Macroparasites of the Eurasian Otter: distribution, life-cycles and population dynamics

A thesis submitted to Cardiff University for the degree of Doctor of Philosophy by

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APPENDIX 1:
Specimen layout for Thesis Summary and Declaration/Statements page to be included in a Thesis

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This work has not been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award.

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Summary

Potential alterations of host and parasite ranges are likely with climate change so an understanding of the host traits and ecological factors that can influence host-parasite interactions is vital for the effective protection of ecosystems. Accidental introductions of non-native species can place elevated stress on native ecosystems so that the examination of key species can act as early warning systems. The Eurasian otter, *Lutra lutra*¸ is a top predator and sentinel species for the health of European freshwater ecosystems and is therefore a suitable model for exploring parasite fauna introductions. In this PhD, the patterns and processes that define macro-parasitic infections were explored using evidence from post-mortems of 587 otters. Specifically, the invasive status of two helminths (*Pseudamphistomum truncatum* and *Metorchis albidus*: Trematoda; Opisthorchiidae) was investigated, both species having been identified in the UK otter populations for the first time within the last 10 years. Genetic variation, however, was similar across Europe indicating neither helminth is likely to have been a recent introduction to the UK., The distribution of both helminths as well as the only ectoparasite, *Ixodes hexagonus* (Arthropoda; Ixodidae), recovered from UK otters, were associated with abiotic factors, particularly temperature. The complexity of the parasite life cycles was investigated; otters act as a definitive host for both helminth species considered in this thesis and early stage intermediate hosts were identified for *P. truncatum* as the snail *Radix balthica* and the roach *Rutilus rutilus.* Metacercariae of *M. ablidus* were detected on chub (*Leuciscus cephalus*), rudd (*Scardinius erythrophthalmus*) and roach. Parasite aggregation and parasite fecundity of the *P. truncatum* populations were influenced by abiotic factors, region and season, whilst *P. truncatum* abundance was defined better by the biotic factors host age-class and condition demonstrating how multiple factors combine to produce parasite population dynamics in wild fauna. Ultimately, the data collated throughout this PhD was used to parameterise a susceptible-infected Susceptible-Infected (SI) model describing the host population dynamics of opisthorchiid trematodes. This model is applied to the *P. truncatum* system to examine which factors might determine the proportion of hosts that become infected.

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1. General Introduction

1.1 Parasites: a global issue

Many species select parasitism as an effective life strategy (Poulin and Morand 2000). Parasites have a significant role in shaping host communities (Poulin 1999a), the individual host and ecosystem properties (Hatcher et al. 2012); not only can they induce changes to the host phenotype but further are considered to have trans-generational impacts on host offspring (Poulin and Frederic 2008). There is limited data on geographic ranges and host population susceptibility, arising in part because of the challenge to achieve comprehensive sampling and the cryptic nature of many parasitic species (Poulin and Morand 2000).

Parasites show an extraordinary capacity to adapt to novel hosts and changing environmental conditions and can even, via progenesis, alter their own life-histories in the absence of definitive hosts (Poulin 2003). This adaptive potential may play a role in colonisation success, which is strongly dependent on host densities (Morand and Poulin 1998, Poulin 1999b). Novel host-parasite interactions often present more serious pathologies for host populations, partly because of naïve immune responses in the host, and the natural equilibrium of the adopted ecosystem may be affected (Torchin et al. 2002). As a result, recognition of the role that anthropogenic movement of living organisms has had in introducing parasites to naïve systems has resulted in more stringent screening and quarantine procedures (see Torchin et al. 2002). Parasites of host populations under such scrutiny are, accordingly, most likely to be discovered.

1.2 Parasite distributions

Identification of parasites through surveillance is challenging because both the geographic distribution and aggregations within the host population are typically variable (Anderson and May 1979, 1991, Poulin and Dick 2007). Parasitic populations tend to be described by the negative binomial distribution such that a minority of the host population are infected with the majority of the parasitic population (Anderson and May 1979, Shaw and Dobson 1995, Shaw et al. 1998, Woolhouse et al. 1997, Perkins et al. 2003, Galvani and May 2005, Lloyd-Smith et al. 2005). Shaw and Dobson's (1995) review on macro-parasite abundance (see Table 1.1 for definitions) and aggregation patterns indicates that mean parasite burdens were log-normally distributed, indicating

that parasite loads must be regulated to a certain degree. Both density independent (for example, environment and host behaviour) and density dependent factors (such as competition and the immune system of the host) act in combination, to a great or lesser extent, as species specific regulatory mechanisms for parasitic populations (Anderson and May 1978, May and Anderson 1978).

Table 1.1 Definitions of common terms used in parasitology adopted in the current thesis (following Bush et al. 1997).

Terminology	**Definition**
Parasite	Any organism that lives on or in another living organism, known as the host, from which it obtains nourishment and benefits at the expense of the other. Parasites include both macroparasites (for example arachnids, helminths and protozoa) and microparasites (including viruses and bacteria). Parasites tend to reduce the biological fitness of their hosts.
Prevalence	The number of hosts infected or infested with one or more parasites of a particular taxonomic group divided by total number of hosts examined for that parasite taxon.
Intensity	The number of individuals of a particular parasite species in a single infected host.
Mean intensity	The total number of parasites of particular species found in a sample divided by the number of hosts infected with that parasite.
Abundance	The number of individuals of a particular parasite in or on a single host, regardless of whether or not that host is infected with the respective parasite
Mean abundance	The total number of parasites of a particular species found in a sample divided by the total number of hosts in that sample.

High parasite intensity (see Table 1.1) within an individual host is not necessarily synonymous with high transmission potential of parasitic infective stages from that host. In a similar manner to parasite distribution patterns, there is inherent variation in the reproductive potential of a parasite and this is affected by parasite and host genetics, parasite age and host immunity (Kaitala et al. 1997, Koehler and Poulin 2012). In addition, certain abiotic factors may result in high reproductive success of parasites. Atypical aggregations, together with an understanding of transmission potential of infective stages from particular sub-sections of the host population, can be advantageous

because particular sub-populations of heavily infected hosts can be isolated for management.

1.3 Abiotic and biotic associations

The interplay between abiotic (both natural and anthropogenic) and biotic factors shape parasite transmission processes (Thieltges et al. 2008). Further, understanding of such interactions is a prerequisite to preparing for the global changes in parasite and host communities that will result from anticipated climate change (Thieltges et al. 2008). All host-parasite systems are dynamic interactions and parasites are theorised to have evolved to infect disproportionately the common phenotypes in the host population (Lively et al. 1990). Underlying genetic mechanisms experience, therefore, continual selection pressures which may be biotic and/or abiotic in nature (Mone et al. 2011). Such dynamic host-parasite interactions are, nevertheless, considered remarkably stable (May 1977, Anderson and May 1979, May and Anderson 1979, Stear et al. 2011). This is, perhaps, a consequence of a long history of co-evolution between hosts and their parasites (see Macnab et al. 2009, Stear et al. 2011). Perturbation through increasing host numbers or weather events will, however, impact on parasite distributions across their range. Identification of dynamic patterns in parasite and host distributions associated with both abiotic and biotic factors are, therefore, integral to our understanding of parasite ecology, particularly where there is a question over the invasive status of a particular parasite.

1.4 Thesis aims

Long-term studies of the parasite fauna of wildlife are rare because of the scarcity of data and the challenges presented in surveying endoparasites. Global climate change will impact host-parasite interactions (Harvell et al. 2002) and therefore, knowledge of the likely perturbations to such systems, will help us anticipate potential damage and so protect the equilibrium of our ecosystems (see Thieltges et al. 2008). Specifically, this thesis aims to investigate and compare the abiotic and biotic factors acting on different parasitic species isolated from otters (*Lutra lutra*) in the UK including endo- (Trematoda) and ecto-parasitic (Ixodida) species. Identifying functionally important factors that impact aggregation, intensity and fecundity, could have a profound effect on our understanding of disease transmission. Therefore, the hypothesis tested was that the patterns of parasite infection across a host population (aggregation) and parasite fecundity may be explained by abiotic (season and geographic region) or biotic (host sex, age-class

and body condition) factors. The two trematodes *Pseudamphistomum truncatum* and *Metorchis albidus* (Opisthorchiida) examined in this thesis have been reported only recently in the UK (see Simpson et al. 2005, Sherrard-Smith et al. 2009). We detailed their multi-host life cycle and examined the host population dynamics for this group of parasites.

Chapter 2 reviews the impact of abiotic and biotic factors on trematode distributions in their hosts, particularly their definitive mammalian hosts. Parallels are drawn between comparable systems and gaps in the literature are highlighted, some of which are explored in the subsequent chapters of this thesis.

In general parasites respond in species-specific manners to external stressors and in this thesis, two groups of parasites are considered. The ectoparasites of otters have been examined rarely. The aim of *Chapter 3* was to assess the extent that inter-annual variations in large-scale weather patterns and host characteristics influence tick prevalence and intensity on otters. A version of this chapter has been published in PLoS One (2012, e47131).

Whilst the majority of work on trematodes focuses on livestock infections, *Chapter 4* aims to define the abiotic and biotic factors contributing to the distribution of *P. truncatum* and *M. albidus* identified recently in wild otter populations in the UK. A version of this chapter has been accepted for publication in International Journal for Parasitology.

There is speculation about the invasive status of *P. truncatum* and *M. albidus* in the UK (see Simpson et al. 2005, Sherrard-Smith et al. 2009). To address this, a molecular analysis of two mitochondrial DNA regions was undertaken in *Chapter 5*, using samples from across Europe, to test the hypothesis that these trematodes have been introduced recently or, alternatively, that they are native to Britain as well as mainland Europe.

The patterns of parasite intensity across a host population and parasite fecundity within different hosts may vary between different groups within a host population. The identification of functionally important groups could have a profound impact on our understanding of disease transmission because hosts that are able to transmit a high

proportion of infective parasitic units (e.g. eggs) can be identified for targeted treatment. The aim of *Chapter 6* was to test whether it was possible to use both aggregation patterns and fecundity (as a proxy for transmission potential), in *P. truncatum* infecting otters, to assess whether parasites aggregate differently within certain hosts distinguished by season, region or host age class, sex or condition.

The distribution of parasites is determined by the distribution of their hosts. The life cycle of the two trematodes examined in this study is complex; three hosts are required, a snail, a fish and a piscivorous mammal. Therefore, a survey of snails, to complement available data on fish infections provided by the Environment Agency, was completed in *Chapter 7* to address the gap in the literature concerning the specific hosts for *P. truncatum* and *M. albidus* at early life stages.

In *Chapter 8,* the empirical data collected during the course of this PhD was brought together in a three host population dynamics model. This chapter aims to i) estimate the proportion of infective stages (eggs) that are successfully transmitted to the first intermediate host; and ii) identify parameters that have a strong influence on the proportion of hosts that are infected at each life stage.

Each chapter in this thesis is written as a self-contained article and therefore there is some overlap with regard to background information and methodology. A final discussion is provided in *Chapter 9* to draw together some of the overall thesis findings, to provide a brief critique of this work and to propose some future directions for the continuation of this study.

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2. Literature Review: How do abiotic and biotic factors determine the host-parasite distribution patterns observed in trematode populations of mammalians hosts?

2.1 Abstract

When parasites are introduced to novel ecosystems, there are sometimes devastating impacts on biodiversity, and recognition of key components influencing host-parasite relationships is essential to increase our ability to react, protect and manage ecosystems. Here abiotic and biotic impacts on trematodes of mammalian definitive hosts are reviewed. As global warming is predicted to promote the proliferation of parasitic infective stages, the first aim is to identify common associations of climate and local weather with host-trematode dynamics. Next, the major biotic factors impacting on host-trematode relationships are discussed. Meteorological trends can be identified such that 1) higher rainfall is often associated with increased transmission and 2) higher temperature tends to benefit trematode populations, but relationships are species specific and complex. Seasonality is a key component in the transmission success of trematodes and acts both directly and indirectly through the impacts on intermediate host density. Host sex can explain the distribution patterns of many parasites but tends not to explain observed patterns for trematodes. Within the definitive host, age-related associations are observed, but are not always apparent, for these trophically transmitted parasites. Evidently, the combined influence of biotic and abiotic stressors drive the population dynamics of parasite systems. This review discusses how multi-disciplinary approaches could greatly improve our understanding of the processes regulating helminth distributions.

2.2 The importance of parasites

Parasites constitute more than half (>50%) of our global biota and parasitism represents one of the most widely adopted life strategies of any living organism (Price 1980, Kuris et al. 2008, Hechinger et al. 2011). Ironically, a high diversity of parasitic fauna is indicative of a healthy ecosystem because of the integral role parasites play in ecosystem functioning (Marcogliese 2005, Hudson et al. 2006). Yet parasites can be devastating to their host populations, particularly when the equilibrium of such systems is perturbed (Kennedy 2009). Consequently, parasite ecology has developed as a discipline concerned with, in part, the harm to host organisms caused by the dynamic nature of parasitism as a consumer strategy. Recognition of the key components influencing host-parasite

relationships is, therefore, essential to increase our ability to react, protect and manage ecosystems.

Helminths are of substantial importance aquaculturally, agriculturally and zoonotically. Some of the principle zoonoses impacting human health are helminthic infections, including fascioliasis and schistosomiasis (trematodes), echinococcosis (cestodes) and trichinellosis (nematodes) (see Robinson and Dalton 2009). More recently, a range of fish-borne trematodiases have been recognized as important zoonoses (Robinson and Dalton 2009) and anthropogenic factors contribute to the distribution and inevitable spread of trematode disease (McCarthy and Moore 2000, Keiser and Utzinger 2005). In addition, parasite or host ranges move in response to global climate change which has, inescapably, contributed to emerging or re-emerging disease. Trematode parasites are particularly responsive to environmental perturbation because of the sensitivity of free-living life stages (Kennedy 2009). It is important however to understand exactly how host-parasite systems respond to abiotic and biotic pressures.

The ultimate prevalence, distribution and intensity of parasites in their definitive hosts is an amalgamation of interacting variables (including climate, weather and season, host sex, age and behaviour) that impact upon each intermediate host, the final host and each life stage of the specific parasite. This review aims to identify general patterns governing host-trematode systems, targeting those digeneans with mammalian hosts in particular. The associations, both abiotic and biotic, between host-parasite interactions are explored here with the objective of increasing our understanding and consequently instructing future research and management. This is particularly important to allow predictions concerning the response of parasitic populations to a changing climate.

2.3 Abiotic interactions

2.3.1 Meteorological conditions

Trematodes have free living stages within their life cycle (see Figure 2.1) during which they are directly exposed to environmental conditions; it is therefore anticipated that the effect of climate change will be significant, especially pertaining to altered cercarial output from intermediate hosts (Mas-Coma et al. 2008, van Dijk et al. 2010). Such elevated sensitivities are highlighted by the associations of climate (long-term trends in meteorological conditions) with *Fasciola* and *Schistosoma* species, both economically

important trematodes that have been studied extensively (see Fenwick et al. 2007, Mas-Coma et al. 2008, Beltran et al. 2009, McCann et al. 2010, van Dijk et al. 2010 and others). More generally, the impact of climate on the free-living stages of trematodes is well-documented. For example, provision of adequate moisture for survival and transmission success is associated with increased geographic range (Kendall and McCullough 1951, Smith and Wilson 1980, Mangal et al. 2008, van Dijk et al. 2010). Certain indirect impacts of climate change on helminth communities are evident through the effects of global warming on intermediate hosts (van Dijk et al. 2010, Lima dos Santos and Howgate 2011) but climate change might not have such obvious effects on defintivie hosts, perhaps because of the range of potential hosts available to generalist parasites (see Jenkins et al. 2006).

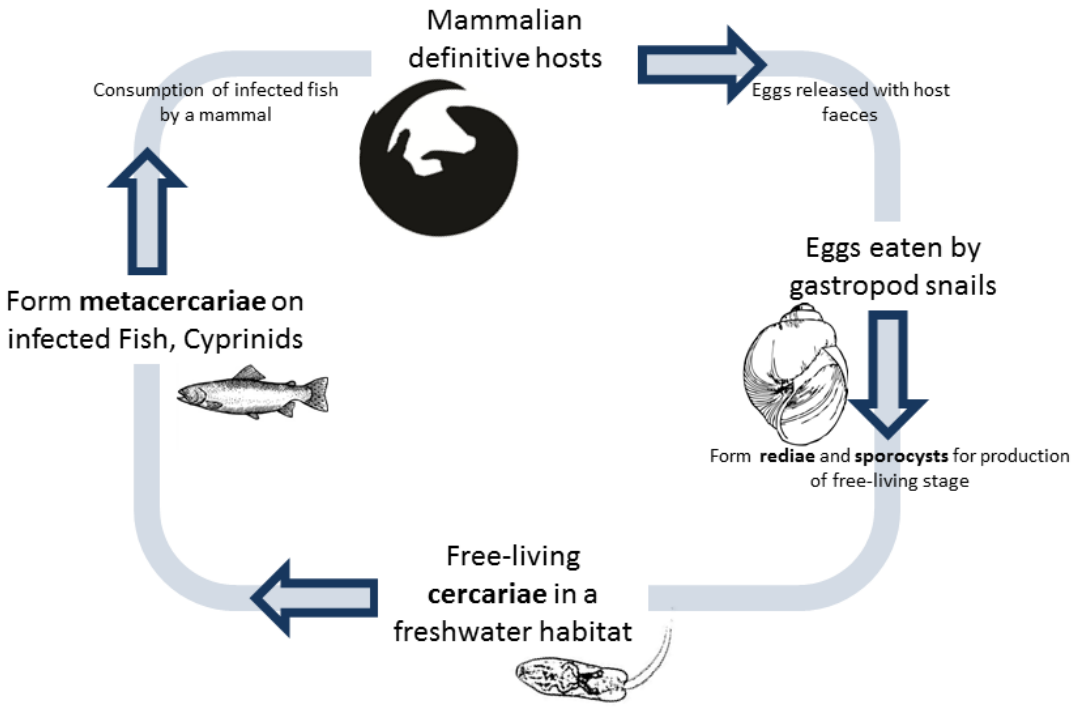

Figure 2.1 An example of the life cycle of three-host trematodes. The trematode releases asexually produced clones (cercariae) from its snail host into freshwater environments in vast numbers (e.g. *Diplostomum spathaceum* 7279 – 37418 mean cercariae shed per host per day, Karvonen et al. 2004). The free-living cercariae attach to fish where they encyst as metacercariae. The life cycle is completed when infected fish are consumed by a vertebrate (a predatory mammal in the current example but this could be any vertebrate

depending on the parasite). The trematode matures in a specific habitat within the definitive host (for *P. truncatum* this is the mammalian gall bladder) and sexual reproduction complemented by occasional self-fertilisation allows the hermaphroditic adult worms to release eggs into the faeces to re-start the cycle.

Where a study considers prevalence and intensity of trematodes in their definitive hosts, associations with climate can be explained, on occasion, by impacts on intermediate hosts and free-living stages of the parasite life cycle so that effects are seen within the definitive host population after a given lag time. Climate primarily explained long-term dynamics of common helminthic infections (but not rare infections) in bank voles, *Clethrionomys glareolus*, and changes in the bank voles were associated with climate-related cycling in intermediate host beetle, flea and free-living mite populations (see Haukisalmi and Henttonen 1990). The comprehensive sampling of parasites from wild fauna would greatly facilitate our current understanding of how global climate change will impact macroparasitic communities (see Cribb 1999, Poulin and Morand 2000, Hotez and Gurwith 2011).

