment for water users who take part in their activities more frequently than once a fortnight.

The inconsistent use of biosecurity messages can lead to confusion, and a lack of compliance, among water users, as demonstrated in Chapter 4 (see also Caffrey *et al.*, 2014). The results of Chapter 3 indicate that the simple use of hot water (45°C for 15 minutes) during the 'Clean' stage of the UK's *Check Clean Dry* biosecurity awareness campaign would greatly enhance biosecurity efforts among water users in the UK. Although further research must be conducted to test the effectiveness of hot water as a 'catch all' treatment to kill aquatic pathogens in addition to INNS, hot water fulfils the criteria of being an effective, safe and environmentally sound biosecurity solution (Beyer *et al.*, 2010; Perepelizin and Boltovskoy, 2011; O'Neill and MacNeill, 1991; Stebbing *et al.*, 2011).

As well as comprising a PhD Chapter, my hot water study also formed a report for Defra (Anderson *et al.*, 2014b) and has been presented the at a number of biosecurity workshops in the UK (including regional workshops for the London Invasive Species Initiative (LISI) and Yorkshire Dales Environment Network (YDEN) as well as the Environment Agency national quagga mussel workshop and the Defra/GB NNSS national *Check Clean Dry* workshop). The use of hot water as a non-toxic, economical and effective biosecurity treatment has received considerable interest from government bodies, environmental management organisations and NGOs, including the GB Non Native Species Secretariat, and the Environment Agency, which now plans to install hot water tanks in its regional offices to decontaminate field equipment (pers. comm. Trevor Renals). As a result of this study, the use of hot water has also been incorporated into national biosecurity advice for use by anglers and water craft users in response to the discovery of quagga mussels in the UK (GB Non Native Species Secretariat, 2014b).

I recognise that the use of hot water as a decontamination treatment for boats may prove more difficult, logistically, and requires further research. However, researchers in the USA are assessing the feasibility of lifting boats into a hot water chamber to prevent upstream movement of zebra mussels, quagga mussels and spiny water fleas in the Fox River, Wisconsin, USA (Beyer *et al.*, 2010) suggesting that the large-scale use of hot water is not unrealistic.

Key recommendations:

- The continued and consistent promotion of hot water (45°C for 15 minutes) as an effective biosecurity treatment for use by recreational water users at the 'Clean' stage of *Check Clean Dry*.

- Further research into the feasibility of using hot water for boats, and as a treatment for aquatic parasites/pathogens.

7.3 Raising biosecurity awareness among water users

New Zealand is widely recognised as a leading example of aquatic biosecurity best practice (Caffrey *et al.*, 2014; Chapple *et al.*, 2013; Meyerson and Reaser, 2002). The country's comprehensive biosecurity strategies are coordinated by a dedicated team in government (Ministry of Primary Industries (MPI)) and supported by unified legislation: the Biosecurity Act 1993. In Chapter 4, which also formed a report for Defra (Anderson *et al.*, 2014c), I conducted questionnaires with water users (boaters and anglers) in New Zealand to compare their biosecurity awareness and compliance with UK water users and to identify what motivated them to/deterred them from taking biosecurity actions. I also conducted semi-structured interviews with key stakeholders to explore the successes and challenges of New Zealand's long running biosecurity programme in order to develop best-practice guidelines for the delivery of similar campaigns in the UK and wider Europe. My results revealed that a significantly higher proportion of New Zealand water users (71%) were aware of the country's biosecurity campaign, compared to water users in the UK (22%, Chapter 2). Moreover, 61% of New Zealand water users checked their boat/equipment after use, 57% cleaned and 50% dried their boat/equipment after every use. In both New Zealand and the UK (Chapters 2 and 4), awareness of a biosecurity campaign was a significant predictor of biosecurity compliance.

As a result of my questionnaire research, I identified that the most cost-effective communication channel for sharing biosecurity information in New Zealand was signage at boat ramps (Chapter 4). I therefore recommend that the UK's *Check Clean Dry* message is disseminated at the access points of high risk water bodies in the UK. Chapters 2 and 6 also highlighted to need to distribute biosecurity awareness materials at the UK border (airports and seaports) to prevent the introduction of stowaways on (terrestrial, marine and freshwater (Chapter 6)) recreational equipment which has been used overseas. The congregation of vectors at national or international angling and watersports events was also identified as a major

biosecurity threat (Chapter 4). Consistent awareness messaging should therefore be used at all stages of the biosecurity continuum to reinforce water users' awareness (Teillac-Deschamps *et al.*, 2009; Caffrey *et al.*, 2014).

Key recommendations:

- The promotion of simple biosecurity advice which remains consistent across the biosecurity continuum (i.e. at the border, at events, at watersports/angling sites).

- The use of multiple communications channels to disseminate biosecurity advice, with a focus on signs at the entry/exit points of waterbodies.

7.4 Improving biosecurity compliance among individuals and organisations

As a result of the questionnaires and stakeholder interviews I conducted in Chapter 4, I discovered that organisations and individuals in New Zealand were equally motivated to comply with biosecurity by the threat of a fine (a 'stick' approach) – something which possible under may become feasible in the EU under new INNS legislation – and by the provision of cleaning stations at lake entry/exit points (a 'carrot' approach).

The new EU INNS legislation may provide an opportunity to penalise individuals and organisations who do not comply with INNS regulations (Beninde *et al.*, 2014; European Commission, 2013). Enforcement has proved even more effective than education in some instances. For example, a study in Colorado, USA, experimentally compared the effectiveness of enforcement (the distribution of written violation notices) and education (volunteers visited households to distribute educational materials and engage with householders) at reducing human-bear conflict (via changes to garbage management) in residential areas (Baruch-Mordo *et al.*, 2011). Despite education being the preferred and widespread management tool used in the country, the study showed that enforcement was more effective at enacting behaviour change.

Regional councils in New Zealand have the authority to impose fines through the Biosecurity Act 1993, acting as a useful deterrent. However fines were rarely distributed in practice because of the logistical difficulty and expense of establishing inspection patrols (Chapter 4, Baruch-Mordo *et al.*, 2011; Keane *et al.*, 2008). The new EU legislation advocates the "polluter pays" principle, whereby individuals will be held

accountable for the negligent introduction or spread of species (Beninde *et al.*, 2014). However, further research will be required to ascertain how such a principle could be enforced in practice.

In New Zealand, watersports organisations were motivated to take biosecurity actions once they understood the potential threat that INNS posed to their livelihoods (Chapter 4). Exchange visits (where business owners visited regions affected by INNS) proved a particularly useful initiative to raise awareness of the potential business impacts of INNS. The results from New Zealand reflect previous research which has indicated that an increased understanding of INNS impacts improves levels of advocacy with INNS management interventions (Bremner and Park, 2007; García-Llorente *et al.*, 2011).

Key recommendations:

- The provision of decontamination stations at high risk sites and watersports events.

- Public engagement initiatives to increase understanding of the need for biosecurity among high-risk groups.

- The promotion of stakeholder exchange visits and the development of eco-certification schemes to encourage best practice amongst water sports and tourism operators.

- The potential use of fines to deter non-compliance with biosecurity.

7.5 Coordinating a national biosecurity strategy

In New Zealand, biosecurity is coordinated nationally, but implemented by local partnerships in each region of the country (Chapter 4). The Ministry of Primary Industries (MPI) establishes national priorities, coordinates a network of biosecurity managers in each region for regular knowledge exchange, and provides an annual sum of NZD $20,000 to each region to assist with biosecurity engagement. In turn, each regional biosecurity manager develops a strategy for the region outlining the objectives for that region, and the roles and responsibilities of each partner organisation, something that has reportedly improved cohesion and motivation, according to the stakeholders interviewed in Chapter 4. Such locally-led approaches enhance communication between parties, encourage participatory decision making, and promote conflict resolution between stakeholders, fostering a collaborative

approach to INNS management (Chapter 4, Sharp et al., 2011). I therefore recommend that a similar hierarchy of regional and national biosecurity managers is established in the UK. As well as coordinating regional biosecurity awareness raising initiatives to prevent the introduction and spread of INNS, the establishment of a network of biosecurity managers would facilitate knowledge exchange and ensure that an infrastructure is in place to quickly deal with new INNS threats. In addition to a UK-wide network, a biosecurity knowledge-exchange network could potentially be established across Europe, however the cross-border nature of European invasions may make such a network more complex to set up (Sambrook *et al.*, 2014). In order to manage INNS in the EU, it will however be critical that member states with shared borders collaborate on such initiatives (Caffrey *et al.*, 2014).

7.5.1 Prioritising management efforts using pathway risk assessments

Pathway risk assessments enable biosecurity interventions (such as cleaning stations, and awareness signage) as well as surveillance efforts to be targeted to the most "high risk" sites. By identifying i) the importance of different recreational vectors, ii) the spatial dynamics of vectors, iii) the environmental suitability of the region and iv) the connectivity of different nodes in the transport network, risk maps can be generated to identify the key hotspots which act as sinks for, or sources of INNS (Hulme, 2009). This approach has already been used to identify hotspots for the introduction of marine INNS in the UK and Ireland and to predict the potential for internal spread of the sea squirt (*Didemnum vexillum)* as a case study (Pearce *et al.*, 2012).

Whilst not within the scope of this PhD, in future, I recommend that a pathway risk assessment is conducted in the UK freshwater environment in order to prioritise biosecurity efforts as part of the new EU legislation. This PhD has provided sufficient data with which to parameterise such a model. Specifically, it has provided data on the movement patterns and current biosecurity actions of different categories of angler and canoeist in the UK (Chapter 2); the survival times of eight high-impact INNS affecting UK freshwater habitats (Chapter 3); the effectiveness and mortality rate of different biosecurity treatments (Chapter 3); and the most effective ways to disseminate biosecurity awareness at high risk sites (Chapter 4). Chapter 6 also highlighted the need to identify hotspots of recreational activity in marine and terrestrial habitats in order to prioritise biosecurity actions.