A number of studies identify long-term meteorological patterns (5 years or more) as better predictors of parasite prevalence in hosts at each life stage than recent weather (1 year or less) conditions (Morley and Lewis 2008, Wimberly et al. 2008, McCann et al. 2010). But weather, the short-term variation in meteorological conditions, influences local parasite ecology (see Morley and Lewis 2008, Wimberly et al. 2008, McCann et al. 2010). The liver fluke, *Fasciola hepatica*, has a global distribution and infects both wild and agriculturally important ruminants (McCann et al. 2010). In Northern Europe, temperature can be a limiting factor in liver fluke distribution and the parasite is endemic only in areas of the UK where day and night temperatures exceed 10°C for more than half the year (Torgerson and Claxton 1999). This is explained by the effects of local temperatures on the intermediate hosts.

Local temperature has a complex association with helminths. Where snails are present, high temperatures can deplete the total abundance of cercariae in a water system (Zbikowska 2001, Koprivnikar et al. 2010). Further, high temperatures can prevent the establishment of snail hosts in some locations (Sturrock 1966, Appleton 1977, Fenwick 2007). Yet generally, temperature increases are correlated with increased cercarial shedding (Poulin 2006, Koprivnikar and Poulin 2009, see Table 2.1). In the UK, sporocyst infections of snails increased following wet summers and warm winters

(Morley and Lewis 2008). There are, however, exceptions to this trend; for example, a decrease in *Maritrema novaezealandensis* cercariae emergence is observed with increasing temperature (Koprivnikar and Poulin 2009) and dicrocoeliid sporocysts decline in UK mollusc populations following hot and dry periods (Morley and Lewis 2008). On the other hand, temperature has no apparent effect on *Euhaplorchis californiensis* (see Koprivnikar et al. 2010). Further, low temperatures can increase the longevity of cercariae whilst high temperatures accelerate cercariae development and maturation (Pechenik and Fried 1995, Measure 1996). So, there are evident system-specific responses to changes in temperature between trematodes and their hosts.

Rainfall and moisture levels can become particularly important where temperatures are, on average, warmer (see Morley and Lewis 2008). When trematodes require water for completion of their development (see Niewiadomska and Pojmańska 2011), heavy rain can have a negative effect on trematode prevalence (see Table 2.1) because of the dilution effect within streams (see Weil and Kvale 1985). Further, high stream velocity as a consequence of heavy rainfall in low level equatorial regions of African countries is considered to result in unfavourable habitat for the snail intermediate hosts of *S. haematobium* (see Wright 1970). Conversely, the prevalence of helminths can be reduced following prolonged periods of drought (see Morley and Lewis 2008) through a decrease in suitable host habitat within the freshwater system. For meteorological associations (climate and weather), the strength of the impact on parasite distribution in the definitive host depends on the scale under consideration. Small scale differences may result from variation in local weather whilst general trends may be caused by associated climatic conditions.

Table 2.1 Trematodes of Mammals in England, Wales and the Isle of Man recorded on the Natural History Museum Host-Parasite Database where associations with abiotic stressors are documented (as of August 2012, excludes Zoo animals); Where available, data from the literature on directional effects of temperature (T), rainfall (R) and season on trematode prevalence (P) abundance (A) or intensity (I) within the definitive mammalian host are presented. *Data additionally incorporated from this thesis. Corresponding references are listed below

	Parasite	**Mammalian Host**	**Effect**		
Class	**Species**		**Temperature**	**Rainfall**	**Season**
Brachylaemidae	*Brachylaima. recurva*	*Apodemus flavicollis*[1] *A. sylvaticus*[2] *Rattus norvegicus*[1] *Vulpes vulpes*[3]			Fewer in Spring[3] Seasonal effects[11]
Dicrocoeliidae	*Corrigia vitta*	*Apodemus flavicollis*[1] *A. sylvaticus*[2]			Two year cycles in P[4]
Fasciolidae	*Fasciola hepatica*	*Bos taurus*[5]	>10ºC increases P[12]		
Heterophyidae	*Cryptocotyle lingua*	*Vulpes vulpes*[3]			P highest in March[3]
Omphalometridae	*Omphalometra flexuosa*	*Talpa europaea*[4]			NS[13]
Opisthorchiidae	*Metorchis albidus**	*Lutra lutra*	Increased P with higher temperature*	Decreased P with increased rainfall*	NS*
	*Pseudamphistomum truncatum**	*Lutra lutra* *Mustela vison*	Increased P with higher temperature*	Decreased P with increased rainfall*	NS*
Paramphistomidae	*Gastrodiscoides hominus*	Mouse deer[8]			(NUK) A high late summer until early autumn[14]
	Paramphistomum sp.	Ruminants			(NUK) High egg output may until october[15]
Schistosomatidae	*Schistosoma haematobium*	*Homo sapiens*[7]		(NUK) Transmission during dry season[16]	
	S. mansoni	*Homo sapiens*[6,7] *Mesocricetus auratus*[9] *Mus musculus*[9] *Rattus rattus*[10]		(NUK) Transmission during wet season[17]	

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2.3.2 Season and annual patterns

Seasonal patterns in parasite fecundity can be observed through faecal egg count studies (for example Hanna et al. 1988) and are often explained by the synchronicity of parasites with the seasonal fluctuations in abundance of their snail hosts (Hughes and Answer 1982, Phiri et al. 2007). Late spring until early autumn tend to be the most successful seasons for trematodes in the UK (Table 2.1). There is evidence of developmental variation between *Paramphistomum leydeni* in reindeer from Finland such that immature worms are recovered only in winter whilst sexually mature worms develop in summer (Nikander and Seppo 2007) suggesting infection occurs sometime during summer or autumn following the release of eggs in summer from mature worms.

Synchrony between egg release and the abundance of the next host is a crucial factor influencing egg production. Many adult helminths elicit a peak egg production during or just after heavy rain (see Rolfe et al., 1991, Madhavi 1979). *Paramphistomum epiclitum* and *Gastrothylax crumenifer,* for example, have peak egg production during the Monsoon season in August and September when grazing definitive hosts are also most abundant (Hanna et al. 1988). Trematodes can be transmitted to their definitive host via encysted vegetation or with predation of infected intermediate hosts. This difference in transmission route (with vegetation or prey) will interplay with the dynamics of host-parasite populations. For instance, seasonality is associated with parasites of the Paramphistomidae such that egg output from infected definitive hosts is greatest over summer months (Table 2.1 and references therein) perhaps because of the use of seasonal vegetation for transmission of cysts to definitive hosts (see Phiri et al. 2007). Similarly, warmer conditions are associated with increased prevalence among the Fasciolidae, transmitted as cysts on vegetation (MacCann et al. 2010). Conversely, cysts can be maintained on fish hosts for long periods, potentially diluting any seasonal impact for trematodes transmitted via intermediate hosts found throughout the year, potentially explaining the lack of seasonality in the Opisthorchiidae (Table 2.1 and references therein). As such, seasonal variation in host diet may affect transmission patterns within host populations.

2.3.3 Land use and habitat ecology

The abundance of the definitive host is inextricably linked to characters of the local habitat, including prey availability, and therefore such characters must be of great

importance for the underlying assemblage of parasites (reviewed in Lafferty and Kuris 1999, Bush et al. 2001, Pietrock and Marcogliese 2003). The strength of ecological factors associated with infection, including interactions with the local environment, are scale dependent (Aukema 2004, Byers et al. 2008). Large scale studies are considered of great importance to recognise trends between land-use, pollution or habitat type and parasite abundance (Holdenrieder et al. 2004, Farnsworth et al. 2006, Byers et al. 2008) but fine scale studies can inform about intricate effects that may impact trematodes (e.g. Lasiak 1993, Lively and Jokela 1996).

Land use and habitat factors that increase host density or transmission potential will have a positive impact on trematode populations (Bustnes and Galaktionov 1999, Lafferty and Kuris 1999, Bush et al. 2001, Pietrock and Marcogliese 2003, Byers et al. 2008). In freshwater systems, infection intensity can decrease with increasing distance from large rivers and this is related, once again, to the use of habitat by host populations (see Hartson et al. 2011). Further, altitude may determine trematode distributions if establishment of snail populations that act as intermediate hosts is not possible at particular altitudes (see Rosenfield 1979, Weil and Kvale 1985).

2.3.4 Anthropogenic impacts

Humans have irreversibly altered global ecosystems and, inevitably, resident fauna and flora communities are disrupted with consequential impacts to parasite distributions in the definitive host. Disturbingly, such human mediated land use change is a contributing factor to disease emergence (Hartson et al. 2011). Parasite populations do not tend to show general responses to altered environmental conditions arising from anthropogenic activities (Lafferty 1997), mainly because each group of parasites demonstrates great variation in their sensitivity to different types of environmental stress. For trematodes specifically, cercariae are vulnerable to anthropogenic environmental conditions, such as the direct exposure to toxins (Pietrock and Marcogliese 2003, Morley et al. 2010, Ariyo and Oyerinde 1990). Pietrock and Marcogliese (2003) review the life stages of digeneans that respond to various stimuli such as toxins resulting from pollution, but also temperature and UV light. Generally, digenean cercarial survival is negatively affected by toxins, which is exacerbated by high temperature, low pH, salinity and water hardness (Morley et al. 2003).

Most laboratory studies have assessed the effects of single or, at most, a few variables on cercarial survival, such as pH (Sawabe and Makiya 1995), cadmium (Cd) or zinc (Zn) concentrations (Morley et al. 2003) but together their impact may differ. Morley et al. (2003) highlighted the need to consider the combined factors that may influence natural systems in the field, specifically in cercarial experiments. This has been demonstrated with *Cryptocotyle lingua* emergence from snails, *Littorina littorea,* where the cercariae located in 'clean' streams had greater survival compared to those emerging from snails in polluted waters (Cross et al. 2001). In addition this study showed that the swimming ability of cercariae from polluted environments was significantly reduced when compared to their 'clean' water sourced counterparts. More specifically, *Zoogonoides viviparous* cercariae survival is reduced when exposed to 0.1% sewage sludge in field experiments (Siddall and Clers 1994). The observed reaction of cercariae to water quality is now used generally as an indicator of freshwater ecosystem health (see Hechinger and Lafferty 2005, Marcogliese 2005). Pollutants can also interact with host susceptibility to parasites, which has been comprehensively reviewed by Lafferty and Kuris (1999).

Schistosomiasis, a trematode induced disease, is estimated to infect 207 million people worldwide (Steinmann et al. 2006). The human activities with greatest impact on Schistosomiasis are reportedly landscape alteration, disease-control measures and host contact with water (reviewed in Weil and Kvale 1985). Trematodes infecting wildlife alone are not subjected to such management or education led changes in host behaviour. In addition, the schistosomes are distinct from other trematodes in that they are dioecious and use only two hosts (a molluscan intermediate host and a vertebrate definitive host) for the completion of their life cycle, but the wealth of research on these trematodes (see Weil and Kvale 1985 and references therein) can be a useful start point for other species.

Another major factor in the spread of parasites is human management of farmed fish, and translocations will have substantial impacts on trematodes using these hosts. The eradication of *Echinostoma echinatum* from the Lindu Valley, Sulawesi, was attributed to the managed introduction of non-native fish, because introduced fish, incapable of hosting the parasite, inadvertently consumed cercariae when competing with native fish for phytoplankton (Carney et al. 1980). This is a particularly clear example to demonstrate how anthropogenic induced changes to an ecosystem can alter parasite prevalence. Equally, the water quality dimensions such as nutrient and organic loading

have massive implications for freshwater systems and may be key drivers of trematode population dynamics.

2.4 Biotic interactions

The role of biotic factors in shaping parasite distributions are well documented and include differences between the host sexes (Poulin 1996, Klein 2000, Arneberg 2002, Zuk 2009) and host age classes (Anderson and Gordon 1982, Anderson and Medley 1985, Pacala and Dobson 1988, Grenfell et al. 1995, Anderson and May 1991) but there is less literature on the role of additional biotic factors such as diet and biodiversity within the habitat of the parasite. Recently however, Thieltges et al. (2008) review the significant impact of biotic factors including biodiversity on parasite transmission success. Below, a number of key interacting biotic factors that are associated with trematode population dynamics in the definitive host are, briefly, outlined.

2.4.1 Diet

For diseases that are trophically transmitted, host diet ultimately defines whether or not an individual will become infected, and differences in parasite loads can be explained therefore by host prey choice. *Clonorchis sinensis* cases in China are more prevalent in men compared to women because males consume more raw fish (Lun et al. 2005). Host diet can have additional repercussions for parasite fecundity (Molan and James 1984), such that the fitness of a parasite increases with the resources available within the host habitat. This is confounded, however, by the increased host resistance that parallels the nutritional status of the host (Bize et al. 2008) and consequently, the success of the parasite is a compromise between these two components (Bize et al. 2008, Heylen and Matthysen 2011). Further, starving parasites at earlier life stages can have repercussions for the success, fecundity and longevity of the parasites once in the definitive host (Davies et al. 2001, Walker et al. 2006). Both the susceptibility of the host, and also the fecundity of the parasite can be impacted therefore by host diet.

2.4.2 Host sex and age

Sex biased parasitism has been reported extensively in the literature (Poulin 1996, Klein 2000, Arneberg 2002). In mammals there is a general trend for males to suffer greater prevalence, intensity of infections and severity of disease (Klein 2004, Zuk 2009), with consequent repercussions for host reproduction depending on mating system and parasite

virulence (see Moore and Wilson 2002, Miller et al. 2007). These sex differences have been related to hormonal differences (Klein 2000), behavioural disparities including home range size (Bundy 1988) and dispersal strategies (Greenward 1980), aggressiveness (Restif and Amos 2010) and foraging strategy (Anderson et al. 2004). All these can affect an individual's ability to compete for resources or a mate and, ultimately result in contrasting risks of infection, disease establishment and / or severity (Lindsey and Altizer 2009). In mammals, polygynous mating systems create morphological and physiological differences between males and females because of competition for mates (Zuk 2009). The resulting male-biased dimorphism (where one sex is larger than the other), on the simplest level, means males become a larger target for parasites (Poulin 1996, Arneberg 2002, Moore and Wilson 2002). There is some evidence that larger parasites carry more eggs (Poulin 1996, Hanelt 2009) and that larger hosts have larger parasites (Loot et al. 2011), so in mammalian systems, males are hypothesised to carry more, and larger, parasites (Poulin 1996) but availability of such data is rare. In addition, an increased body mass is linked to decreased leucocyte counts (Semple et al. 2002) causing the larger sex to suffer from increased pathology. Such variation in response to disease appears to result in differences in parasite infra-population size (Klein 2000), transmission rate (Perkins et al. 2003, Ferrari et al. 2004), and the genetic structure of the parasitic population (Caillaud et al. 2006). Recently it has been shown that male mammalian hosts may also shed more infective particles (for example, eggs) than their female conspecifics (Lin et al. 2006). Yet *F. hepatica,* which lacks prevalence differences between the host sexes (Mas-Coma et al. 1999), is most fecund within female hosts (children *Homo sapiens* in Peru) such that females produced c. 400 eggs per gram (epg) whilst parasites in male hosts produced only c. 100 epg (Esteban et al. 2002).

The prevalence of infections in mammals from the UK tended to show no difference between the sexes (Table 2.2) perhaps because diet is similar among the sexes for most mammals. Additional space and resources for parasites within the host habitat in the larger males, alongside a greater consumption of potentially infected fish, could explain infection intensity differences between male and female hosts where apparent (see Table 2.2; Frankland 1959). Predominantly male biases were observed in trematode infections of definitive hosts for studies outside the UK (see Klein 2004) so perhaps habitat and weather related factors cause increased differences between male and female behaviours elsewhere resulting in increased exposure to helminths of males but this geographic difference requires further study.

Table 2.2 Trematodes of Mammals in England, Wales and the Isle of Man recorded on the Natural History Museum Host-Parasite Database (as of August 2012, excludes Zoo animals); The directional effects of host sex, host age and host health on trematode prevalence P, abundance A, intensity I or fecundity F within the definitive mammalian host is presented where available in the literature. *Data additionally incorporated by the authors. NUK = Relationship identified in a study on a non-UK mammal. NS = No significant difference reported).

Parasite		Mammalian Host	Effect		
Class	**Species**		**Host Sex**	**Host Age**	**Host health**
Brachylaemidae	*B. recurva*	*Apodemus flavicollis*[1]			
		A. sylvaticus[2]			
		Rattus norvegicus[1]			
		Vulpes vulpes[3]	NS[3]	NS[3]	
	Ityogonimus lorum	*Meles meles*[1]			
		Talpa europaea[4]	NS[21]		
	I. talpae	*Talpa europaea*[4]			
Dicrocoeliidae	*Corrigia vitta*	*Apodemus flavicollis*[1]			
		A. sylvaticus[2]	NS[22]	Increased P with age[2,22]	
		Arvicola terrestris[1]			
		Clethrionomys glareolus[1]			
		Microtus agrestis[1]			
	Dicrocoelium dendriticum	*Myocastor coypus*[1]			
		Ruminants[20]	P higher in females[23]	Increased P and I with age[23]	F increases if host stressed[24]
Fasciolidae	*Fasciola hepatica*	*Bos taurus*[5]			Compromised health with respect to additional infection[25]
Heterophyidae	*Cryptocotyle lingua*	*Vulpes vulpes*[3]	NS[3]	NS[3]	
Microphallidae	*Microphallus pygmaeus*	*Mus musculus*[18]	I higher in males[26]	F higher in older conspecifics but NS[26]	
Omphalometridae	*Omphalometra flexuosa*	*Talpa europaea*[4]	NS[13]		
Opisthorchiidae	*Clonorchis sinensis*	*Homo sapiens*[19]	P higher in males[27]		

	*Metorchis albidus**	*Lutra lutra*	NS*	I higher in adults*	Males show greater pathology*
	*Pseudamphistomum truncatum**	*Lutra lutra* *Mustela vison*	NS*	I higher in adults*	Males show greater pathology*
Schistosomatidae	*Schistosoma haematobium*	*Homo sapiens*[7]	(NUK) NS in P[28]	(NUK) P increases with age until c.15 years[28], but reduced egg counts from adults (>24 years) observed[29]	Associated with diseases of the bladder and urinary tract[30]
	S. japonicum	*Mus muculus*[6]			Associated with biochemical changes affecting blood serum, liver and pancreas[30]
	S. mansoni	*Homo sapiens*[6,7] *Mesocricetus auratus*[9] *Mus musculus*[9] *Rattus rattus*[10]			Associated with biochemical changes affecting blood serum, liver and pancreas[30]

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In addition to host sex differences, parasite prevalence and intensity can be typically and positively associated with host age through the cumulative risk of exposure with time (Anderson and Gordon 1982, Anderson and Medley 1985, Pacala and Dobson 1988, Grenfell et al. 1995, Anderson and May 1991). Essentially, where parasites across the host population become less aggregated with host age, then density-dependent effects such as acquired immunity are acting on the parasite population (see Quinnell et al. 1995). Trophically transmitted parasites tend to accumulate with host age but parasite fecundity decreases as the parasite ages (see Table 2.2; Shaw et al. 1999). This has implications for disease spread because hosts that have been infected for a longer period may have more parasites but release less infective units (eggs) than younger conspecifics carrying fewer parasites. If the host continuously acquires more parasites, however, the overall fecundity of the individual hosts' parasite population (and release of eggs into the environment) will remain high. Further, host resistance against parasites is heritable resulting in generational differences in parasite load and / or fecundity across the host population (Smith et al. 1999).

2.4.3 Parasite competition and co-infection

Trematode populations may be regulated further through density-dependent mechanisms such as competition (Madhavi et al. 1998). Both intra- and inter- specific competition can impact the life history strategy (Jackson et al. 2006, Lagrue and Poulin 2008) and transmission success (Pederson and Fenton 2007) of co-habiting species. For example, a higher proportion of *Coitocaecum parvum* exhibited progenesis when the molluscan host was co-infected with *Microphallus* sp. (see Lagrue and Poulin 2008). Competitive exclusion within the snail intermediate host limits the number of multiple helminth infections (Soldanova et al. 2012). In addition, pre exposure can limit the intensity of trematode infection where the host immune system is primed and this acquired immunity can protect the host from secondary species infection (Luong et al. 2011). Processes such as intraspecific competition are perhaps less important for trematodes because a large proportion of early life-stage parasites (for example, cercariae) die prior to transmission which results in naturally low recruitment rates (see Underwood 1979, Keough and Chernoff 1987).

The likelihood of co-infection can change depending on the life-stage of the parasite species. Trematodes have different requirements at different life stages: trematodes reproduce in the first intermediate and definitive hosts, whilst the second intermediate host (fish) can be viewed as a transmission vehicle. The resulting intensity of competition between parasites at each life stage will therefore differ considerably (Karvonen et al. 2012 but see Niewiadomska and Pojmańska 2011). Karvonen et al. (2012)'s recent hypothesis suggests that co-infections will be less common in snails than in fish because resources in the snail are limited so that trematodes would be negatively affected at this life stage. Conversely, resources are not limited in the fish when the parasite is dormant. In addition, there is a benefit to co-infection from the parasite's perspective because species specific responses to cysts by the host are less likely to develop as a consequence of continual exposure to generic species. This hypothesis was supported by observed low levels of co-infection in snails and high levels in fish (Karvonen et al. 2012). Alternatively, such a trend may result from sheer numbers: in snails, a single parasitic unit (sporocyst) produces thousands of clones (cercariae) perhaps restricting space for further trematode infections so that the first parasite to infect a snail becomes dominant. Conversely, the low impact metacercariae, encysting on fish, have little impact on the space or resources of the fish host. Under natural conditions, co-infections are common and considered the norm (for example Lello et al. 2004, Pederson and Fenton 2007, Telfer et al. 2010). In mice, co-infection with multiple strains of *Trypanosoma brucei* mitigated the negative pathological effects caused by single strain infections (Balmer et al. 2009) and perhaps this acts to alleviate the major effects of infections in natural populations.

The parasite genotype may interact with the number or type of parasites infecting a single host to influence the overall distribution and assembly of parasites. For example, the probability of multiple infections of *Microbotryum violaceum* apparently depends on the genotypes of the particular interacting strains (Koskella et al. 2006). In the dioecious schistosomes, genetic structure differs between the sexes (see Prugnolle et al. 2003) with c. 50% of genes biased to a particular sex (Beltran and Boissier 2010). Schistosomes are monogamous and demonstrate male biased sex ratios in wild populations (Beltran et al. 2009), although this ratio can be altered by ecological variables (see Mone 1997). As a

result, separation of monogamous couples ('divorce') increases with increasing male-biased parasite ratios and this allows genetic diversity to increase across the population because more males would be able to contribute to the gene pool (Beltran et al. 2009). Further, the immune defence of the host is more successful against genetically more similar male worms than more distinct ones to those experienced during previous infection which is again tailored toward greater genetic diversity in schistosomes (Beltran et al. 2011).

2.4.4 Trematodes as generalists

Trematodes are able to infect a range of host species at each life stage. This combination of hosts may serve to dilute the overall parasite abundance within any single species host population (Weil and Kvale 1985). Both the nature of a parasite's life-cycle and niche breadth (the specific host species available) can explain interspecific and latitudinal variation in geographic range size of parasites (Poulin et al. 2011). The diversity of the ecosystem under consideration will have additional impacts on the success of parasite populations in their definitive host. For example, the presence of predatory fish can reduce cercarial populations (see Weil and Kvale 1985). An understanding of the parasitic fauna within an ecosystem through comprehensive sampling can ultimately contribute to an analysis of ecosystem health (see Hechinger and Lafferty 2005).

Parasites can influence their hosts in several ways and are integral in shaping their host community (Poulin 1999a). Direct impacts include a decrease in the ability of an infected individual to compete or survive predator-prey interactions within the host environment (Mouritsen and Poulin 2002). In some cases, native parasites are able to increase in abundance following the arrival of a novel host species; a mechanism known as 'parasite spillback' (Kelly et al. 2009). This may apply equally to cases where a native species recovers from a population crash so that the increase in host numbers benefits the parasite community by increasing their abundance and potential to infect other host populations.

2.5 Combined impacts of abiotic and biotic factors

The huge variation in different trematode-mammalian host interactions is evidence of the multitude of interacting variables that can define parasite distributions. The advance in

statistical methodologies (for example General Linear Models GLMs, Mixed-Effects GLMs) has allowed researchers to consider the combined impact of abiotic and biotic factors (see Nelder and Wedderburn 1972; e.g. Lord et al. 2012). Yet the concept of mixed effects has been considered since Anderson and May (1979) identified that: i) increasing host density leads to an increase in hosts contacting infective stages but no proportional change in parasite prevalence, whilst; 2) climate acts as a mechanism to generate changes in prevalence across the system because of the interplay between temperature and rainfall. Investigating the relationships between trematodes and mammalian hosts (particularly in the lesser-studied wild mammal systems) will ultimately allow comprehensive predictions of future parasite distributions in response to a changing world (e.g. Sripa 2010, Hotez and Gurwith 2011).

2.6 Conclusions and future directions

The impact of abiotic stressors on transmission success and population dynamics of host-parasite systems is significant but often system specific (for example, Dobson and Carper 1992, Lafferty and Kuris 1999, Bush et al. 2001, Pietrock and Marcogliese 2003). Parasites are an integral unit of any ecosystem but stochastic changes that dramatically change the abundance of either host or parasite population can alter the dynamics of the host-parasite interaction. The combined impact of both abiotic and biotic factors ultimately dictates the host-parasite dynamic of parasite systems and is considered less often in the literature. The lack of opportunities to survey the parasitic fauna of medium-large wild mammals is a significant challenge to our understanding of the dynamics of host-helminth systems. Further, to predict the response of parasitic populations to a changing climate we must first understand the current nature of host-parasite interactions and utilise surveys that can monitor long-term patterns defining such systems. Much work on parasite ecology expresses the species-specific nature of these organisms but there may be general principles that are applicable across host taxa. The incorporation of multi-disciplinary approaches – long-term studies, deterministic models and survey work – to consider fully the multiple factors operating, will be most beneficial to understand further the processes underpinning the dynamic nature of helminth distributions.

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CHAPTER 3

3. Abiotic and biotic factors associated with tick population dynamics on a mammalian host: *Ixodes hexagonus* infesting otters, *Lutra lutra*

3.1 Abstract

The Eurasian otter, *Lutra lutra,* hosts several parasites with zoonotic potential. As this semiaquatic mammal has large ranges across terrestrial, freshwater and marine habitats, it has the capacity for wide dispersion of pathogens. Despite this, parasites of otters have received relatively little attention. Here, we examine their ectoparasite load and assess whether this is influenced by abiotic or biotic variables. Climatic phenomena such as the North Atlantic Oscillation (NAO) affect weather conditions in northern Europe. Consequently parasite distributions, particularly species with life stages exposed to the external environment, can be affected. We assessed the extent to which inter-annual variations in large-scale weather patterns (specifically the NAO and Central England (CE) temperatures) and host characteristics influenced tick prevalence and intensity. Ectoparasites consisted of a single species, the nidiculous tick *Ixodes hexagonus* (prevalence = 24.3%; mean intensity = 7.2; range = 1-122; on n = 820 otter hosts). The prevalence, but not intensity of infestation, was associated with high CE temperatures, while both prevalence and intensity were associated with positive phases of the NAO. Such associations indicate that *I. hexagonus* are most abundant when weather conditions are warmer and wetter. Ticks were more prevalent on juvenile than sub-adult or adult otters, which probably reflects the length of time the hosts spend in the holt where these ticks quest. High tick number was associated with poor host condition, so either poor condition hosts are more susceptible to ticks, or tick infestations negatively impact on host condition. Otters are clearly an important and common host for *I. hexagonus*, which has implications for vector-borne diseases. This work is the first to consider the impacts of long-term weather patterns on *I. hexagonus* and uses wild-animal cadavers to illustrate the importance of abiotic and biotic pressures impacting parasitic populations.

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3.2 Introduction

Current change in climate (the long-term average meteorological conditions of a region IPCC 2007) is associated with increases in temperature and precipitation, especially in Northern temperate zones (IPCC 2007). This influences parasite distributions both directly (Kutz et al. 2004, Mangal et al. 2008) and indirectly, for example via impacts on host range (Patz et al. 1996, Harvell et al. 2002). Weather (short-term variation in meteorological conditions) can cause variations in parasite distributions whilst synchronously influencing host abundance (Cattadori et al. 2005) but will affect specific host-parasite interactions differently (Patz et al. 1996, Kovats et al. 2001, Lafferty 2009, Moller 2010). Weather patterns are influenced by climatic phenomena such as the North Atlantic Oscillation (NAO). The NAO affects European climate such that, when in positive phases, northern Europe experiences warmer and wetter conditions (Hurrell et al. 2003, Lopez-Moreno and Vicente-Serrano 2007). Identifying associations between climate and the distribution of vectors over time (e.g. Mills et al. 2010, Jongejan and Uilenberg 2004) is an essential pre-requisite to understanding public and wildlife health risks resulting from vector-borne infection.

Ixodid ticks are vectors for a range of pathogens causing diseases including Lyme disease, Boutonneuse fever and tick-borne encephalitis (Hillyard 1996). *Ixodes hexagonus* is an efficient vector of *Borrelia burgdorferi*, the causative agent of Lyme disease (Gern et al. 1991) but in the UK, *I. ricinus* has received most attention because of its ubiquitous nature and association with transmission of pathogens to humans and livestock (Hillyard 1996). The distribution of *I. ricinus* is influenced by weather (Randolph et al. 2002, Hancock et al. 2011) and the presence of suitable hosts and habitat (Gray et al. 1992). The increasing population density and geographic range of *I. ricinus*, a European tick (Hancock et al. 2011, Lindgren et al. 2000), and other tick species such as the North American species *I. scapularis*, are associated with increasing temperatures (Ogden et al. 2006). The majority of ixodid ticks require >80% relative humidity for survival off the host (Macloed 1934, 1939, Medlock et al. 2008) and as such, positive phases of the NAO may benefit ixodid ticks by creating suitably humid weather. Landscape, habitat use and local weather conditions have been associated with tick distributions previously (Randolph et al. 2002, Hancock et al. 2011, Lindgren et al. 2000,

Ogden et al. 2006, Arthur 1953, Hoberg and Brooks 2008). The impact of such environmental variables on host-parasite interactions is, however, highly variable (Tylianakis et al. 2008). Mustelids have been associated with the nidiculous (burrow or nest dwelling) tick *I. hexagonus* (Arthur 1953) but the relationship between *I. hexagonus* and weather conditions has not been examined previously.

The Eurasian otter, *Lutra lutra*, is a top predator in the UK and a sentinel of freshwater health (Chadwick et al. 2011). Otters are wide ranging opportunistic predators that feed in terrestrial, freshwater and marine habitats (Kruuk 2006). They are therefore potentially exposed to a wide diversity of pathogens and a great deal can be learned about the distribution of parasites in UK ecosystems by screening such a generalist host. Here, we identified the tick species that use otters as a host. Next, we investigated how weather patterns and host characteristics are associated with tick infestations of otters in England and Wales. Specifically, we hypothesised that tick occurrence (prevalence and intensity) would be positively correlated with temporal variation in: i) the NAO (associated with warmer and wetter weather in the UK), and; ii) higher Central England (CE) temperatures (a long-term record of temperature in central England, see Materials and Methods). Based on these findings we hypothesised that spatial variation in tick counts among meteorologically distinct regions of the UK would correlate positively with rainfall and temperature.

3.3 Materials and Methods

3.3.1 The host

The Cardiff University Otter Project receives dead otters, *Lutra lutra*, from across England and Wales. Most (86% of the current study) have been killed by road traffic and are stored subsequently at -20°C. The location (British National Grid Reference) and date of death (month and year), sex, age-class (juvenile (N = 25), sub-adult (238) and adult (312)) and size (weight (kg) and length (m)) were recorded for each otter collected between 2004 and 2010. A condition index K was calculated controlling for the dimorphism of otter sexes, following (Kruuk et al. 1987). Such that:

Equation 3.1

$$K = \text{weight} / [a \times \text{length}^{n}]$$

where a = 5.02 and n = 2.33 for females, and a = 5.87 and n = 2.39 for males (Kruuk et al. 1987). Seasons were defined as winter: December-February, spring: March-May, summer: June-August, and autumn: September-November. Very decomposed otters were excluded from the analysis. Remaining otters included in the model (and excluding those with missing data; n = 575) were distributed across seasons and years as follows: spring = 137; summer = 83; autumn = 179; and winter = 176; 2004 = 12; 2005 = 48; 2006 = 70; 2007 = 122; 2008 = 146; 2009 = 116; and 2010 = 61.

3.3.2 Parasite identification

Ticks were removed (via pelt searching and fur combing) and stored in 90% molecular grade ethanol prior to immersion in 0.1% saline solution for microscopic examination (x30 magnification) using a Nikon dissecting microscope with fibre optic illumination, and identified to species using morphological features (Snow 1979, Hillyard 1996). *Ixodes hexagonus* was the only tick species present and species identification of 15 specimens (5 adults, 5 nymph and 5 larvae) was confirmed by the Natural History Museum. Occasionally, damage or desiccation prevented morphological identification so for these specimens we sought confirmation using mitochondrial DNA cytochrome oxidase sub-unit 1 (COX1) analysis as follows:

DNA was extracted from 13 specimens, three adults, three nymphs and seven larvae, from four geographically separate hosts. Ethanol was evaporated fully from each sample. Extractions were conducted using a QIAGEN kit as per the manufacturer's protocol (QIAGEN DNeasy Blood and Tissue Handbook 2006) with the additional step of manually crushing each tick body with a sterile pin tip at the start of the process. PCR followed standard procedures (QIAGEN DNeasy Blood and Tissue Handbook 2006). Novel primers (IHEXCO1F: 5'- TCATAAAGACATTGGGACT-3', IHEXCO1R: 5'-TGGTAAAGAATGGGGTCT-3') were designed by alignment of COX1 mtDNA from 8 reference tick species (GenBank: *Dermacentor reticulatus* AF132829, *Haemaphysalis punctata* FN394339.1, *Hyalomma aegyptium* AF132821, *Ixodes uriae* NC006078, *I.*

hexagonus AF081828.1, *I. lividus* GU124743, *I. ricinus* FN394342 and *Rhipicephalus sanguineus* NC002074). The PCR reaction conditions were carried out in a 50µl final volume, with 10x PCR buffer II (Applied Biosystems, UK), 50mM MgCl (Applied Biosystems, UK), 2.5mM of each dNTP, 10pmol/µl of each primer, 0.5U Taq DNA polymerase (Invitrogen) for each 10 µl DNA template. PCR conditions (GenAmp PCR System 9700, Applied Biosystems, UK) were: 95°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 53°C for 1 min and 72°C for 1 min, with a final extension of 72°C for 10 min. PCR products produced identical sized bands for all tick samples on a 1.5% agarose gel. Four larvae, one nymph and one adult were sequenced (QIAGEN, Genomic Services, Germany) using both forward and reverse primers. All 592 bp sequences from the current study were identical and showed 99% similarity to the corresponding region of GenBank *I. hexagonus* AF081828.1. This reference sequence, AF081828.1, was obtained from laboratory maintained ticks over ten years ago (Black and Roehrdanz 1998), perhaps explaining the 3bp discrepancy (at position 130 transition T to C, and at positions 172 and 188 transversions A to C). The next closest sequence match was 82% with *Ixodes asanumai* Kitaoka 1973 (GenBank: AB231674.1).

3.3.3 Data preparation

Temporal variation in weather was quantified using mean monthly temperatures (°C) for Central England (CE temperature) (Parker et al. 1992) and North Atlantic Oscillation (NAO) phases (http://www.cpc.ncep.noaa.gov/products/precip/CWlink/pna/nao.shtml, data provided by the Climate Prediction Centre of the U.S. National Oceanographic and Atmospheric Administration website). The mean of each was calculated for: i) the month of host death; ii) the sixth month period preceding host death; and iii) the year preceding host death, for each otter. These time periods were selected based on literature indicating that populations may be influenced by conditions during the previous season or year (Randolph et al. 2002, Ruiz-Fons and Gilbert 2010).

Spatial variation in climate was quantified using long-term averages, which are a useful tool to describe the state of the climate in a particular region (Perry and Hollis 2005). Long-term yearly average (1971-2000) temperature (maximum and minimum, ºC) and rainfall (mm) measures for meteorologically distinct regions of England and Wales were

collated (Perry and Hollis 2005). These regions are defined as East and Northeast England, East Anglia, Southeast England and Central South, Northwest England and North Wales, South Wales and Southwest England, and Midlands (Figure 3.1) and are used by the Meteorological Office UK Climate Impacts Programme (UKCIP) to summarise weather patterns in the UK (http://www.metoffice.gov.uk/climate/uk/averages/19712000/). To determine the abiotic conditions for each sampled carcass, otters were assigned the regional average for climate data depending on their geographic location at time of death. Associations between these measures of climate and tick prevalence (the number of hosts infested with specific parasitic species, in the current study ticks, divided by the total number of hosts examined, Bush et al. 1997), intensity (the number of individuals of a particular parasite species on a single infested host, Bush et al. 1997) and tick count (the total number of ticks within a population) were examined from otters found between 2004 and 2010.

3.3.4 Data analysis

Ectoparasites are thought to abandon dead hosts (Nelder and Reeves 2004). We tested initially, therefore, whether there was a difference in tick abundance between fresh (collected within 24 h of death, N = 610) and not fresh (otters characterised as slightly or moderately decomposed, N = 210) otters. We found no significant difference in tick presence/absence ($\chi^2_{1,820} = 0.515$, $p = 0.473$) or median intensity (Kruskal-Wallis $H_{1,195} = 0.35$, $p = 0.556$) and subsequently pooled all data for further analyses.

The NAO and CE temperatures, for each time period examined, and host factors (sex, age, condition, season and year of death) were combined in a general linear model fitted to the tick presence/absence data with a binomial error distribution (N = 575 hosts, individuals were omitted where data was missing). A generalised additive model incorporating these explanatory variables was fitted to the tick intensity data (tick counts per otter excluding zero counts) with negative binomial error distribution. Relationships between explanatory variables and tick intensity were non-linear. A generalised additive model (GAM) was applied therefore with splines fitted appropriately. Final models were selected using Akaike Information Criterion (AIC).

Tick counts were compared between the meteorologically distinct regions of England and Wales (described above) by calculating regional mean tick intensities and testing for a correlation with the long-term yearly average maximum and minimum temperature (ºC) and total rainfall (mm) in each region.

The spatial distribution of infested otter carcasses (N = 199) was examined to look for clustering within the host distribution (N = 820) by calculating a modified Ripley's K statistic, K[i.](r), using Ripley's isotropic edge correction (Ripley 1988) with a simplified border of England and Wales as a boundary (for further details of methodology, see Sherrard-Smith et al. 2009). All statistical analyses were conducted using R version 2.12 (R Development Core Team 2008).