7.5.2 Critical evaluation of management effectiveness

It is important to critically appraise conservation management and education strategies to ensure they are meeting their objectives and – in the case of education – enacting behaviour change (Baruch-Mordo *et al.*, 2011). However, this will require conservation scientists to adopt the widespread use of repeated, randomised and controlled experiments – as used in healthcare science – to evaluate success (Sutherland *et al.*, 2004; Pullin and Knight, 2009; Stewart, 2010).

In Chapters 4 and 5, I encountered obstacles relating to ecological survey designs. In Chapter 4, the absence of a control site or baseline information from before the biosecurity campaign made it impossible to tease apart which aspects (i.e. education vs. control measures) of the Bay of Plenty's regional INNS strategy (2011) had been the most effective (and most cost effective) at preventing the introduction and spread of aquatic INNS. The selection of a valid control site (with no biosecurity awareness campaign) was impossible due to widespread biosecurity messaging at waterways across the country. Similarly, Chapter 5 revealed that many INNS studies do not provide sufficient data, or lack the necessary control-impact design to be included in a meta-analysis – commonly used in medicine to systematically review the effectiveness of a particular treatment (Pullin and Knight, 2009; Stewart, 2010). The need for ecologists to adopt the use of meta-analyses (Stewart, 2010) and to report conservation science results in a format suitable for inclusion in meta-analyses (i.e. providing means, sample sizes and variability in control and treatment sites) are increasingly being recognised (Haddaway, 2015).

With biosecurity programmes in their infancy, Europe is afforded an unparalleled opportunity to use Before-After-Control-Impact (BACI) studies to evaluate the effectiveness of (and subsequently adapt) different elements of forthcoming INNS management strategies. The assessment and adaptation of future initiatives will help to improve management effectiveness, gain the support of funding bodies and policy makers, and the acceptance – and continued compliance – of the public (Pullin and Knight, 2009; Sutherland *et al.*, 2004; Baruch-Mordo *et al.*, 2011).

Key recommendations:

- The formation of regional biosecurity partnerships across the UK with overarching national support (legislation and coordination).

- The development of integrated regional INNS management strategies to improve cohesion between partner organisations.

- The use of BACI studies to evaluate and adapt INNS management initiatives.

- The identification of priority sites using pathway risk assessments.

7.6 Understanding the interplay between INNS and introduced parasites and pathogens

In Chapter five, I investigated the role that parasites and pathogens can play in the success of freshwater invasions, focusing on the invasion of the American signal crayfish (*P. leniusculus*) in the UK. Histological analysis revealed that relative to native white-clawed crayfish (*A. pallipes*)**,** invasive non-native signal crayfish were affected by very few parasites or pathogens. The only pathogen that they were infected by, an intranuclear bacilliform virus, is considered to be an innocuous generalist pathogen and is not known to cause any gross pathological or behavioural effects (Longshaw 2011). These results provide support for the concept of enemy release (Colautti et al 2004) and further understanding about why the signal crayfish has been so successful at replacing white-clawed crayfish in the UK. The results of this Chapter also reinforce the need to consider the role of pathogens in the invasions process, both as free-living stages (such as *Aphanomyces astaci***,** the causative agent of crayfish plague) which require the same biosecurity prevention measures as INNS, and as host-infections (e.g. microsporidian infections) which may affect the competitive dynamics of both native and invasive non-native species.

7.7 Concluding remarks

Biological invasions are one of the greatest threats to global biodiversity, particularly in freshwater environments (Mack, 2000; Dudgeon *et al.*, 2006; WWF, 2014). Preventing the introduction and secondary spread of INNS through effective pathway management is universally accepted as the most effective form of control, as promoted in both international and European agreements (Caffrey *et al.*, 2014; European Commission, 2013; Convention on Biological Diversity, 2014; Roy *et al.*, 2014). Effective biosecurity is a key component of pathway management and has the potential to simultaneously prevent the introduction of a whole assemblage of INNS while requiring little effort or knowledge by the user. In the UK, biosecurity is a relatively new concept. However, it warrants further promotion among recreational water users having identified that their movement patterns, coupled with low biosecurity awareness and compliance pose a potential biosecurity threat. Evidence from New Zealand demonstrates that the development of biosecurity awareness

campaigns can be a successful and highly cost-effective way to improve the behaviours of high-risk groups and slow the spread of freshwater invaders. With investment in biosecurity awareness promotion, a programme of close monitoring and adaptation, and the support of new legislation, biosecurity initiatives could be equally as effective at slowing the spread – and the unrelenting impacts – of INNS in Europe. The evidence collected throughout this PhD will hopefully provide a useful foundation to achieving such a goal.

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9 APPENDICES

APPENDIX A: DATA REQUIREMENTS TABLE FOR ONLINE QUESTIONNAIRE ADMINISTERED TO UK ANGLERS AND CANOEISTS (CHAPTER 2).

Demographic information	
Age	- under 18 18-24 25-34 35-44 45-54 55-64 65+
Sex	Male/Female
Which type of canoeing/angling do you do most frequently?	Selection from a list
Movement patterns	
How frequently do you go angling/canoeing?	- More than once a week - Once a week - Once every 2 weeks - Once every 3 weeks - Once every month - Once every 2 months - Once every 3 months - More than once every 3 months
Please enter the first three or four digits of your postcode (This will enable us to estimate how far different water users travel to take part in their activities. Your location will remain anonymous)	Open answer
Please list the 3 sites you went angling at **most recently**.	Site name_____ Nearest town_____ County_____ Site name_____ Nearest town_____ County_____ Site name_____ Nearest town_____ County_____
Please list the 3 sites that you go angling/canoeing at **most frequently**	Site name_____ Nearest town_____ County_____ Site name_____ Nearest town_____ County_____ Site name_____ Nearest town_____ County_____

Have you ever used your own angling equipment/canoe outside the UK?	Yes/No
If yes, which countries?	Open answer
Equipment use	
Which of the following items of equipment do you use?	Multiple selections from list – specific to angling/canoeing
Where do you store your equipment between trips?	- Indoors - Outhouse or garage - Outdoors
If you use waders or a keep net, how long do you typically keep them in the water for? (anglers only)	__hours (waders) __hours (keep net)
Do you ever clean you equipment between trips?	Yes/No
If yes, how frequently?	- After every trip - After 2-5 trips - After 6-10 trips - After 11+trips
If yes, what do you use?	- Water - Detergent - Disinfectant - Other (please state)
Do you ever dry your equipment completely between trips?	Yes/No
If yes, how frequently?	- After every trip - After 2-5 trips - After 6-10 trips - After 11+trips
Anglers only	
Do you ever use live bait?	Yes/No
If yes, what type of bait do you use?	Multiple selections from list
If yes, where do you source your bait from?	Multiple selections from list
If yes, what do you do with your bait at the end of your angling trip?	Multiple selections from list
Canoeists/kayakers only	
How important are the following factors when deciding whether to clean your kayak/canoe and equipment after a trip.	*Please rate the following from 1 to 5 (1 = not at all important, 5 = extremely important)* - The availability of a hose/cleaning station - The cost of cleaning equipment - The time it takes to clean equipment - The availability of information about what to do

|	
	• How clean your kayak/canoe looks at the end of your trip
Check Clean Dry	
Have you heard of the '*Clean Check Dry*' campaign in the UK?	Yes/No
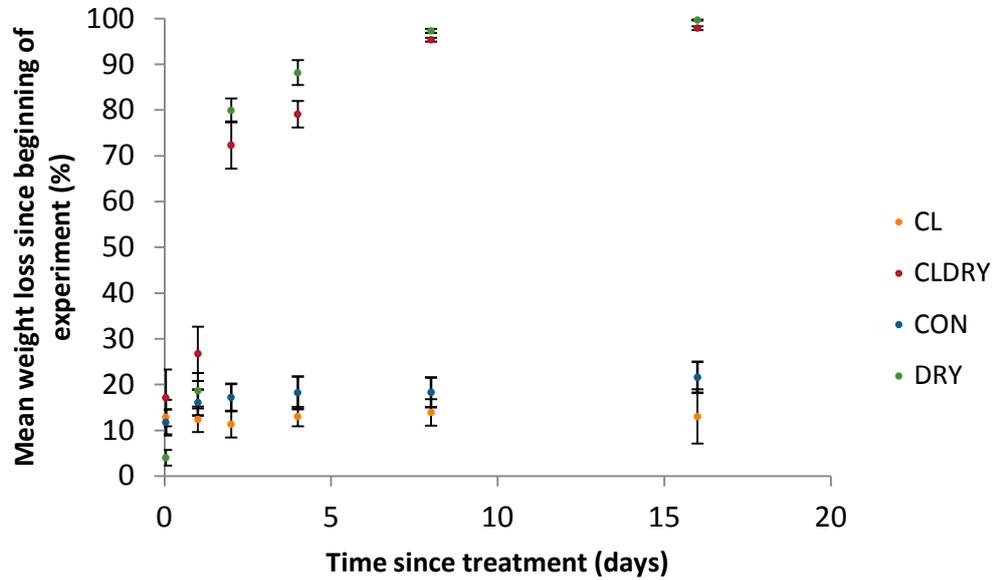
FIGURE C1. THE GRAPH SHOWS THE MEAN WEIGHT LOSS MEASURED IN A SUBSET OF TWENTY
RANDOMLY SELECTED NETS FROM EACH TREATMENT AT EACH TIME POINT. ERROR BARS SHOW
STANDARD ERROR AROUND THE MEAN. CL=HOT WATER ONLY TREATMENT; CLDRY=HOT WATER
TREATMENT FOLLOWED BY DRYING TREATMENT; DRY = DRYING ONLY TREATMENT; CON=CONTROL
GROUP.