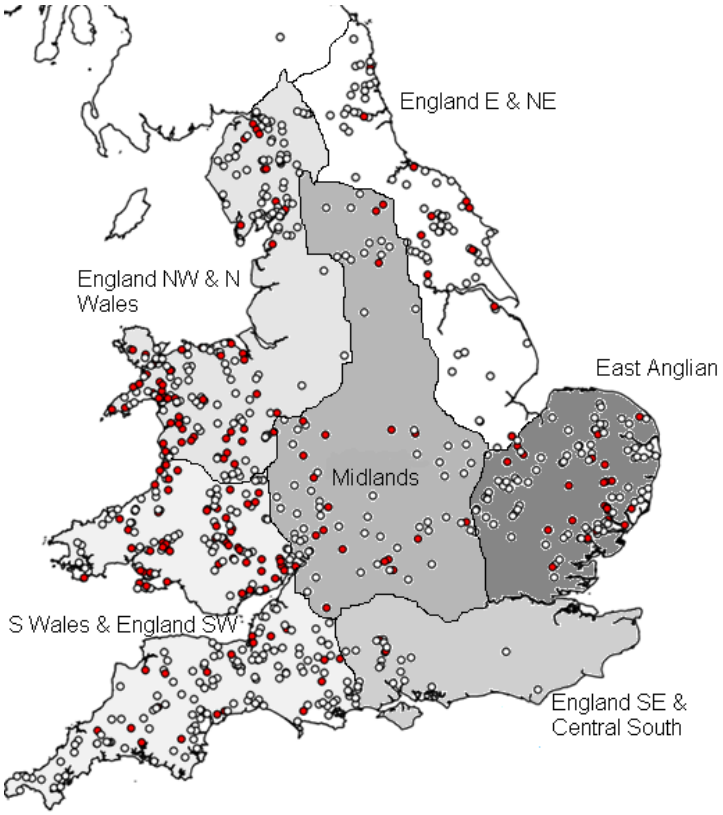

Figure 3.1 Distribution of *Ixodes hexagonus* infested (red circles) and uninfested (clear circles) otters in England and Wales. Meteorologically distinct regions (East and Northeast England, East Anglia, Southeast England and Central South, Northwest England and North Wales, South Wales and Southwest England, and Midlands) defined by the Meteorological Office UK Climate Impacts Programme (data available online).

3.4 Results

3.4.1 Tick species

Tick (*Ixodes hexagonus*) prevalence on Eurasian otters, between 2004 and 2010, was 24.3% (199 out of 820) (Figure 3.1). On some hosts, all post-hatch tick life stages (larva, nymph and adults) were recovered (18 cases), but almost 40% of hosts had only one life stage present at collection (Larvae = 13 cases, Nymph = 40 cases, Adult = 26 cases) (Table 3.1). Infested otters were widespread across England and Wales (Figure 3.1) with no evidence of clustering of infestation within the otter distribution (Ripley's K analysis at the 95% confidence level using radii ranging from 1 km-130 km).

Table 3.1 Summary of *Ixodes hexagonus* on otters: *Ixodes hexagonus* infestations of *Lutra lutra* in England and Wales between 2004 and 2009 (N = 820); showing prevalence, parasite count, mean intensity with upper and lower 95% bootstrap confidence interval (10000 iterations), and maximum intensity for each tick life stage.

Parasite stage	Prevalence (%)	Count (/ 820 hosts)	Mean Intensity (95% CI)	Range
Any stage	24.3	199	7.2 (5.5-9.2)	1-122
Larva	9.3	76	7.7 (4.7-11.7)	1-112
Nymph	15.1	124	4.0 (2.9-5.2)	1-44
Adult	11.8	97	2.7 (2.1-3.5)	1-26

3.4.2 Abiotic factors

Tick prevalence on otters was associated with higher Central England (CE) temperatures for the 12 month period preceding host death (GLM_{569}: $t = 2.594$, $p < 0.01$), and more positive phases of the North Atlantic Oscillation (NAO) over the 12 month period preceding host death (GLM_{569}: $t = 2.099$, $p < 0.05$) (Figure 3.2). Tick intensity was not significantly associated with CE temperatures over any period preceding host death (GAM_{155}: $t = 1.445$, $p = 0.15$). Tick intensity was, however, positively associated with the NAO at month of host death (GAM_{155}: $t = 2.670$, $p < 0.05$) (Figure 3.3).

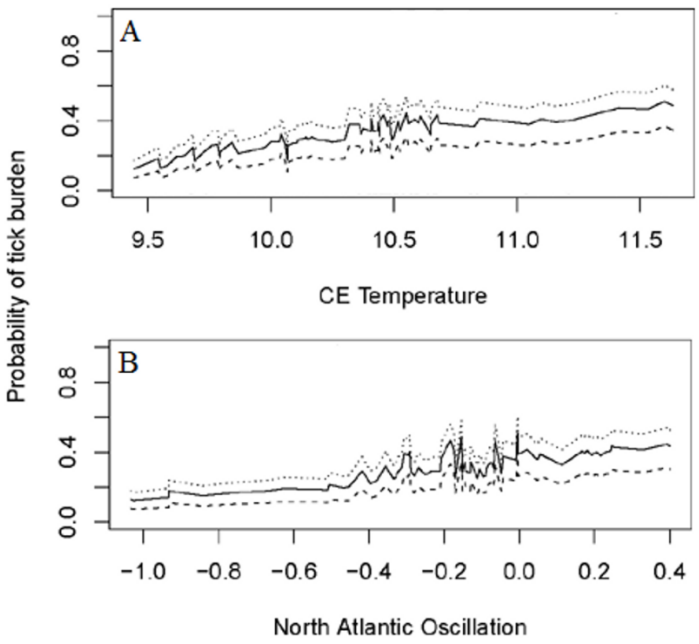

Figure 3.2 Probability plot for a model of the association between tick prevalence and the explanatory variables A) Central England Temperature for the 12 month period preceding host death, B) North Atlantic Oscillation for the 12 month period preceding death for each host age class: Dotted line = juvenile hosts; Solid line = Adult hosts; Dashed line = Sub-adult hosts.

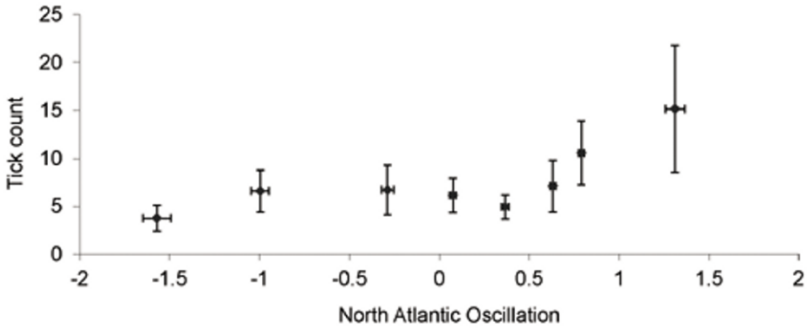

Figure 3.3 Relationship of tick count to mean North Atlantic Oscillation at month of host death. Standard error bars shown.

The South Wales and Southwest England region (Figure 3.1) had significantly higher tick counts on otters than all other regions, while East and Northeast England had significantly lower tick counts than all other regions; these two regions contributed most strongly to the statistically significant difference in counts between regions (χ^2_5 = 302.169, p < 0.001). Mean intensities for each region did not, however, correlate with maximum or minimum temperature, or mean rainfall for the long-term yearly average (1971-2000) regional data (Correlations, all p > 0.1) (Figure 3.4). There were no associations with season between larval, nymph or adult stage ticks on otters (GLM, p > 0.1).

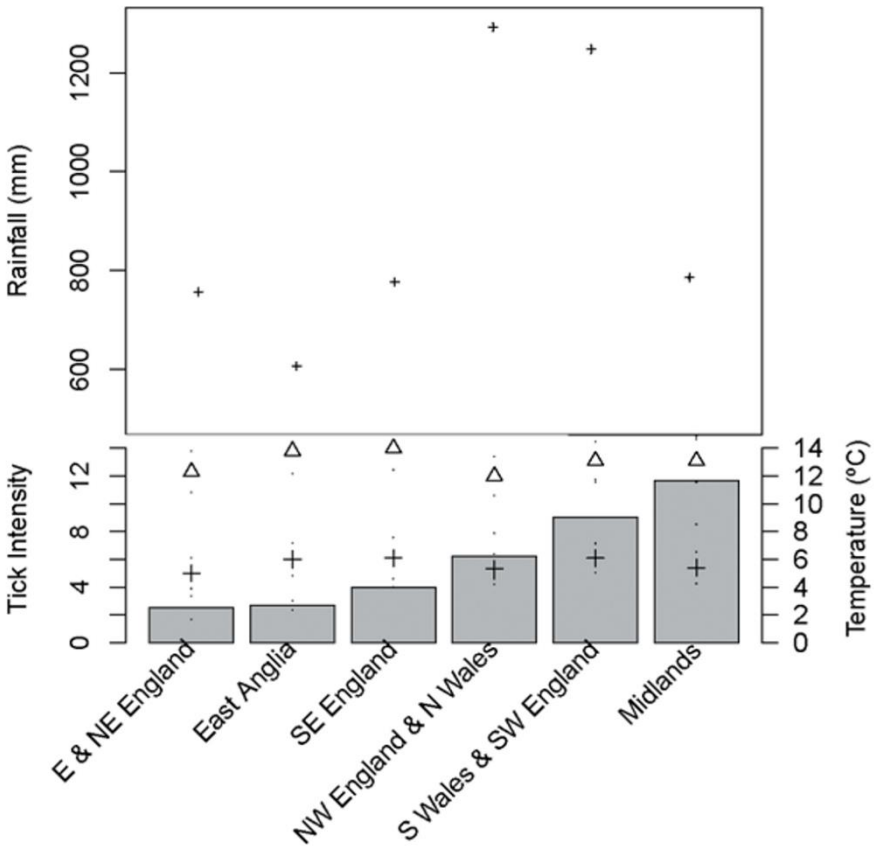

Figure 3.4 Mean tick intensity (grey bars) in each meteorologically distinct region (East and Northeast England, East Anglia, Southeast England and Central South, Northwest England and North Wales, South Wales and Southwest England, and Midlands) and corresponding 30 year average (1971-2000) summed mean rainfall (mm) (upper Y-axis), maximum (triangle) and minimum (cross) 30 year (1971-2000) average temperature (°C) for each region (lower Y-axis). Standard error marks for rainfall, maximum and minimum temperature correspond to variability in monthly averages.

3.4.3 Biotic factors

More juvenile otters were infested than older age-classes (GLM: $p > 0.01$; Figure 3.2). The mean host condition 'K' for the sampled population was 1.0286. Tick intensity was inversely related to otter condition so that as otter condition increased, tick intensity decreased (GAM_{155}: $t = 3.137$, $p > 0.01$) (Figure 3.5).

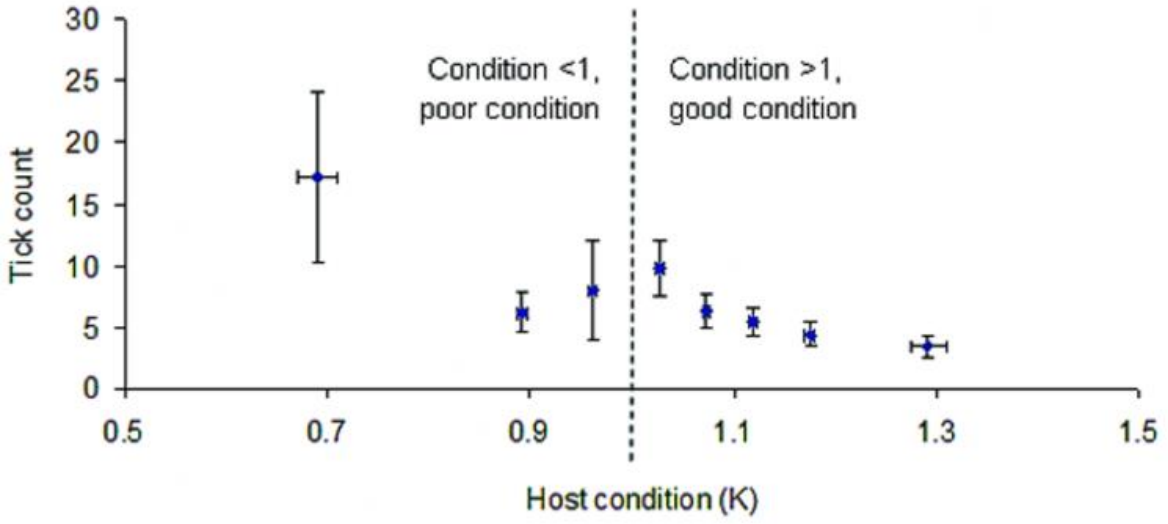

Figure 3.5 Relationship of tick intensity to host condition (K). Standard error bars shown.

3.5 Discussion

Ixodes hexagonus is the only tick species reported from the Eurasian otter (Kelly et al. 2001, current study). *Ixodes hexagonus* can complete its life cycle on the European hedgehog (Arthur 1953), fox (Harris and Thompson 1978) and American Mink (Page and Langton 1996). As all three post-hatch tick life stages were found on the otter in the current study, it appears that *I. hexagonus* can also potentially complete its life cycle on this mammal. The prevalence of *I. hexagonus* on otters (24.3%) is lower than that reported on European hedgehogs, which are the preferred host for this tick (Arthur 1953, Pfäffle 2010) (53.3% prevalence on hedgehogs from Western Europe, Pfäffle 2010). *I. hexagonus* is encountered by domestic dogs and cats in urban areas (Ogden et al. 2000) illustrating the close proximity of this particular tick to human populations. The prevalence of *I. hexagonus* on otters is, however, high in comparison to its prevalence on the domestic dog in the UK (5.6%, N = 3534, Smith et al. 2011). Further, the mean

intensity (the total number of parasites of a particular species found in a sample divided by the number of infested hosts, Bush et al. 1997) of ticks is higher on otters (7.2 per host) than on hedgehogs (3.8, Pfäffle et al. 2011) despite examination of cadavers in the current study and live hosts in the hedgehog study. This suggests that otters are a noteworthy host for *I. hexagonus*. The association between otters and *I. hexagonus* populations may be important for pathogen transmission, particularly if otters act as either reservoir or amplifier hosts, or reduce pathogen abundance through the dilution effect (Schmidt and Ostfeld 2001). Further, otters have large home ranges (Green et al. 1984, Kruuk 2006) indicating that this host has the potential to transfer ticks between habitat islands. The nocturnal and aquatic nature of otters may deter other tick species from utilising such a resource, explaining the absence of diversity in tick species.

Positive phases of the North Atlantic Oscillation (NAO) were associated with increased prevalence and intensity in tick populations on otters. Strong positive phases of the NAO are linked with above average temperature and precipitation across northern Europe. Together with the elevated humidity produced, such weather conditions may lead to increased abundance of *I. hexagonus*, as reported for *I. ricinus* and *I. scapularis* (Lindgren et al. 2000, Ogden et al. 2006, Hancock et al. 2011). This may be related to the weather conditions causing changes in the behaviour of either the parasite or the host thereby altering infestation rates (see Kerr and Bull 2006). No previous literature was found relating NAO to *I. hexagonus*. In *I. ricinus* however, the NAO did not correlate with intensity of tick infestation but negative winter NAO phases (associated with warmer and wetter winters) corresponded to increased Borreliae infections (Hubalek et al. 2003). Further investigation into the underlying pathogenic infections of otters would be useful to examine whether this association holds for *I. hexagonus.*

Both NAO and CE temperatures are indices that can be used to describe temporal variation in weather; they do not provide spatially explicit weather data within the UK. The significant relationships found in the current study therefore describe an association between otter ticks (prevalence and intensity) and temporal variation in weather. We tested subsequently whether warmer and wetter regions were associated with higher tick infestations of otters, and found significantly more ticks in South Wales and Southwest

England – a region associated with higher rainfall (Perry and Hollis 2005). Overall, however, at the regional scale we identified no significant correlations between the mean intensity of ticks and either temperature or rainfall. This may be because at the regional scale temperature and rainfall are negatively correlated, so a more detailed analysis of local weather is necessary to clarify their interaction. Other factors such as the distribution of non-otter hosts, and variation in habitat type, may also heavily influence spatial variation in tick abundance. In preliminary investigations we explored the impact of local weather, alternative hosts, and habitat on *I. hexagonus* distribution, but subsequently removed these from our analyses because: i) Restricted availability of data meant that inclusion of both spatial and temporal variation in weather reduced the size of the dataset considerably, rendering conclusions less robust; ii) Information on the reported distribution of alternative hosts (hedgehog and fox) and of *I. hexagonus* were obtained from the National Biodiversity Network (NBN). Hedgehogs and foxes are both widespread and abundant in the UK and therefore availability of alternative hosts seems unlikely to limit *I. hexagonus* distribution at the regional scale. Further, *I. hexagonus* records from the NBN are concentrated in the London area of South East England, but because this database relies heavily on records submitted by members of the public this is likely to represent bias due to distribution of the human population. The NBN records map presence only (and not absence on screened hosts), so it was not possible to test for clustering within the host distribution as we did for *I. hexagonus* on otters. Comparisons were therefore uninformative; iii) Data on land use (arable, broadleaf and coniferous woodland, improved and semi-natural grassland, and upland habitat) were obtained from the Countryside Information System (CIS version 8, available online). ArcMap GIS (version 9.2) was used to interrogate these data and to assign percentage cover of each land-use within a 20 km radius of each otter. Significant negative associations were revealed (between tick prevalence and arable land, improved grassland, and conifer woodland), but interpretation is questionable because of the heterogenous and patchy nature of habitat data, the relatively large areas examined which may not accurately reflect the nature of real otter ranges (these tend to be linear along water courses, and vary considerably in length from a few to 40km, Kruuk 2006), and the difficulty in defining where, within this unknown range, an otter may have become infested.

Tick prevalence, but not intensity on otters, was associated with CE temperature. As far as we are aware, there are no previous records of temperature effects on *I. hexagonus* and the only long-term study on population dynamics of *I. hexagonus* indicates little seasonal variation and low-level abundance (on hedgehogs, Pfäffle et al. 2011). In general, however; temperature has a key role in driving tick development rates (Lindgren 2000, Hancock et al. 2011) and so affects population dynamics (Randolph et al. 2002, Hancock et al. 2011). Additionally, temperature tends to be associated with length of diapause, larval activity and adult interactions (Randolph 2004). Particularly strong associations are found between *I. ricinus* and temperature (Hancock et al. 2011). Stochastic temperature variations across the year are predicted to alter population dynamics of *I. ricinus* with subsequent impacts on the transmission of vector borne diseases (Hancock et al. 2011). The contrasting impact of temperatures on *I. hexagonus* and *I. ricinus* may be attributable to the ecological differences between the two species. The most important of these is likely to be habitat choice. *I. hexagonus* is nest dwelling, and so to some extent insulated from changes in ambient temperatures. In contrast, *I. ricinus* uses open areas for questing (Hillyard 1996), so is likely to be exposed to wider fluctuations in air temperature.