ANOVA tests were performed in R version 2.15 (R Development Core Team, 2012) to determine whether the differences in weight in the nets exposed to drying treatments or non-drying treatments were significantly different. At each time point, 80 bags (40 from drying treatments and 40 from non-drying treatments) were due to end at that time point were randomly selected and weighed. From day 1 onwards, there was a significant difference in the weight of the bags exposed to drying vs. bags which were prevented from drying (Table C1).

TABLE C1. RESULTS OF ANOVA TESTS TO COMPARE THE WEIGHTS OF NETS WHICH WERE SUBJECTED TO DRYING TREATMENTS (HOT WATER AND DRY TREATMENT; DRY ONLY TREATMENT) AND THE NETS WHICH WERE PREVENTED FROM DRYING BY BEING STORED IN UNSEALED PLASTIC BAGS (HOT WATER TREATMENT; CONTROL). N = 80 NETS AT EACH TIME POINT.

Time point	Results of ANOVA test
1h	$F_{1,78} = 11.79$, p<0.01
1 day	$F_{1,78} = 46.95$, p<0.0001
2 days	$F_{1,78} = 50.25$, p<0.0001
4 days	$F_{1,78} = 61.43$, p<0.0001
8 days	$F_{1,78} = 79.04$, p<0.0001
16 days	$F_{1,78} = 130.9$, P<0.0001

WATER USERS SURVEY

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This survey aims to find out about the recreational activities you did today in the Rotorua Lakes and your awareness of plants and animals in the lakes. It will take no more than five minutes to complete. The answers you give are completely anonymous and will help improve public awareness about nature conservation both here and in other parts of the world. Thank you for agreeing to complete this survey.

Unless otherwise asked, please choose the ONE answer that best represents your view by placing a tick in the appropriate box.

A. About your trip to the lake/river today

1. Which of the following water-based activities did you mainly do today? *If you did more than one activity, please select the main one you did.*

- Waterskiing — *used my own tow boat*
- Wakeboarding — *used my own tow boat*
- Biscuiting — *used my own tow boat*
- Jetskiing
- Boating (motorised)
- Boating (non-motorised)
- Sailing
- Fishing *from a boat?*   *from the shore?*
- Canoeing/kayaking
- Rafting
- Stand up paddleboarding
- Other *please state*

2. During a typical month, how frequently do you take part in this activity?

- More than once a week
- Once a week
- Once a fortnight
- Once every three weeks
- Once a month
- Less than once a month

3. Did you carry out your activity today as part of a competition or tournament?

- Yes
- No

B. About the boat/equipment you used for your main activity today

4. Do you ever use your boat/equipment at other sites beside this one?

- Yes
- No *[please go to Question 6]*

5. If yes, where did you last use your boat or equipment?

Site name

Nearest town

Country

6. After you last used your boat or equipment, which of the following - if any - did you do? *Please tick all that apply.*

- Checked it for weeds/plants
- Checked it for dirt/mud
- Cleaned it with lake/river water
- Cleaned it with tap water
- Cleaned it with detergent/soap
- Cleaned it with disinfectant
- Allowed it to dry for at least 48 hours (2 days)

- None of the above

7. How regularly do you take the actions that you ticked above?

	After every trip	After every 2-5 trips	After every 6-10 trips	After 11+ trips	Never
Check it for weeds/plants					
Check it for dirt/mud					
Clean it with lake/river water					
Clean it with tap water					
Clean it with detergent/soap					
Clean it with disinfectant					
Allow it to dry for 48 hours					

8. How important are the following factors when deciding whether to clean your equipment or boat at the end of your activity?

	Extremely important	Very important	Somewhat important	Not very important	Not at all important
The availability of a hose or cleaning station					
The expense of buying cleaning products					
The time I have available to clean everything					
The availability of information about how to clean my equipment					
Whether there are signs up reminding me to clean it					
How dirty my boat or equipment looks					

Please turn over >>

9. If the following initiatives were put into place, how likely would you be to clean your boat/equipment after *every* use? *For each option, please circle a number from 1 (highly likely) to 5 (highly unlikely)*

	Highly likely				Highly unlikely
Free cleaning stations with hoses/jet washers	1	2	3	4	5
An information board showing you how to clean your boat or equipment	1	2	3	4	5
The prospect of a $500 spot fine if you were found arriving at or leaving a lake without having cleaned your kit	1	2	3	4	5
Signs up reminding you to clean your boat or equipment before leaving	1	2	3	4	5
More information about how you would be helping the environment by doing so	1	2	3	4	5

C. Your knowledge of aquatic pest species

10. Are any of the following species present in this lake?

	Yes	No	Don't know
Elodea *(Elodea canadensis)*	☐	☐	☐
Didymo *(Didymosphenia geminata)*	☐	☐	☐
Brown bullhead catfish *(Ameiurus nebulosus)*	☐	☐	☐
Mosquito fish *(Gambusia affinis)*	☐	☐	☐