Juvenile otters were more frequently infested with *I. hexagonus* than adult hosts. Host age, in general, influences the intensity of infestations, but can also affect parasite-induced mortality, and the distribution of the parasite among host individuals (Hawlena et al. 2006). Several hypotheses (Sol et al. 2003, Hawlena et al. 2006) predict that juveniles will carry heavier infestations than older hosts, either because: i) adult hosts develop immunity and/or behavioural adaptations to avoid or remove parasites; and/or ii) heavily infested juveniles die before adulthood (selection hypothesis, Sol et al. 2003) although this is very unlikely as a direct cause of death. Grooming is a learned activity in otters (Kruuk 2006) and may contribute to lower tick numbers on older otters (Kruuk 2006). Additionally, young otters spend the majority of their time in holts, the resting place of otters (Kruuk 2006) so are disproportionately exposed to such parasites. Effects may be underestimated here, however, because road killed samples tend to reflect the healthier section of the population (Nusser et al. 2008).

Finally, we found a relationship between host condition and tick intensity such that a better host condition is associated with decreased intensity. This is not a reflection of the elevated infestations on juvenile hosts because the host condition index used here (Kruuk et al. 1987) controls for size and therefore age, in addition to sexual dimorphism. This positive relationship could imply that otters in better condition are more efficient at grooming and thereby rid themselves of ticks, or that ticks have a negative impact on otter condition.

We acknowledge that data from road-killed hosts are likely to underestimate tick counts and recognise that road-kill samples are a stochastic sub-sample of a population and may lead to bias in terms of the proportion of the host population examined. For protected species, however, road-kill samples remain the only way to obtain large sample sizes for analysis. The absence of tick species other than *I. hexagonus,* in concordance with the only other report of otter tick infestations (Kelly et al. 2001), could reflect differences in emigration patterns when abandoning a dead host, while tick emigration rates from dead hosts may interact with local microclimate. Our analysis of recently killed versus decomposed otters (see Materials and Methods), however, reveals no significant difference in infestation levels or species diversity, suggesting that observed associations are robust. Such data can therefore successfully illustrate associations between inter-annual variations in weather patterns, host characteristics and *I. hexagonus* populations.

To our knowledge this work is the first to consider the impacts of weather on *I. hexagonus*, and reveals that inter-annual variations in large-scale weather patterns, together with host characteristics, combine to affect the distribution, prevalence and intensity of *I. hexagonus* on Eurasian otters. Associations were identified between positive NAO phases, CE temperatures and tick prevalence, suggesting that the predicted change in climate in northern temperate zones may cause an increase in *I. hexagonus* populations. Although the associations highlighted here may not necessarily parallel what is observed on other hosts for this tick, *I. hexagonus* is common on domestic cats and dogs (Ogden et al. 2010) and we suggest that tick research should, perhaps, target species other than *I. ricinus* in the future. This study illustrates how surveys of wild-animal cadavers can be hugely informative about parasitic populations.

3.6 Acknowledgements

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4. Climate variables are associated with the presence of biliary trematodes in otters

4.1 Abstract

Parasites with complex life cycles are expected to be disproportionately affected by climate change. Knowledge of current associations with weather and host-parasite interactions is therefore essential for the inference of future distributions. The Eurasian otter, *Lutra lutra,* is exposed to a range of parasites due to its large home range and use of terrestrial, freshwater and marine habitats. As such, it can act as a sentinel species for generalist parasites. Here we consider two biliary parasites recently reported in the United Kingdom, *Pseudamphistomum truncatum* and *Metorchis albidus* (Trematoda, Opisthorchiidae), and ask whether there are associations between abiotic factors (season, temperature, rainfall and the North Atlantic Oscillation) and the prevalence and intensities of these parasites in otters (n = 586). To control for biotic interactions we first examined whether particular sub-groups of the otter population (grouped by sex, age-class and condition) are more prone to infection and whether any damage is associated with the presence of these parasites. Even though mean intensities of the smaller trematode, *P. truncatum* (28.3 worms/host), were much higher than *M. albidus* (4.1), both parasite species had similar impacts on the otter. The distributions of parasites on host sexes were similar, but males suffered greater damage and regardless of sex, parasite intensity increased in older hosts. The probability of infection with either parasite was negatively associated with ground frost, minimum temperatures and rainfall, but was positively associated with warm long-term average temperatures. Although it is widely accepted that multiple variables influence parasite distributions, to our knowledge this is one of only a few studies to examine the combined impact of biotic and abiotic variables on parasites with complex life cycles within their wild definitive host. Identifying such associations can give greater accuracy to predictions concerning the distribution and spread of trematodes with future climate change.

4.2 Introduction

Abiotic factors have direct impacts on parasites, particularly during free-living life-stages (eggs, cysts, larvae), but can also exert indirect impacts via their hosts. Parasites with

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complex life-cycles are particularly vulnerable to variations in weather conditions (Harvell et al. 2002). Digenean trematodes infect between two and five hosts during their life-cycle, interspersed with free-living phases, at which time the organism is directly exposed to external environmental conditions (Combes 2001). Predictable responses to certain weather stressors have been observed in digeneans and, generally, the highest abundance of larval helminths coincides with heavier rainfall (Rolfe et al. 1991, van Dijk et al. 2010, Martins et al. 2011). Within the definitive host there may be differences in resource availability, space, oxygen levels and subtle differences in chemistry due to diet or host age impacting on metabolism. It follows that where parasite populations have similar biotic interactions with their hosts, we would also expect responses to abiotic stressors to be similar. Knowledge of current associations between parasite distribution and climate is essential to understand how parasite distributions may change in the future, thereby facilitating conservation of native susceptible fauna.

The Eurasian otter (*Lutra lutra*) is a sentinel species of freshwater health and, due to population crashes in the recent past and the near threatened status of this mammal, extensive data on otter anatomy and health have been collected in the United Kingdom (UK) (Chadwick 2007, Simpson 2007). This host can be used, therefore, as a model system to examine the interaction between generalist parasites and climate. *Pseudamphistomum truncatum* (Platyhelminthes: Opisthorchiidae) was recently recovered from otter carcasses in Somerset (UK) and, despite detailed post mortem examinations since 1988 ($n > 400$), records did not pre-date 2000 (Simpson et al. 2005). Consequently, it was assumed that this was an invasive parasite (Simpson et al. 2005). In a subsequent study on additional otters from across the UK, Sherrard-Smith et al. (2009) identified a second biliary opisthorchiid, *Metorchis albidus*. These were, respectively, the first reports of each parasite in otters in the UK and in both cases there is evidence of some associated damage to the gall bladder (Simpson et al. 2005, 2009, Sherrard-Smith et al. 2009). There is, however, anecdotal evidence that both parasite species have been recovered from other British mammals (fox, *Vulpes vulpes*; grey seal, *Halichoerus gryphus*; and domestic cats and dogs; whilst only *Pseudamphistomum truncatum* has been reported in the common seal, *Phoca vitulina*, and harp seal, *Phoca groenlandica*) since the early 1900s (Nicoll 1923) but details of host origins are ambiguous and it remains unclear whether they are, in reality, recent invaders. The reported native range of

these parasites spans continental Europe to Asia (Loos-Frank and Zeyhle 1982, Shimalov et al. 2000, Torres et al. 2004). The life-cycle of *P. truncatum* is complex; parasite eggs are consumed by gastropod intermediate hosts (reviewed in Sukhdeo and Sukhdeo 2004). Cercariae emerge from these snail hosts and encyst on the second intermediate cyprinid host (see Skov et al. 2008, Hawkins et al. 2010) before they are trophically transmitted to piscivorous mammals, where they mature within the gall bladder (Dunn 1978).

Examination of endoparasites usually requires destructive sampling (although estimates can be made via faecal egg counts; Anderson and Schad 1985, Knopp et al. 2008), which presents ethical issues and is not a viable approach when the host organism is of conservation concern. Long-term surveys of road-killed otters in the UK (Simpson 2007, Chadwick 2007) provide, therefore, an invaluable resource for the study of mammalian parasites. Here, we examined first how sub-groups of the otter population (grouped by host sex, age-class and condition) were associated with the biliary trematodes, *P. truncatum* and *M. albidus*. The distribution of each parasite in the UK, within otter hosts was described; the damage caused by these trematodes and extent of both spatial and temporal clustering was assessed. The dynamic nature of these parasite distributions were explored in terms of their range over time. Next, the effects of abiotic factors (temporal variation in weather, described using monthly averages of meteorological data), and spatial variation in climate (described using spatially explicit long-term 40 year averages of meteorological data) on each trematode population was considered. Although many studies consider climate, environmental or biotic factors, to our knowledge, this represents one of the first studies (also see Haukisalmi and Henttonen 1990) on wildlife to have examined the effects of both abiotic and biotic variables on parasites with complex life cycles within their definitive host.

4.3 Materials and methods

4.3.1 Sample preparation

Necropsies were performed on Eurasian otter cadavers (n = 586), predominantly road-killed samples collected between 2004 and 2010 from across England and Wales (273 included in Sherrard-Smith et al. (2009) and 313 samples collected subsequently). Data on otter location (National Grid References) were recorded and each specimen was assigned to a Region (using UK Environment Agency Regions which are based on

groups of river catchments: Wales, Southwest, South, Midlands, Thames, Anglian, Northeast and Northwest, see Sherrard-Smith et al., 2009). Gall bladders were removed, stored at -18°C and subsequently screened for the presence of parasites. Otter weight (kg), length (m), sex and age-class were determined. A condition index was calculated:

$$K = \text{body mass (kg)} / [a \times \text{length}^n]$$

where a = 5.02 and n = 2.33 for females, and a = 5.87 and n = 2.39 for males (Kruuk et al. 1987). Prevalence (the number of hosts infected with specific parasitic species divided by the total number of hosts examined (Bush et al. 1997)) and mean intensity (the mean number of parasites per host excluding those individuals without infection; Bush et al. 1997) were recorded.

4.3.2 Species identification

Gall bladders were defrosted, immersed in 0.6% saline in a Petri dish and examined under a Nikon dissecting microscope at 30x magnification with fibre optic illumination. Parasites were counted to calculate prevalence, mean and median intensity (excluding uninfected hosts) of infections, and identified morphologically (Yamaguti 1971). Species identification was confirmed molecularly on a subset of parasites (*n* = 17 *P. truncatum* and 13 *M. albidus*) selected from across the geographic range found in the UK. For each worm the Internal Transcribed Spacer sub-unit II region (ITS2) ribosomal DNA sequence was amplified as follows: for each sample, an entire parasite was digested in 15 µl of TE buffer (4 parts 1M Tris-Hydrochloric acid, 10 parts 0.5M EDTA, 986 parts de-ionised water) containing 0.45% Tween 20 and 2 µg of Proteinase K (modified from Faria et al. 2010) for 3 h at 55°C. The total PCR volume of 10 µl was comprised of 2 µl of DNA extract with 10x PCR buffer II (Applied Biosystems, USA), 50 mM $MgCl_2$ (Applied Biosystems), 2.5 mM of each dNTP, 10 pmol/µl of each primer (ITS2 rDNA: Ophet F1 5'-CTCGGCTCGTGTGTCGATGA-3' and Ophet R1 5'-GCATGCARTTCAGCGGGTA-3' following Müller et al. (2007); GenBank Accession numbers: *P. truncatum* JF710315, and *M. albidus* JF710316) and 5U Taq DNA polymerase (Invitrogen, USA). PCR conditions were: 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 53°C for 1 min and 72°C for 1 min, with a final extension step of 72°C for 10 min (GenAmp PCR System 9700, Applied Biosystems). The samples were run on a 1.5% agarose gel and produced 388 bp and 403 bp (ITS2) amplicons for *P. truncatum* and *M. albidus*, respectively. Sequencing was conducted by Macrogen

[Netherlands] and alignment of forward and reverse sequences performed in Sequencher™ (version 4.9) and species were confirmed by matching consensus sequences with those in GenBank (Accession numbers: *P. truncatum* EU483073.1 and *M. albidus* JF710316).

4.3.3 Gall bladder damage

Opisthorchiid parasites cause pathological changes to the gall bladder and bile ducts of their hosts (Sripa et al. 2007). Histo-pathological examination was attempted in the current study but cells were too damaged following freezing. Condition scores of 1 to 5 (1 corresponding to a normal gall bladder lining, 2 characterized by low level inflammation and 3-5 representing progressively more fibrous tissue) were therefore visually assigned and used to describe the internal lining of the gall bladder (following Sherrard-Smith et al. 2009). I asked whether there was an association between the intensity of parasitic infection and host condition and/or gall bladder damage, for each parasite species and host sex category (generalized linear model (GLM) with negative binomial error distributions). Damaged gall bladders lacking adult trematodes were recorded as uninfected. Although not known in otters, clearance of trematode infection might be possible but other factors (such as gall stones) related to dietary or genetic conditions may cause similar thickening and inflammation of this tissue.

4.3.4 Spatial and temporal trends

Clustering in space and time was assessed independently for each parasite species, using a Bernoulli Model (see Kulldorff and Nagarwalla 1995, Kulldorff 1997) with the log-likelihood ratio calculated for 999 Monte Carlo simulations in the statistical package SaTScan (http://www.satscan.org/). SaTScan uses a cluster detection method to identify and test the significance of clusters using presence/absence data. The number of actual infection cases in an area is compared with the expected number if cases were distributed randomly (Kulldorff and Nagarwalla 1995). Statistical significance is then based on the log-likelihood ratio test where the alternative hypothesis is a significant increase in risk within, compared with outside, an area (Odoi et al. 2004, http://www.satscan.org/). Locations were defined as counties in England and Wales and the mean X and Y co-ordinate for each county was used to describe the area centroids for analysis. Clusters in recent time, but previously absent, indicate increasing infections. SaTScan identifies

significant and secondary clusters in defined areas (counties) over specific time periods (http://www.satscan.org/) and therefore it is possible to examine both spatial and temporal shifts in range simultaneously. To assess whether the mean intensity of either parasite species varied over the study period 2007-2010 (time scale in months with sufficient consecutive data), time (in months) was fitted to log transformed mean intensity using linear regression. This enabled season to be considered and provided an indication of how robust the parasite population was across a consecutive time period. Spatial clustering in parasite intensity was examined for each parasite independently (using ANOVA for *P. trucatum* and Kruskal-Wallis for *M. albidus* due to smaller sample sizes).

4.3.5 Abiotic associations

Spatial variation in climate can be described using long-term average data (Perry and Hollis 2005). To determine whether climate is associated with biliary parasite prevalence and intensity, a buffer with a 20 km radius (otter ranges can reach 40 km; Kruuk 2006) was fitted to each otter location and plotted using ArcGIS (version 9.2). Otter polygons were then joined to climate data for each respective location to provide a measure of the typical climate in the area where each host was found. In the current study, long-term average data were taken from the UK Climate Observations, UKCIP09 (http://www.metoffice.gov.uk/public/weather/climate/?tab=climateTables) which models weather variables on a 5 km^2 gridded area across the UK. The mean values of each 5 km^2 measure, falling within the 20 km radius buffer describing each otter location, for each climate variable, were taken as the climate for that location over the past 46 years. Four measures of climate were included here: mean rainfall (mm), mean days of ground frost, mean temperature (°C) and average minimum temperature (°C) across a 46 year period (1961-2006). These variables were chosen to give a broad indication of the effects of climate. Specifically, rainfall has been shown to affect the distribution of intermediate life stages of trematodes (e.g. Rolfe et al. 1991, van Dijk et al. 2010, Martins et al. 2011) while understanding the effects of temperature could be a first step in predicting the change in the distribution of these opisthorchiids with predicted climate change (e.g. Harvell et al. 2002, Pachauri and Reisinger 2007). Further, ground frost was included because previous work has reported declines in the viability or survival of egg stages at low temperatures (Hunter and Hunter 1929) and we wanted to investigate whether a similar pattern might be apparent here.

Temporal variation in local weather conditions (daily variation within the 20 km radius of each otter) and variation in habitat type may influence spatial variation in biliary parasite load of otters. These variables were included in preliminary analyses but restricted the dataset considerably, rendering conclusions less robust. Habitat at the location where the otter was killed was discarded because the habitat at the site of death may not accurately reflect the habitat where that otter became infected. Location-specific weather data were discarded because limited availability reduced sample size. Equally, local weather is very likely to have an effect on parasite distributions but detailed analysis of these effects was beyond the scope of the current data: i) the current data include no information on when the host was infected and; ii) the definitive host can retain an infection for an extended period. Instead, mean monthly Central England (CE) temperature (°C) (Parker et al. 1992) and North Atlantic Oscillation (NAO) values (http://www.cpc.ncep.noaa.gov/products/precip/CWlink/pna/nao.shtml) for the UK were used to examine the general response of trematode populations to short-term temporal variation in temperature patterns. The mean CE temperature fluctuates across the year in parallel with local measures of temperature whilst the NAO is a climatic phenomenon that, when in positive phases, is associated with warmer and wetter winters in northern Europe. Mean monthly CE temperature and NAO values were assigned to each otter, from each of three periods prior to that otter's death: (i) the month of host death, (ii) the 6 month period preceding host death, and (iii) the year preceding host death (see Sherrard-Smith et al. 2012). Typically, the otters collected as road-killed individuals are 1-2 years old (Sherrard-Smith and Chadwick 2010) so infection is most likely within this period and other parasites have been reported to respond to past weather patterns fitting the above lag times (see Randolph et al. 2002).

Each parasite species was considered independently. Both biotic (host sex, age, condition) and abiotic (season and year of death, CE temperature and NAO as measures of weather, 40 year average mean and minimum temperature, mean rainfall and days of ground frost as measures of climate) factors were included as predictive terms in GLMs fitted to prevalence and, initially, intensity data with appropriate error distributions (binomial and negative binomial error distributions, respectively). Models were, however, a poor fit to intensity data regardless of any transformations or error distributions assessed and were therefore uninformative, and were subsequently

discarded. Binomial models for prevalence data were simplified using the Akaike Information Criterion (AIC) and residual plots were checked visually for normality where appropriate. All statistical analysis was conducted using ArcGIS 9.1 and R version 2.12.1 (R Development Core Team 2008), a *P* value of less than 0.05 was considered significant.

4.4 Results

Identification of *P. truncatum* and *M. albidus* was confirmed morphologically and molecularly (100% similarity to GenBank: JF710315 and JF710316, respectively). There was no intraspecific variability within the ITS2 rDNA sequences for either parasite. *Pseudamphostomum truncatum* and *M. albidus* were found in 13.5% (79 out of 586) and 7.85% (46 out of 586) of hosts, and the mean intensities of infection were 28.3 (range 1 – 302) and 4.1 (1 – 34), respectively (Table 1). Only two otters were co-infected with both parasite species (see Fig. 1).

Table 4.1 Parasite load of gall bladders from otters, *Lutra lutra*, collected in England and Wales between 2004 and 2010 (*n* = 586); showing prevalence, mean intensity with upper and lower 95% bootstrap confidence intervals (CI) (10,000 iterations), median intensity and main geographic range.