11. Which of the attached images shows hornwort (*Ceratophyllum demersum*)? *Please see the laminated sheet for the three images and tick your answer below*

Image A ☐
Image B ☐
Image C ☐

Don't know ☐

12. Which of the attached images shows rudd (*Scardinius erythropthalmus*)? *Please see the laminated sheet for the three images and tick your answer below*

Image D ☐
Image E ☐
Image F ☐

Don't know ☐

D. Awareness campaign

13. Have you heard of a campaign called *STOP THE SPREAD!* in the Rotorua Lakes region?

☐ Yes
☐ No *[please go to Question 16]*

14. If yes, where did you hear about the *STOP THE SPREAD!* campaign? *Please tick ALL that apply*

☐ Hotel
☐ Campsite
☐ Shop
☐ Sign or poster at lake
☐ Leaflet
☐ Internet
☐ TV
☐ Newspaper/magazine
☐ Word of mouth
☐ Information provided at a competition
☐ Spoke to member of staff at boat ramp
☐ Other (please state) _____

15. If yes, what is the campaign about? *Please tick ALL that apply.*

☐ Reporting sightings of aquatic pests
☐ Cleaning your boat before moving to another site
☐ Cleaning your angling kit before moving to another site
☐ Preventing the movement of didymo
☐ Preventing the movement of other aquatic pests
☐ Preventing people from moving their boats to different sites

☐ Don't know

E. About you

16. Are you...

☐ Male?   ☐ Female?

17. How old are you?

☐ 18-24   ☐ 45-54
☐ 25-34   ☐ 55-64
☐ 35-44   ☐ 65+

18. What is your nationality? *Please write in*

_____

19. Where do you usually live? *Please write in your country*

_____

20. If you live in NZ, what is your nearest town/city?

_____

21. If you have any comments about the *STOP THE SPREAD!* campaign, or aquatic pests in the lakes you use, please write them in the space below.

Thank you for completing this questionnaire. Your help is much appreciated. Please hand the questionnaire back to the interviewer.

Time_____ Date_____ Site:_____ LA/SR

11. Which of the following images shows **hornwort** (*Ceratophyllum demersum*)?

Image A

Image B

Image C

12. Which of the following images shows **rudd** (*Scardinius erythropthalmus*)?

Image D

Image E

Image F

Key Informant Interviews
Participant Consent Form

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Please initial the box if you agree with the statement to the left

- I have read and understood the Study Information sheet provided. I have been given the opportunity to ask questions and have had these questions answered to my satisfaction ☐

- I understand that my participation is voluntary, that I can withdraw from the study at any time and that I will not be asked any questions about why I no longer want to take part. Should I not wish to answer any particular question or questions, I am free to decline. ☐

- I understand that taking part in the study will include being interviewed and audio recorded ☐

- I understand that my responses will be kept confidential and that my personal details such as name and employer address will not be revealed to people outside the project. My words may be quoted in publications, reports and other research outputs but my name will not be used without my prior approval. ☐

Name of participant_____Date _____

Signature of participant_____

Researcher signature_____Date _____

Study Information
Key informant interviews

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- Thank you very much for agreeing to participate in this study. This sheet provides information about what the study is about and how we would like you to participate in it. Please take time to read the information carefully and discuss it with others if you wish. Please feel free to ask any questions you have about the study or ask for more information.

- The purpose of this study is to investigate the effectiveness of an aquatic pest management programme which has been running in the Rotorua Lakes for the past 9 years. We are particular interested in finding out which aspects of the programme are working well and which may need improvement. The results of this research will not only be used to improve conservation in this region, but form a basis for developing similar programmes in other parts of the world where aquatic pests are a problem, such as freshwater lakes and rivers in the UK.

- There are two components to the research: i) self-completion questionnaires examining recreational water users' knowledge and understanding of the awareness programme, their knowledge of aquatic pests and the actions that they take to prevent the movement of aquatic pests and ii) semi-structured interviews with key informants i.e. representatives from tourism, water-sports and conservation organisations to see how aquatic pests have affected their activities.

- In order to hear your views, we would like to interview you as part of this research. If you agree to this, an interview of approximately 30 minutes will be arranged at a time to suit you and will be audio-recorded.

- The information provided by you will be used for research purposes. It will not be used in a manner which would allow individual responses to be identified without your prior approval.

- If you are happy to participate in the study, we would be grateful if you could read and sign the consent form.

Lucy Anderson
PhD student
University of Leeds
bslga@leeds.ac.uk
NZ Mobile 02040316713

APPENDIX D: SUPPLEMENTARY INFORMATION ACCOMPANYING CHAPTER 4

TABLE D1. BREAKDOWN OF RESPONDENTS BY DEMOGRAPHIC GROUP, LOCAL/VISITOR AND ACTIVITY TYPE.

Age class	% (n)	Activity	% (n)	Local/ Visitor	% (n)	Sex	% (n)
18-24	6.5 (15)	Motorboating	55.7 (128)	Local	61 (143)	M	77 (178)
25-34	9.1 (21)	Angling	20 (46)	Visitor	39 (87)	F	23 (52)
35-44	30 (69)	Jetskiing	10.4 (24)				
45-54	34.4 (79)	Rowing	7.4 (17)				
55-64	13.5 (31)	Canoeing	5.2 (12)				
65+	6.5 (15)	Sailing	1.3 (3)				

Calculations used to estimate the number of recreational water users in the Rotorua Lakes.

Approximately 13,000 households in the Bay of Plenty region own a pleasure boat (Marine Safety Authority of New Zealand, 1999) and figures suggest that of the 500,000 domestic tourists visiting Rotorua each year, 2500 go kayaking, 5000 go pleasure boating and 5000 do other water sports (The Ministry of Tourism, 2004). A further 19,807 (including locals, domestic tourists and international tourists) purchase either full or part season fishing licenses from Fish & Game in the Eastern (Bay of Plenty) region each year (Unwin, 2009). International pleasure boaters/kayakers/water sports participants were excluded as they are unlikely to have transported their own vessels to New Zealand.

References

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The Ministry of Tourism, 2004. Waters of National Importance for Tourism.

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TABLE D2. CLEANING PRACTICES AND AWARENESS OF BIOSECURITY CAMPAIGN AMONG DIFFERENT WATER USERS IN THE ROTORUA LAKES.

Activity	Awareness of Stop the Spread (%)	Visit >1 site	Of those visiting >1 site			Check AND clean OR dry every time (%)
			Check for weeds every time (%)	Dry every time (%)	Clean every time (%)	
Motor-boating	79.7	83.6	67.3	45.8	Tap 51.4 Deter 27.1	50.5
Angling	76.1	80.4	75.68	48.7	Tap 64.9 Deter 43.2	67.6
Jetskiing	41.7	91.7	36.4	27.3	Tap 31.8 Deter 27.3	36.4
Rowing	35.3	100	35.3	17.6	Tap 23.5 Deter 11.8	29.4
Canoeing	75.0	91.7	36.4	18.2	Tap 18.2 Deter 18.2	27.3
Sailing	100	66.7	100	100	Tap 100 Deter 100	100
All	71.7	85.21	61.22	40.82	Tap 48.1 Deter 29.1	49.5

Note: Tap = tap water alone used to clean equipment. Deter = detergent used to clean equipment

APPENDIX E: EXPLORATION OF PUBLICATION BIAS (CHAPTER 6)

a)
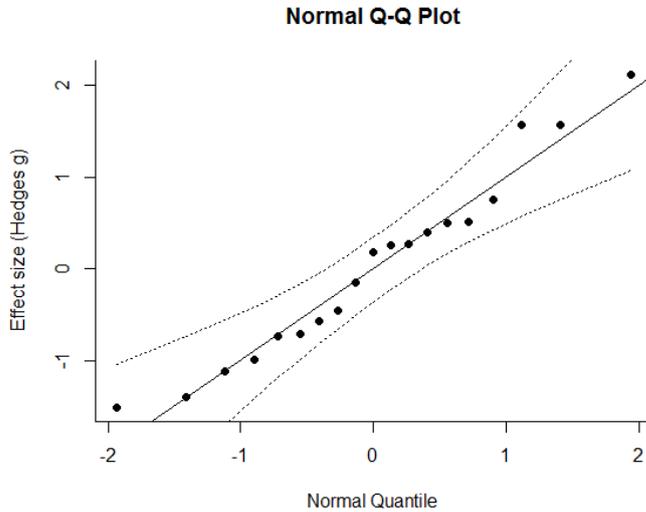
b)
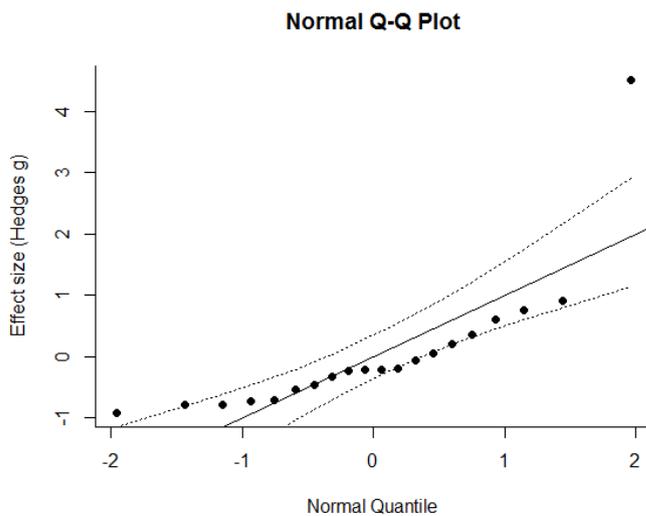
FIGURE 8.1 NORMAL QUANTILE PLOTS OF THE STANDARDIZED EFFECT SIZES (*HEDGE'S G*) AGAINST NORMAL QUANTILES FOR THE DATA SETS USED IN META-ANALYSES ASSESSING THE RESPONSES OF NON-NATIVE SPECIES (A) RICHNESS AND (B) ABUNDANCE IN SITES DISTURBED BY TOURISM/RECREATION. ALL POINTS BUT ONE FALL WITHIN THE CONFIDENCE INTERVALS, INDICATING THAT THE DATA ARE NORMALLY DISTRIBUTED.