Parasitic species	Prevalence (%)	Mean Intensity (95% CI)	Median Intensity	Geographic Range
Pseudamphistomum truncatum	13.5	28.3 (17.4-41)	9	Southeast Wales, Southwest England
Metorchis albidus	7.85	4.1 (2.3-6.4)	2	East Anglia, across England and Wales

4.4.1 Spatial and temporal geographic trends

The Bernoulli model highlighted a number of distinct counties as important locations for *P. truncatum* and *M. albidus* (Fig. 1). Important locations for *P. truncatum* were Avon, Devon, Dorset, Gloucestershire, Gwent, Hampshire, Hereford and Worcestershire, Mid-Glamorgan, Powys, Somerset, South Glamorgan, West Glamorgan and Wiltshire (Wales Region: $Z_{7,582} = 2.894$, $P < 0.005$, and Southwest Region: $Z_{7,582} = 3.252$, $P < 0.001$), but there were no detectable temporal trends in clustering over the study period (Cluster time period: December 2008 until March 2010; Ll ratio = 18.98, Number of cases = 34/98, *P* <

0.0001). One of the limitations of this approach is that the Bernoulli model works on concentric circles and therefore, although there were no actual cases in West Glamorgan it is identified as an important location for *P. truncatum* here because it is adjacent to the main cluster of cases in Somerset and South Glamorgan and therefore falls within the definitive circle. Equally, important locations for *M. albidus* were Bedfordshire, Cambridgeshire, Essex, Hertfordshire, Norfolk and Suffolk (Anglian Region: $Z_{7,582}$ = 3.57, $P < 0.005$) and similarly to *P. truncatum*, no trend in time was observed (Cluster time period: July 2008 until March 2010 Ll ratio = 27.52, Number of cases = 18/39, $P < 0.0001$). Although these differences in parasite prevalence were observed, there were no large-scale regional differences in intensity for either parasite (*P. truncatum* ANOVA: $F_{4,69} = 1.18$, $P = 0.327$; *M. albidus* Kruskal-Wallis test: $H_{3,33} = 5.59$, $P = 0.134$). There was no overall increase in parasite mean intensity with time (months) for *P. truncatum* ($F_{30,32} = 0.6208$, $P = 0.4369$) or *M. albidus* ($F_{18,20} = 0.03885$, df = 18, $P = 0.846$).

4.4.2 Biotic associations

Adult otters had a higher prevalence of both parasites than sub-adults (*P. truncatum* $GLM_{507,517}$: $t = 2.482$, S.E. = 0.302, $P < 0.05$; *M. albidus* $GLM_{508,517}$: $t = 2.296$, S.E. = 0.519, $P > 0.05$). Host sex was not important for predicting infection with either parasite.

Hosts with better condition scores (K) were associated with a higher prevalence of *P. truncatum* (GLM: $t_{9,506} = 3.701$, S.E. = 1.08, $P < 0.001$). Host condition increased with both *P. truncatum* and *M. albidus* intensity (GLM: $F_{66,72} = 2.66$, $P < 0.01$, and $\chi^2_{510,516} = 2.493$, $P = 0.013$, respectively). Both *P. truncatum* and *M. albidus* cause significant damage to the gall bladders of otters so that heavier infections resulted in increased gall bladder damage (ANOVA: $F_{4,72} = 3.53$, $P < 0.01$; and $F_{4,39} = 7.97$, $P < 0.001$ for *P. truncatum* and *M. albidus*, respectively). The mean condition of a gall bladder infected with *P. truncatum* was 3.1, compared with 1.7 for uninfected otters whilst the mean gall bladder condition of an otter infected with *M. albidus* was 2.4 compared with 1.7 for the uninfected individuals. For uninfected otters, there was no difference in gall bladder condition between the sexes (GLM: t = 1.129, S.E. = 0.127, $P = 0.259$) and, although there was no difference between the sexes in likelihood of infection, once infected with either trematode, males presented with more severe pathological changes, however this relationship was not significant (ANOVA: $F_{1,119} = 3.56$, $P < 0.06$). There was no

relationship between host condition and gall bladder damage for either parasite (*P. truncatum*: ANOVA: $F_{4,72} = 0.6554$, $P = 0.625$; *M. albidus*: ANOVA: $F_{4,44} = 0.5813$, $P = 0.678$).

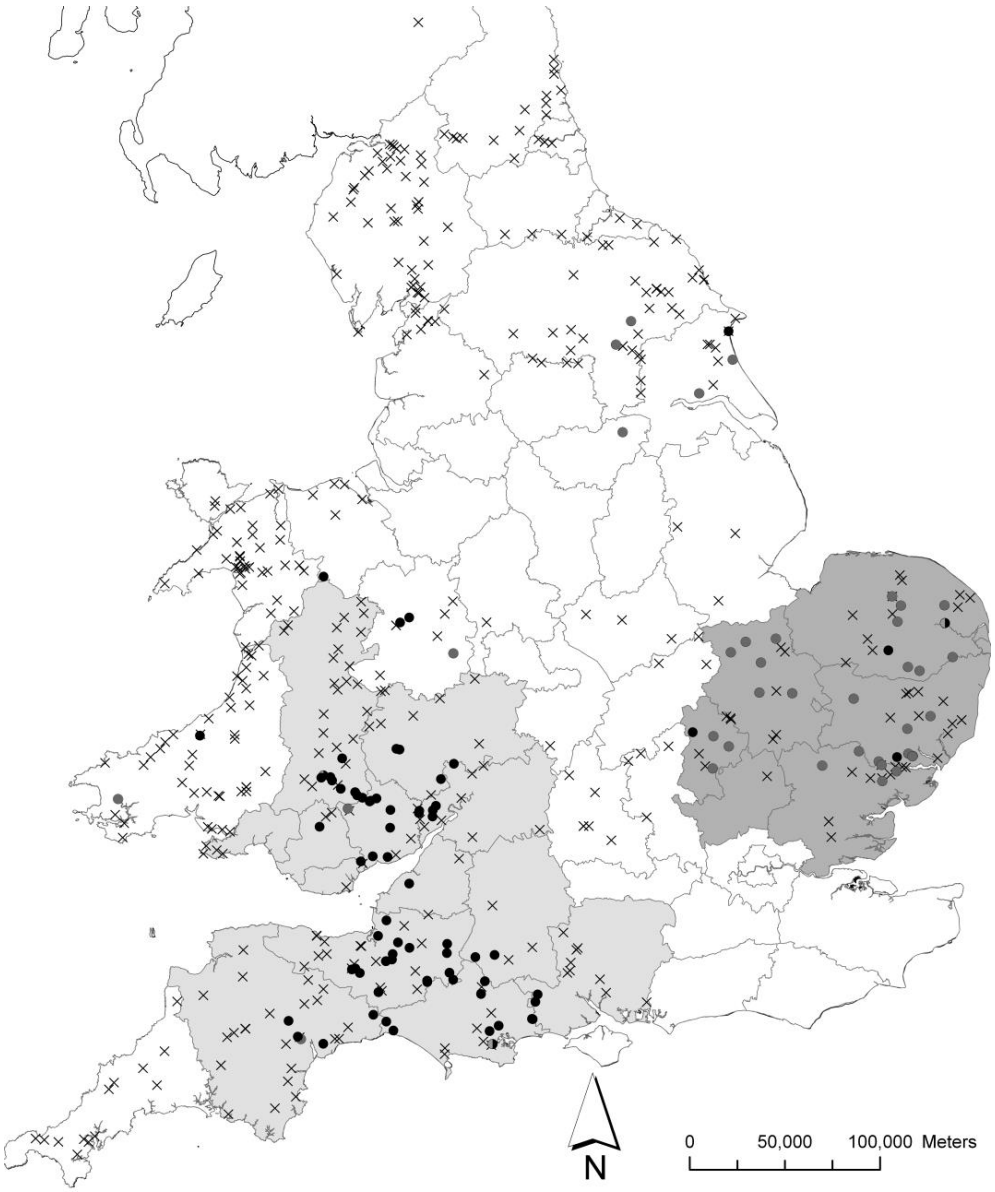

Figure 4.1 Location of infected and uninfected otter *Lutra lutra* across England and Wales: *Metorchis albidus* infections are shown in grey circles, *Pseudamphistomum truncatum* infections are shown in black circles, co-infections are represented by black and grey circles, and uninfected hosts are marked with a cross. Significant hotspots of *Pseudamphistomum truncatum* in the West (lighter grey, $p < 0.001$) and *Metorchis albidus* in the East (darker grey, $p < 0.001$) as defined by the Bernoulli model are shown, with County boundaries indicated.

4.4.3 Abiotic Associations

The probability of infection with either parasite was negatively associated with long-term average days of ground frost (*P. truncatum* $GLM_{507,517}$: $t = 3.102$, SE = 0.14, $p < 0.01$ and *M. albidus* $GLM_{508,517}$: $t = 2.411$, SE = 0.28, $p < 0.05$), minimum temperatures (*P. truncatum* $GLM_{507,517}$: $t = 3.295$, SE = 2.7, $p < 0.001$ and *M. albidus* $GLM_{508,517}$: $t = 3.163$, SE = 5.4, $p < 0.05$) and rainfall (*P. truncatum* $GLM_{507,517}$: $t = 3.119$, SE = 0.16, $p < 0.005$ and *M. albidus* $GLM_{508,517}$: $t = 3.252$, SE = 0.08, $p < 0.01$) but, in the case of *P. truncatum*, positively associated with warm long-term average temperatures (*P. truncatum* $GLM_{507,517}$: $t = 4.555$, SE = 0.54, $p < 0.001$; *M. albidus* was not significant $GLM_{508,517}$: $t = 1.61$, SE = 0.71, $p = 0.108$; see Table 4.2, Figure 4.2). Trematode prevalence was not associated significantly with temporal variation in weather, represented by mean monthly Central England Temperatures and North Atlantic Oscillation measures (GLM for both parasites, $p > 0.1$).

Table 4.2 Mean climate measures (40 year average mean and minimum temperature (°C), mean monthly rainfall (mm), and mean ground frost (days per year)) for otter *Lutra lutra,* populations in the UK either infected with *Pseudamphistomum truncatum* or *Metorchis albidus,* or free from infection (standard error shown in parentheses). Data summarised from UK Climate Projections, UKCIP09 (*data available online*).

Parasitic species	Presence in hosts	Mean temperature	Minimum temperature	Mean Rainfall	Mean Ground frost
Pseudamphistomum truncatum	Infected	9.588 (0.075)	0.087 (0.064)	79.4 (2.368)	101 (1.07)
	Uninfected	9.198 (0.037)	-0.024 (0.039)	84.6 (1.561)	104 (0.7)
Metorchis albidus	Infected	9.782 (0.068)	0.029 (0.062)	56.5 (2.854)	102 (1.13)
	Uninfected	9.208 (0.036)	-0.011 (0.037)	86.2 (1.433)	104 (0.67)

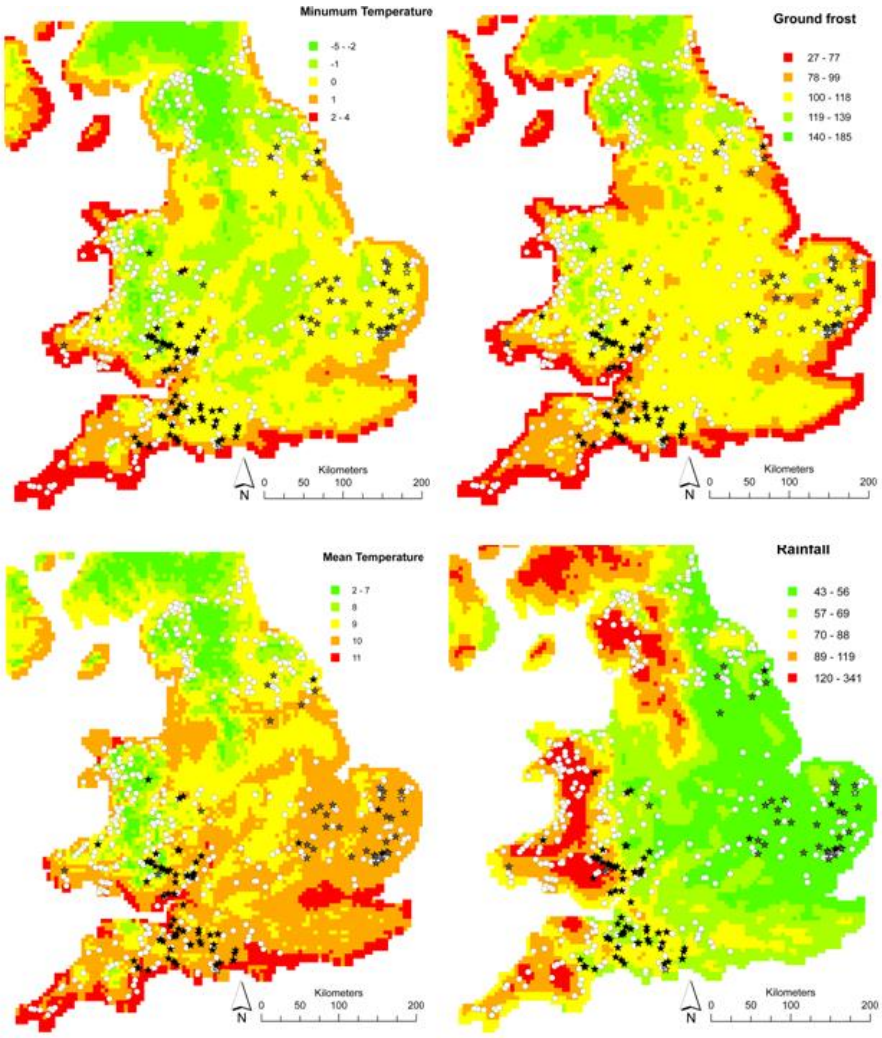

Figure 4.2 Prevalence of *Pseudamphistomum truncatum* (black circles) and *Metorchis albidus* (grey circles) in otters *Lutra lutra* (unifected otters marked as white circles) across the UK with long-term meteorological measures associated with parasite distribution including mean 40 year minumum temperature, ºC, mean temperature, ºC, number of days of ground frost per year and rainfall, mm per year.

4.2 Discussion

The ultimate prevalence, distribution and intensity of parasites in their definitive hosts are determined by interacting variables that impact upon each intermediate host, the final host and each life-stage of the specific parasite. In the current study, climate was associated with distributions of the biliary parasites, *P. truncatum* and *M. albidus*, in their definitive host, the Eurasian otter. Although the two trematodes have distinct geographic distributions, within those regions their respective responses to climate were similar. For both parasites warmer temperatures, lower rainfall and fewer days of ground frost were associated with an increased probability of infection. In northern Europe there is a trend

towards a warmer and wetter climate (Nakićenovíc and Swart 2000, Pachauri and Reisinger 2007, Murphy et al. 2009) with milder winters and warmer springs expected in the UK (Pachauri and Reisinger 2007). These observations support previous research on trematodes suggesting that warmer conditions will benefit these parasites (via the increased transmission success between intermediate hosts; Poulin, 2006, Paull and Johnson 2011). Yet the current study is, to our knowledge, one of the first to consider the combined interaction of meteorological variables and host factors for complex life-cycle parasites in a definitive wild host (also see Haukisalmi and Henttonen 1990).

In general, the response of trematodes to rainfall appears to be species-specific (Morgan and Wall 2009) and most studies relate trematode success to the response of intermediate hosts to wetter conditions (Gray et al. 2008, Morgan and Wall 2009, van Dijk et al. 2010). Yet, whilst adequate moisture is an essential requirement for most stages of the trematode life-cycle including egg survival, ability to infect snails, cercarial release and survival of encysted metacercariae (Kendall and McCullough 1951, Smith and Wilson 1980, van Dijk et al. 2010), excessive rainfall may be inhibitory. Both biliary trematodes considered here were negatively associated with rainfall (see Table 2) to such an extent that no otters residing in a location with an average of > 151 mm of rainfall per year were infected. In the UK, river levels are extremely responsive to rainfall (Marsh and Dale 2002) so increased precipitation, resulting in flooding, will increase space availability in freshwater habitat. This could reduce the likelihood of contact between eggs and snails, and then cercariae and fish, with consequent reductions in transmission to the definitive host – ultimately resulting in the observed reduced prevalence at locations with relatively high rainfall. Parasite dispersal patterns are affected by rainfall; in Trinidad, for instance, infected fish are more likely to be washed downstream during seasonal flooding (van Oosterhout et al. 2007).

Fewer days of ground frost per year were associated with an increased probability of infection with either *P. truncatum* or *M. albidus*. In addition, lower minimum temperatures, reaching freezing or below, were associated with lower prevalence for both parasite species. Both findings suggest that cold spells have a significant negative impact on survival of free-living stages of the biliary trematodes and perhaps their intermediate hosts. There is little reported in the literature about the effect of ground frost events on helminth populations; however, the strength of the association between *P. truncatum* and

M. albidus and days of ground frost suggest that this variable is, perhaps, an overlooked but vital aspect of climate influencing helminth populations. Egg development of *Fascioloides magna* is retarded by approximately 1 month in conditions below freezing but, even at -25°C, eggs still hatched and released viable larvae (Erhardova 1965). The tapeworm *Proteocephalus ambloplitis* is, however, more sensitive to frost and does not survive freezing (Hunter and Hunter 1929). Once again, the impacts of cold temperatures on helminths appear to be species-specific and although some studies report that, as suggested by our observations, cold conditions may have an adverse effect on the various life-stages of helminths (see Anderson and Levine 1968, O'Connor et al. 2006), others report positive impacts of cold weather relating to reduced immune function of the host (Huebert et al. 1990).

The availability of hosts is fundamental to the underlying parasite distributions and, to a large degree, host distributions are also determined by climate. Indirect impacts of climate change on helminth communities are often detected through their effects on intermediate hosts (MacCann et al. 2010, van Dijk et al. 2010, Lima dos Santos and Howgate 2011) but have been reported only occasionally in the definitive hosts, where they relate most often to range shifts in livestock (see Jenkins et al. 2006, van Dijk et al. 2010) rather than observations from wild hosts. Climate was shown, however, to explain long-term dynamics of common (but not rare) helminthic infections in bank voles, *Clethrionomys glareolus* (see Haukisalmi and Henttonen 1990). Sampling of parasites from wild fauna, such as in the current study, will facilitate our understanding of how global climate change will impact the macroparasitic community (see Cribb 1999, Poulin and Morand 2000, Hotez and Gurwith 2011).

Together, the apparent lack of ongoing range expansion in either trematode and their adaptation to local climate suggest that *P. truncatum* and *M. albidus* have been long established, even though both parasites were formally identified in the UK only recently (Simpson et al. 2005, Sherrard-Smith et al. 2009). Given the short time period over which range expansion has been tested, however, it is recognised that gradual expansion may be occurring. The similarity in response to meteorological variables exhibited by both species makes climate an unlikely explanation for the spatially distinct distributions observed. An alternative explanation may be host density and geographic distribution (Arneberg et al. 1998, Tsai and Manos 2010, but see Shaw and Dobson 1995, Gaston

2003). Both parasites use *Bithynia* spp. (Gastropoda) and cyprinids as intermediate hosts (see Dunn 1978) but alternative hosts may be involved and it is unknown whether the two parasite species use the same intermediate hosts. Generally, the ranges of cyprinids do not extend to the far west of Cornwall or further north in the UK than the England - Scotland border (for suitable cyprinid examples – Rudd, *Scardinius erythrophthalmus*, Roach, *Rutilus rutilus* and Tench, *Tinca tinca* – see NBN Gateway, http://data.nbn.ork.uk/) and present a possible reason for the current limited distribution of both parasites in the far west and north of England. The snail, *Bithynia* sp., and a second, previously unreported host, *Radix balthica* (Sherrard-Smith et al. Chapter 7), have ranges that are also limited within Cornwall but would not prevent spread of either parasite further north where these snails are common. Host availability alone does not appear to explain the geographic separation of the two trematodes examined here.