APPENDIX F: CHARACTERISTICS OF STUDIES USED IN META-ANALYSIS

TABLE F1. CHARACTERISTICS OF THE STUDIES INCLUDED IN THE META-ANALYSIS.

Study	Intervention	Taxon	Continent	Type (Design)	Metric	EcoRegion
Airoldi *et al.*, 2005	Recreational harvesting	Invertebrates	Europe	Exp (CI)	Ab	Marine
Allen *et al.*, 2008	Visitors	Plants	North America	Obs (other)	SR	Terrestrial
Banks and Baker, 2011	Trails	Plants	North America	Obs (CI)	Ab	Terrestrial
Baret and Strasberg, 2005	Trails	Plants	Europe (Indian Ocean)	Obs (BA)	SR	Terrestrial
Barros and Pickering, 2014	Trails	Plants	South America	Obs (CI)	Both	Terrestrial
Campbell and Gibson, 2001	Horses	Plants	North America	Obs (CI)	Ab	Terrestrial
Capers *et al.*, 2009	Boats	Aquatic plants	North America	Obs (CI)	SR	Freshwater
Clark and Johnston, 2009	Boats	Invertebrates	Australasia	Exp (CI)	Ab	Marine
Cowie and Werner, 1993	Visitors	Plants	Australasia	Obs (CI)	SR	Terrestrial
Cushman and Meentemeyer, 2008	Trails	Pathogen	North America	Obs (CI)	Ab	Terrestrial

Study	Intervention	Taxon	Continent	Type (Design)	Metric	EcoRegion
Dickens *et al.*, 2005	Trails	Plants	North America	Obs (CI)	Both	Terrestrial
Gower, 2008	Horses	Plants	North America	Obs (CI)	SR	Terrestrial
Hill and Pickering, 2006	Trails	Plants	Australasia	Obs (CI)	SR	Terrestrial
Johnston *et al.*, 2011	Boats	Invertebrates	Europe	Obs (CI)	Ab	Marine
LaPaix *et al.*, 2012	Trails	Plants	North America	Obs (CI)	Both	Terrestrial
McKinney, 2002	Visitors	Plants	North America	Obs (Other)	SR	Terrestrial
Molina-Montenegro *et al.*, 2014	Visitors	Plants	Antarctica	Obs (Other)	Ab	Terrestrial
Morgan and Carnegie, 2009	Visitors	Plants	Australasia	Obs (CI)	SR	Terrestrial
Peters *et al.*, 2014	Boats	Aquatic plants	Africa	Obs (CI)	SR	Marine
Potito and Beatty, 2005	Trails	Plants	North America	Obs (CI)	Both	Terrestrial
Ranasinghe *et al.*, 2005	Boats	Aquatic plants	North America	Obs (Other)	Ab	Marine
Rodgers and Parker, 2003	Visitors	Plants	North America	Obs (CI)	SR	Terrestrial
Ros *et al.*, 2013	Boats	Invertebrates	Europe	Obs (CI)	Ab	Marine
Sikorski *et al.*, 2013	Visitors	Plants	Europe	Obs (CI)	SR	Terrestrial
Solomon *et al.*, 2010	Boats	Invertebrates	North America	Obs (CI)	Ab	Freshwater
Stasko *et al.*, 2012	Boats	Zooplankton	Europe	Obs (CI)	SR	Freshwater

Study	Intervention	Taxon	Continent	Type (Design)	Metric	EcoRegion
Tamburello *et al.*, 2014	Boats	Aquatic plants	Europe	Exp (CI)	Ab	Marine
Törn *et al.*, 2009	Horses	Plants	Europe	Exp (CI)	Ab	Terrestrial
Wells *et al.*, 2012	Trails	Plants	North America	Obs (CI)	SR	Terrestrial
Whinam *et al.*, 1994	Horses	Plants	Australasia	Exp (BA)	Ab	Terrestrial
Willette *et al.*, 2014	Boats	Aquatic plants	Central America	Obs (Other)	Ab	Marine
Wolf and Croft, 2014	Trails	Plants	Australasia	Obs (CI)	Ab	Terrestrial
Wu *et al.*, 2009	Trails	Plants	Asia	Obs (CI)	SR	Terrestrial

Note: Exp = experimental study, Obs = observational study. CI = Control/Impact, BA = before/after. Ab = abundance, SR = species richness.