Indirect impacts may combine to determine parasite distribution. In northern Europe, the effect of local temperature is a limiting factor in liver fluke (*Fasciola hepatica*) distribution due to the responsive nature of snail populations to fluctuating temperatures (Torgerson and Claxton 1999) and a similar trend is apparent elsewhere (see Sturrock 1966, Appleton 1977, Fenwick et al. 2007). Although cercarial shedding tends to increase with warmer temperatures (Poulin 2006, Koprivnikar and Poulin 2009), once released into the water, high temperatures can decrease the survival time of the cercarial stage (Zbikowska 2004, Koprivnikar et al. 2010). More locally, sporocyst infections of snails in the UK increased following wet summers and warm winters (Morley and Lewis 2008) but on a finer scale particular species, such as *Maritrema novaezealandensis* in New Zealand (see Koprivnikar and Poulin 2009) and dicrocoeliid parasites in the UK (Morley and Lewis 2008), showed the opposite trend. So, there are evident system-specific responses to changes in meteorological stress between trematodes and their hosts including those identified here between the opisthorchiids and otter hosts.

There was no evidence of seasonality in the prevalence or intensity of either *P. truncatum* or *M. albidus* in otters. In the literature, seasonality can be observed on occasion in faecal egg deposits from omnipresent definitive hosts (for example Hughes and Answer 1982, Phiri et al. 2007) or immature parasitic stages in seasonally abundant mollusc and fish intermediate hosts (e.g. Sturrock et al. 2001, Rinchard and Kestemont 2005, Phiri et al. 2007). Many parasites with complex life cycles synchronise events, such as release of

eggs or larvae, to coincide with host availability (see Altizer et al. 2006). Gastropod communities vary significantly over time, with large seasonal fluctuations in species abundance (Gerard et al. 2008) causing subsequent impacts for their trematode populations (see Karvonen et al. 2006, Ben Abdalah et al. 2010), but this trend may not persist in the second intermediate host. For example, although more cercariae were released from the snail host *Bithynia tentaculata* between September and February, there was no seasonality in metacercarial infections of fish in the Lower Thames region (Morley et al. 2004). The long life of opisthorchiid adults in definitive hosts (see Dunn, 1978) may additionally mask any seasonal patterns in exposure risk.

Taking an alternative perspective, otter diet varies spatially in the UK with more salmonid remains found in otters from the west and more cyprinids, the reported second intermediate host for these parasites (Dunn 1978) in the east (Chadwick et al. unpublished data, but see Hughes et al. 2001, Copp and Roche 2003, Britton et al. 2006). This indicates potential differences in diet which may determine parasite assemblages in the definitive host. The otters have themselves recently recovered from population crashes following the expansion of remnant populations in Wales, the southwest and east Anglia (Strachan and Jefferies 1996, Jones and Jones 2004, Crawford 2010). It is, perhaps, unsurprising that higher parasite prevalence and intensities are found in those regions where otter populations also have greatest densities. Where host populations are expanding, parasites and pathogens tend to lag behind the host population at the expanding edge of the host range (Phillips et al. 2010). This is due to stochastic events that may lead to local extinction of parasites, especially in low density frontal populations and where parasite transmission is density-dependent (Colautti et al. 2004, Liu and Stiling 2006, Phillips et al. 2010). Otter populations are currently increasing in England and Wales (Crawford 2010) and perhaps the populations of *P. truncatum* and *M. albidus* are lagging behind the frontal edge of the expanding otter range, due to stochastic local extinctions.

The abundance of all hosts is inextricably linked to characteristics of the local habitat and therefore such traits must be of great importance for the underlying assemblage of parasites (reviewed in Lafferty and Kuris 1999, Bush et al. 2001, Pietrock and Marcogliese 2003). For example, the prevalence of *Cryptocotyle lingua*, predominantly a trematode of seabirds, was determined by habitat characteristics synonymous with high

gull abundance (Byers et al. 2008). Terrain differs in the east and west of the UK, which contributes to contrasting river flows (Poff 2002, Bower et al. 2004, Monk et al. 2006) and this may drive differences in host and trematode populations. Furthermore, altitude is thought to determine trematode distributions because snail populations are better adapted to low altitudes (see Rosenfield 1979, Weil and Kvale 1985). Factors including the velocity of river systems, proximity to standing pools, light and shade ultimately impact the abundance of hosts and have consequences on the transmission success of the cercarial life-stage that drive distribution patterns of trematodes in the otters. The benthic community diversity is apparently more important than the presence of competent hosts for determining aquatic parasite prevalence (Anderson and Sukhdeo 2010). This indicates that, perhaps, the underlying community structure and diversity dictates the different geographic distributions of the trematodes investigated.

The host condition index in the current study was positively correlated with both parasite prevalence and intensity, indicating that parasitized otters have a better diet, the two trematodes are associated with nutritionally high quality prey, or that these parasites are more successful in otters in good condition. The latter two suggestions are supported by the observation that parasites will grow larger in hosts (fish) that are growing at the highest rate (Barber 2005). In otters, the host condition index was not correlated with gall bladder damage, although the organ itself had significant damage and this was positively associated with parasite prevalence and intensity. Both species use the same habitat within their definitive hosts (the gall bladder and biliary ducts) and as such, inter-specific competition may limit further range expansion. Co-infections are possible (0.34%) but the limited instances where both parasites were present are consistent with that expected for host populations with the observed geographic segregation. The damage caused to otter gall bladders is similar for *P. truncatum* and *M. albidus* (also see Schuster 2010, Simpson et al. 2005, 2009). There was no difference in infection prevalence or intensity between host sexes and without infection, there was no difference in gall bladder damage between males (mean condition index = 1.622) and females (1.716). Males, however, suffered greater damage to the gall bladder when parasites were found. Sex differences in host responses to parasites are well documented (for example, Pickering and Christie 1980, Fox et al. 2003, Klein 2004, Dozières et al. 2010, McClelland and Smith 2011) and in general, there is a trend toward higher prevalence and severity of parasitic infections in

male hosts compared with female conspecifics (Goble and Konopka 1973, Degu et al. 2002, Klein 2004).

Changing climate is likely to have an effect on specific host-parasite interactions (Harvell et al. 2002, Thomas et al. 2004, Rohr et al. 2011). Here, biotic and abiotic factors interacted in a predictable manner for two biliary trematodes in a definitive host and with further studies we will be able to determine whether this is a true trend for this type of fauna. Although male and female otters have similar prevalence and intensity of infections, male otters exhibit greater damage once infected. Further, adult otters accumulate more parasites than younger conspecifics. High temperatures, less rainfall and fewer days of ground frost are conditions associated with higher prevalence in these systems. The associations observed here suggest that the warmer conditions in the UK following predicted climate change will lead to a greater risk of infection with these biliary parasites but, in contrast, increased rainfall and cold spells will limit the parasites. Climate has a huge influence on host-parasite systems but multiple conditions must be considered in parallel to better understand the ultimate effects on distribution patterns.

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CHAPTER 5

5. Distribution and molecular phylogeny of biliary trematodes (Opisthorchiida) infecting fish-eating mammals (*Lutra lutra* and *Mustela vison*) across Europe

5.1 Abstract

Invasive parasites often present serious risks to novel host populations. The recent identification of *Pseudamphistomum truncatum* and *Metorchis albidus* (Trematoda: Opisthorchiidae) in the UK caused concern because of the biliary damage associated with both digeneans coupled with speculation over their invasive status in Britain. Here, we assess the prevalence, intensity and phylogeography of these trematodes across mainland Europe (Czech Republic, Denmark, France, Germany, Norway, Poland and Sweden) to determine whether or not these species are recent introductions into the UK. Both parasites are found across Europe but hotspots are evident; the Saxony Region of Germany has comparatively high prevalence (73%) of *P. truncatum* whilst the parasite appears to be absent from both Scotland and Norway. Otters in Brittany (France) were only found to be infected with *M. albidus*. Sequencing of the cytochrome c oxidase subunits I and III (COXI and COXIII, mitochondrial DNA) revealed similarities in genetic heterogeneity across Britain and mainland Europe, suggesting that neither parasite is a recent introduction to the UK. The wide distribution of both parasites probably reflects relatively unrestricted movements (both natural and anthropogenic) of fish and snail intermediate hosts across Europe.

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5.2 Introduction

When widespread species become isolated geographically, a lack of gene flow and different environmental pressures can lead to the divergence of populations (for example see Coyne and Orr 2004, Cadena et al. 2007, Losos and Ricklefs 2009, Nakazato et al. 2010, Miura et al. 2011). Certain geological events are proposed as exceptional periods of speciation because of climate cycles (see Hewitt 2000). The Pleistocene speciation model, for instance, suggests that populations of widespread species became fragmented and isolated geographically in available glacial refugia. Such isolation led to the formation of multiple sister species via allopatric speciation (Bermingham et al. 1992, Zink and Slowinski 1995 but see Near et al. 2003). This process can be reflected in the genetic structure of populations and therefore, comparison of genetic diversity can indicate the timing of separation between distinct populations. Recent evidence suggests that in the southern parts of the British Isles, cryptic glacial refugia may have been habitable by thermophilic biota (Stewart and Lister 2001, Stewart et al. 2009). This is illustrated by some earthworm populations, suggesting that the extant British fauna could be the result of both migration from mainland Europe following the retreating ice-sheets, and the survival of some isolated populations in favourable climatic pockets during the Pleistocene glaciations (Sechi et al. in preparation).

Parasites play an integral role in ecosystem functioning (Poulin 1999; Torchin et al. 2002; Poulin and Frederic 2008; Hatcher et al. 2012). There is, however, incomplete knowledge of existing parasitic species, their geographic and host ranges. This arises in part because of the morphologically cryptic nature of many parasitic species and, specifically, the challenge of detection and identification (Poulin and Morand 2000; Cribb and Bray 2011). The recent identification of *Pseudamphistomum truncatum* and *Metorchis albidus* (Trematoda: Opisthorchiidae) in the UK (Simpson et al. 2005, Sherrard-Smith et al. 2009) caused concern because of the biliary damage associated with both digeneans and speculation over their invasive status in Britain (Simpson et al. 2009, Sherrard-Smith et al. 2009). Invading parasites often present serious risks to novel host populations, partly because of naïve host immune responses coupled by disruption of the ecosystem equilibrium (Torchin et al. 2002). These two digeneans occur across Europe and have been found in various animal taxa (Table 5.1). During their life cycle, the parasites initially infect freshwater snails; a free-living cercarial stage is then released that encysts on a

freshwater fish intermediate host, before completing development once consumed by a mammalian definitive host. Anecdotal evidence from the early 1900s reports both parasite species from British mammals (fox *Vulpes vulpes*, grey seal *Halichoerus gryphus*, domestic cats *Felis catus* and dogs *Canis lupis familiaris*; *Pseudamphistomum truncatum* has also been reported in common seal *Phoca vitulina* and the harp seal *P. groenlandica*; see Nicoll 1923). Details of host origins are ambiguous however – while the mammals in question are British species, it is unclear whether the specimens reported were found in Britain – and so it remains unclear whether these trematodes are, in reality, recent invaders. The damage associated with host-parasite interactions that follow recent invasions makes the identification of novel species particularly crucial for successful conservation efforts (see Abdelkrim et al. 2005, Nieberding et al. 2005).

The current study aims to establish whether *Pseudamphistomum truncatum* and *M. albidus* are recently introduced from mainland Europe or whether their origin is more ancient. To address this, we collected samples from across Europe, confirmed species identification using conserved internal transcribed spacer region II (ITS2) ribosomal DNA sequences and compared genetic diversity using mtDNA (COXI and COXIII). Although two major clades were identified across Europe for each species, using the mtDNA markers the parasite genetic structure did not support the hypothesis that these trematodes are recent introductions to the UK.

Table 5.1 Locations across Europe and Asia, intermediate and definitive hosts of *Pseudamphistomum truncatum* and *Metorchis albidus*

Location	Host	Reference
	Pseudamphistomum truncatum	
Belarus	Wolf *Canis lupis,* American mink *Mustela vison,* European polecat *M. putorius,* Stoat *M. erminea,* Weasel *M. nivalis,* Red fox *Vulpes vulpes,* Siberian raccoon dog *Nyctereutes procyonoides*	Shimalov and Shimalov 2000, 2001a, b, 2002a, b,
Denmark	Red fox, American mink, roach *Rutilus rutilus,*	Guildal and Clausen 1973, Saeed et al. 2006, Skov et al. 2008
England and Wales	Eurasian otter *Lutra lutra,* American mink, Roach	Simpson et al. 2005, 2009, Sherrard-Smith et al. 2009
France	European mink *Mustela lutreola,* European polecat, American mink	Torres et al. 2008
Germany	Red fox, Eurasian otter	Saar 1957, Schuster et al. 1988, 2000
Ireland	Eurasian otter, Mink	Hawkins et al. 2010
Italy		Simpson et al. 2005
Poland	Eurasian otter	Hildebrand et al. 2011
Portugal	Red fox	Eira et al. 2006
Russia	American muskrat *Ondatra zibethicus,* American mink, Siberian raccoon dog,	Ivanov and Semenova 2000
Spain	European mink, American mink	Torres et al. 2003
Ukraine	Carp *Cyprinus carpio*	Davydov et al. 2011
Yugoslavia	Red fox	Loos-Frank et al. 1982
Wadden Sea, Caspian Sea	Common Seals *Phoca vitulina,* Caspian Seals *P. caspica*	Strauss et al. 1991, Kuiken et al. 2006
	Metorchis albidus	
Belarus	Otter, Polecat *Mustela putorius* Mink *Mustela lutreola*	Anisimova 2002, Bychkova and Sidarovich 1994
Bulgaria	Insectivorous mammals	Genov 1979
England and Wales	Otter *Lutra lutra*	Sherrard-Smith et al. 2009
Norway	Domestic cats *Felis catus*	Nielsen and Guildan 1974
Novosibirsk Region, Russia	Bithyniidae snails, *Codiella troscheli*	Serbina and Yurlova 2002
Serbia	Foxes *Vulpes vulpes*	Pavlovic and Kulisic 2001
Shanghai, China	Domestic dogs *Canis lupis familiaris*	Andrews 1937
Siberia	Cyprinids	Fattakhov 1990
Spain	Foxes	Gortazar et al. 1998
Tura-Pyshma	Cyprinids	Krivenko and Filatov 1990

5.3 Materials and Methods

5.3.1 Sample collection

Gall bladder samples of fish-eating mammals, predominantly otters (*Lutra lutra*) and some American mink (*Mustela vison*) were sourced from across Europe. Samples were donated from European countries (Britain, Denmark, Czech Republic, France, Germany, Norway, Poland, Scotland, and Sweden). Gall bladders were examined microscopically at 20x magnification and parasites were morphologically identified according to Yamaguti (1971). A sub-sample of parasites (N = 65, see Table 5.2) was selected for molecular analysis. For British samples, stratified random sampling was used to select hosts previously identified (Chapter 4) from across their known ranges (*P. truncatum* from Counties of Somerset Dorset, Gwent and Powys; *M. albidus* from Bedfordshire, Cambridgeshire, Essex, Hertfordshire and Suffolk). For continental samples DNA was performed on at least one parasite per host sample.

Table 5.2 Summary of otter, *Lutra lutra*, or mink, *Mustela vison* (specified where appropriate) biliary parasite data from across Europe. The country of origin, total number of hosts examined (N), prevalence and intensities of the two species (*Pseudamphistomum truncatum* and *Metorchis albidus*) isolated from European piscivorous mammals.

Country	**N (otters unless otherwise specified**	***Pseudamphistomum truncatum***		***Metorchis albidus***	
		Prevalence (%)	**Mean intensity (N for DNA analysis: Total = 37)**	**Prevalence (%)**	**Mean intensity (N for DNA analysis: Total = 28)**
Czech Republic	*	-	- (2)	-	- (1)
Denmark	52	5.8	2.3 (4)	30.8	3.4 (8)
France, Brittany	22	0	0 (0)	18.2	2 (4)
France,Poitou-Charentes	19	5.3	4 (1)	0	0 (0)
Germany, Lusatica	11	72.7	29 (8)	18.2	1 (1)
Norway	21	0	0 (0)	0	0 (0)
Poland	*	-	- (2)	-	- (0)
Scotland	40 (mink)	0	0 (0)	0	0 (0)
Sweden	12	16.7	96 (3)	16.7	13.5 (2)
England and Wales	586	13.5	28.3 (17)	7.8	4.1 (12)
England and Wales	104 (mink)	8.7	222 (0)	0	0 (0)

**Parasites provided directly, one specimen of each species donated.*

A general linear model (GLM), with a binary error distribution, was used to compare the parasite prevalence (the infection status of an individual regardless of the number of parasites present; infected = 1, uninfected = 0) between European regions where the sample size was equal to or larger than 10.

To investigate intensity (the number of parasites infecting each individual, excluding those without infection) differences across host populations in Europe, a GLM (with a negative binomial error distribution) was fitted to the intensity data for regions where sample size of infected hosts was greater than or equal to 4. This analysis of intensity was limited however because of the small sample size available (Table 5.2).

5.3.2 DNA analysis

A conserved gene, the internal transcribed spacer sub-unit II (ITS2) ribosomal DNA region was used for species discrimination (see Chapter 4; Müller et al. 2007), while COXI and COXIII mtDNA fragments were used to examine genetic variation of *P. truncatum* and *M. albidus* populations across Europe. We reviewed previous phylogenetic studies of trematodes to identify which genes might provide a suitable target to examine genetic divergence across a geographic range (Appendix 5.1).

Genomic DNA was extracted from whole individuals using the protocol of Faria et al. (2011) with some modifications. Tissue was digested for 3 hours at 55°C in 15 µl TE buffer containing 0.45% Tween 20 and 2 µg Proteinase K, followed by 10 min at 95°C to denature the proteinase K. The PCR reaction was conducted in a final volume of 10 µl, 2 µl of DNA extract with 10x PCR buffer II (Applied Biosystems), 50 mM $MgCl_2$ (Applied Biosystems), 2.5 mM of each dNTP, 10 pmol/µl of each primer (depending on reaction: ITS2 rDNA: Ophet F1 5'-CTCGGCTCGTGTGTCGATGA-3' and Ophet R1 5'-GCATGCARTTCAGCGGGTA-3' following Müller et al. 2007; COXI mtDNA: ThaenCO1F 5'- CGGGTTTTGGAGCGTCATTC3' and ThaenCO1R 5'-ACAGGCCACCACCAAATCAT -3'; and COXIII mtDNA: CO3FTremat 5'-ATGAGWTGATTACCKTT -3' and CO3RTremat 5' –ACAACCACACATAATCCACAAAATG-3') and 5U Taq DNA polymerase (Invitrogen). PCR conditions were: 95°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 53°C (ITS2 rDNA), 55°C (COXI mtDNA) or 53°C (COXIII mtDNA) for 1 min and 72°C for 1

min, with a final extension step of 72°C for 7 min (GenAmp PCR System 9700, Applied Biosystems). The samples were run on a 1.5% agarose gel and produced 387bp and 403bp (ITS2), 388bp and 435bp (COXI) and 370bp and 394bp (COXIII) amplicons for *P. truncatum* and *M. albidus*, respectively. Sequencing was conducted by Macrogen (Macrogen Inc., Seoul, South Korea). For *P. truncatum* 27 individuals were sequenced for ITS2 rDNA, 17 for COXI and 22 for COXIII mtDNA while for *M. albidus* 22 individuals were sequenced for ITS2 rDNA, 15 for COXI and 14 for COXIII mtDNA (see Figure 5.1 and 5.2). Alignment of forward and reverse sequences was performed in Sequencher™ (version 4.9) and species were confirmed by matching consensus ITS2 sequences to those on GenBank (Accession numbers *P. truncatum* EU483073.1, and *M. albidus* JF710316).

Mitochondrial DNA amplicons were considered independently and also concatenated to create a total 758bp (*P. truncatum*) and 829bp (*M. albidus*) sequence for the population comparisons (conducted using MEGA 4.0; Tamura et al. 2007). Identical sequences were collapsed into haplotypes leaving a total of N = 13 *P. truncatum* haplotypes and N = 14 *M. albidus* haplotypes for phylogenetic analyses. For those samples where only one of the mtDNA fragments was successfully amplified we extended the sequence of the missing gene with missing data (i.e. "?") to complete the concatenated alignment length. Maximum-Likelihood (ML) and Bayesian inference (BI) methods were used to reconstruct the phylogenetic relationships among the mtDNA haplotypes for each species using PhyML version 3 (Guindon et al. 2010) and MrBayes version 3.2 (Ronquist et al. 2012) respectively. MrModeltest version 2 (Nylander 2004) was used to estimate the adequate model of sequence evolution of these datasets. For both datasets in each species the inferred model of evolution was HKY with invariant sites, two rate categories and a transition-transversion ratio ~2.3. The human liver fluke *Clonorchis sinensis* was used as outgroup for both datasets. Node support was determined by calculating 100 bootstrap replicates for the ML method and with posterior probabilities for the BI method. These models and phylogenetic trees were run by Pablo Orozco-terWengel.

5.4 Results

In total, 723 otters and 144 mink gall bladders were dissected from samples of 8 European countries (see Table 5.2, Figure 5.3). Otters from Germany had, by far, the highest prevalence (72.7%) of *P. truncatum* (GLM binomial error distribution: $\chi^2_{769,757} = 4.188$, SE

= 0.901, p < 0.001) and those otters in Denmark had the highest prevalence (30.8%) of *M. albidus* (GLM with a binomial error distribution: $\chi^2_{769,757}$ = 2.333, SE = 0.6061, p < 0.05). There were, in contrast, no parasites in any of the gall bladders from Norwegian or Scottish samples, whereas every other country had biliary trematodes in their otter populations (Table 5.2). There was no significant difference in the intensity of *P. truncatum* among infected otters from Britain or Germany (the only two countries with large enough sample sizes to compare statistically, GLM negative binomial error distribution: $F_{88,86}$ = 0.3167, p = 0.57). Equally, the intensity of *M. albidus* infection did not differ between comparable data sets from France, Sweden or England and Wales (GLM negative binomial error distribution: $F_{65,62}$ = 2.42, p = 0.097).

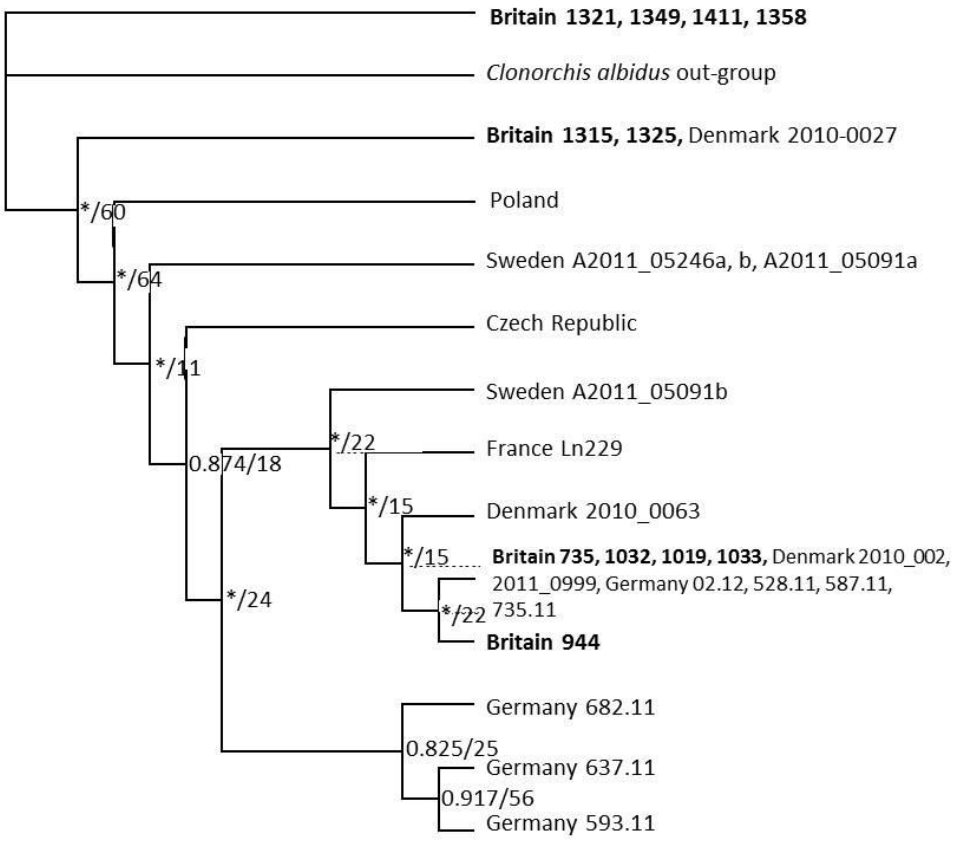

Figure 5.1 Phylogram for *Pseudamphistomum truncatum* (Bayesian Inference /Maximum likelihood, 100 bootstraps) based on the combined cytochrome c oxidase sub-unit I (COXI) and III (COXIII) mitochondrial DNA regions (758bp) from European samples. British samples are referred to by individual numbers. * indicates a BI probability of <0.2

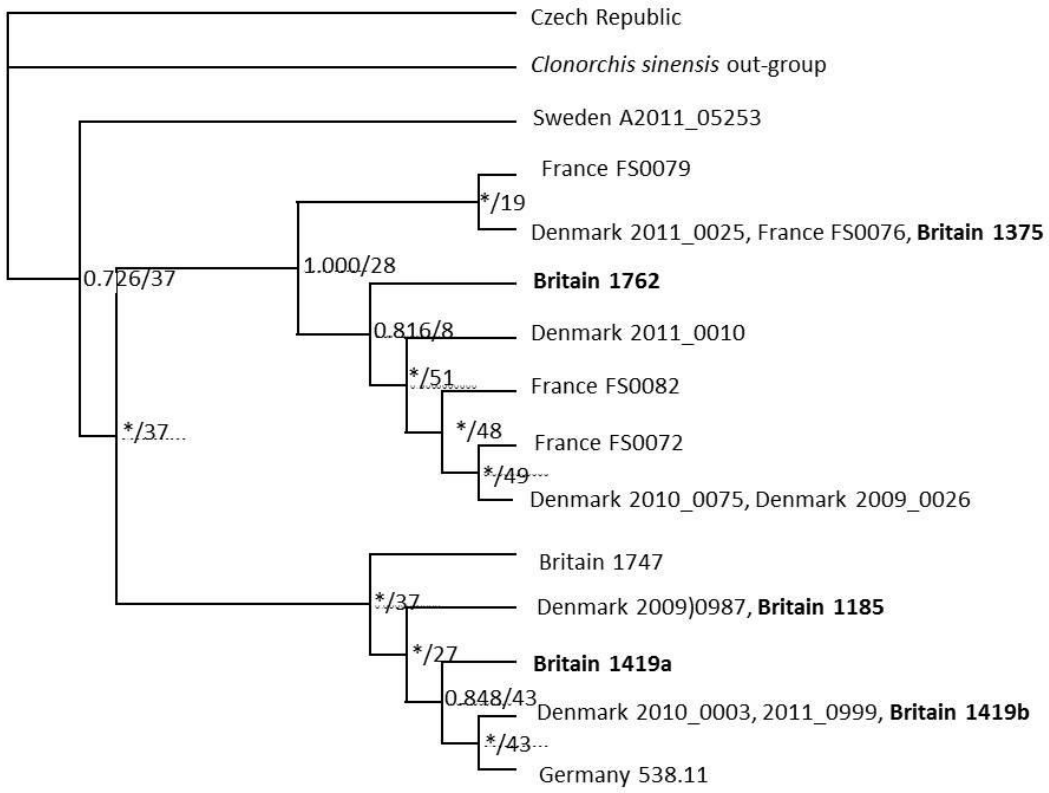

Figure 5.2 Phylogram for *Metorchis albidus* (Bayesian Inference /Maximum likelihood, 100 bootstraps) based on the combined cytochrome c oxidase sub-unit I (COXI) and III (COXIII) mitochondrial DNA regions (829bp from European samples. British samples are referred to by individual numbers.* indicates a BI probability of <0.2

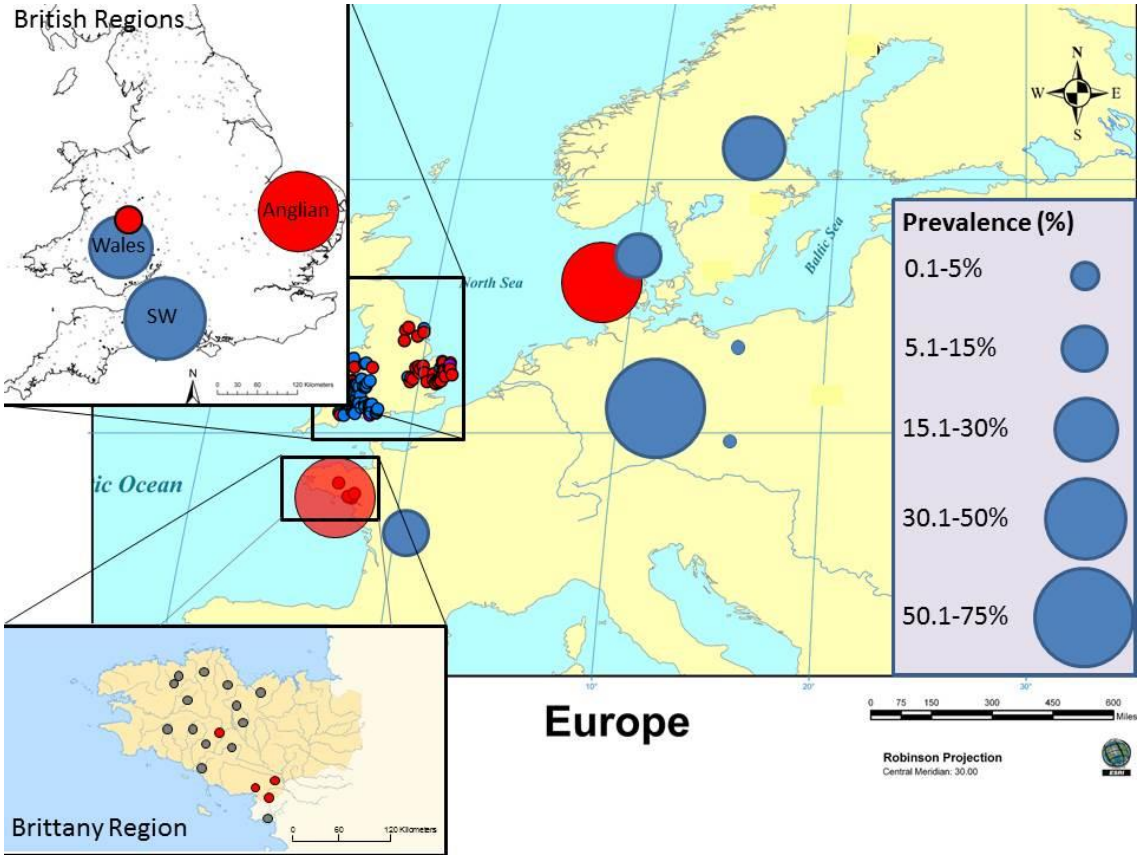

Figure 5.3 Prevalence of *Pseudamphistomum truncatum* (blue) and *Metorchis albidus* (red), Opisthorchiidae, across Europe. The increasing size of circles corresponds to the increasing prevalence within a particular region. Inserts show the regional prevalence within the UK and locations of *M. albidus* infected (red) and uninfected (grey) otters in Brittany.

The number of haplotypes per region is given in Table 5.3. Only a single ITS2 haplotype was identified across Europe for each parasite species; the two species differed by 2% across 387bp (Kimura-2-Parameter). Across the mtDNA (COXI and COXIII) phylogeny (758bp and 829bp for *P. truncatum* and *M. albidus* respectively), there was only 1.8% and 3.6% difference among *P. truncatum* and *M. albidus* haplotypes, respectively. These differences correspond to 14 segregating sites in *P. truncatum* (8/388 for COXI, 6/370 for COXIII) and 30 in *M. albidus* (19/435 for COXI and 11/394 for COXIII). The resolution of the mtDNA trees was poor for both the ML and MrBayes analyses. However, for both species the British samples were scattered across the phylogenies (Figures 5.1 and 5.2) instead of clustered together as expected under a scenario of a recent introduction to Britain. The populations appear fairly heterogeneous across Europe with no significant structure based on the geographic locations (Table 5.3).

Table 5.3 Summary of the phylogenetic data on *Pseudamphistomum truncatum* and *Metorchis albidus* across Europe: Samples size (number of parasites sequences), the gene sequenced, the population (country of origin), the evolutionary distance among the population showing the pairwise difference, the fraction of sequence positions that differ by a transition and PI, the fraction of sequence positions that differ by a transversion, and the number of haplotypes within the population.

Parasite	**Sample size**	**Gene**	**Population (country of origin)**	**Mean within population pairwise difference (PI)**	**Number of haplotypes**
Pseudamphistomum truncatum	6	COXI	Britain	0.005 (0.0026)	2
	10		Mainland Europe	0.006 (0.0023)	7
	3		Sweden	0.000	1
	5	COXIII	Britain	0.0011 (0.001)	2
	17		Mainland Europe	0.0027 (0.001)	4
	4		Sweden	0.0014 (0.001)	2
	3		Denmark	0.0018 (0.0017)	2
	7		Germany	0.0026 (0.0018)	2
Metorchis albidus	5	COXI	Britain	0.0200 (0.0051)	5
	11		Mainland Europe t	0.0187 (0.0043)	8
	3		Denmark	0.0211 (0.0052)	3
	4		France	0.0052 (0.0027)	3
	4	COXIII	Britain	0.0013 (0.0012)	2
	10		Mainland Europe	0.0138 (0.0045)	2
	6		Denmark	0.0156 (0.005)	2
	3		France	0.000	1

5.5 Discussion

The phylogenies of *Pseudamphistomum truncatum* and *Metorchis albidus* across their European range suggest that neither digenean is a recent introduction into Britain. Despite anecdotal reports of the potential for *P. truncatum* and *M. albidus* to infect British mammals (see Nicoll 1923) the two parasite species were identified only in 2005 (Simpson et al. 2005) and 2009 (Sherrard-Smith et al. 2009) respectively, leading to their status as suspected invaders. Until recently, susceptible wildlife was not screened systematically (Sainsbury et al. 2001, and see Simpson 1997, Chadwick 2007); therefore the likelihood of identifying such parasitic fauna was low, perhaps explaining the recent reports of these digeneans. There is often ambiguity over the native or non-native status of parasites in parts of their known range (Blakeslee et al. 2012). Identification is particularly challenging for inconspicuous or cryptic species (see Nei et al. 1975, Sakai et al. 2001) and may explain why neither trematode was recognised in Britain prior to 2005 (Simpson et al. 2005 but see Nicoll 1923).

Both *P. truncatum,* but *M. albidus* in particular, have similarly poor resolution across both mainland Europe and British populations. The resolution of both ML and Bayesian phylogenetic trees was poor as indicated by the low node support values inferred with the current data. On one hand, the current phylogenies are not well resolved as a result of the lack of an adequate outgroup for our data. Nevertheless, alternative outgroups were tested in both ML and Bayesian analyses (including *M. albidus* as outgroup for *P. truncatum* and vice versa) but the tree resolution remained poor. On the other hand, the lack of geographic resolution in our dataset may reflect a mechanism that operates to maintain a relatively homogeneous population across mainland Europe and Britain. Legislation is operative to protect fish from disease (e.g. EU Council Directive 2006/88/EC) but does not apply to most digeneans. In part, this relaxed approach to screening for digeneans stems from their reported low level impact on fish and it is therefore impractical to restrict fish movements on this basis. The widespread translocation of fish stocks, alongside movement of snails and parasite eggs with plants, gravel or water, across the continent and within Britain almost certainly contributes to a widespread distribution of digenean species.

The variation in prevalence of *P. truncatum* between countries (from zero in Scotland and Norway, to >70% in part of Germany) may be indicative of patchy distribution

across Europe, although more extensive sampling would be required to examine this further. Scotland and Norway may be, at present, beyond the geographic range of both parasites but the presence of both species in Sweden shows that both countries are within the northern latitudinal limit for the parasite. Further, the predicted warmer conditions across Europe with climate change may suit both species and encourage a northerly movement of their current distributions (Chapter 4). The distribution of host species provides no apparent barrier to their spread within Europe: the first intermediate hosts are members of the gastropod Families Lymnaeidae and Bithyniidae and second intermediate hosts, Cyprinidae (Dunn 1978; Chapter 7); both host stages are found throughout Europe.

The density-dependent processes that determine the distribution of host populations can result in spatial aggregation of parasite populations (Shaw and Dobson 1995) and has been used to explain the co-existence of species (e.g. Hassell et al. 1991, Dobson et al. 1992, Shaw and Dobson 1995). The presence of two dominant biliary parasites in the otter population across Europe may contribute to the observed patchiness in distribution via interspecific competition, or some level of acquired host immunity, acting to separate *P. truncatum* and *M. albidus*. It is noteworthy that across the entire study, only 5 co-infections were observed; 2 from the UK (out of 586 otters) and 3 in Denmark (out of 52 otters), but none elsewhere (867 otters in total). Specifically, a distinction between the geographic distributions of *M. albidus* and *P. truncatum* was observed in France. Recent studies report a modification in otter diet observing a switch from eels to introduced crayfish (Beja, 1996) so differentiation in diet might explain differences in the likelihood of infection. The otter population of France is divided between the Massif Central Region and the Atlantic Coast which is probably connected to the Poitou-Charentes Region and from here the otter population is expanding west (Robitaille and Laurence 2002). Only a single *P. truncatum* specimen was recorded in the Poitou-Charentes Region whereas the dominant parasite in Brittany was *M. albidus*. The rarity of *P. truncatum* and *M. albidus* in the same geographic location is observed across continental Europe but also in Britain where sample sizes are large enough (N = 586) to make conclusions robust (see Sherrard-Smith et al. 2009).

Ultimately, this study does not support the hypothesis that *P. truncatum* and *Metorchis albidus* were recent introductions to Britain, although further work would be required to conclude this definitively. The significant heterogeneity among the combined COXI and

COXIII *M. albidus* mtDNA indicates population mixing, perhaps the result of the translocation of fish stocks. Although legislative controls are in place to restrict fish movements they rarely consider trematode infection (see EU Council Directive 2006/88/EC) because the pathology associated with trematode larvae is often negligible on many fish intermediate hosts. The current study provides an insight into the genetic structure, but also geographic heterogeneity, of a widespread digenean of threatened wild mammals.

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Appendix 5.1 Genetic diversity among trematodes; rationale for choosing the mtDNA COXI and COXIII regions

The haplotype diversity, sample size and amplicon length were compared among studies that consider genetic variation within the trematodes (GLM with suitable error distributions, see Table 5A). The mtDNA NAD1 region presented slightly higher haplotype diversity than COXI – the most studied DNA sequence – but this was not significant and either gene seems equally informative (Figure 5A). In addition, our empirical data shows the mitochondrial cytochrome c oxidase sub-unit (COXIII) is perhaps more variable than sub-unit 1 (COXI) indicating its suitability for intraspecific population analyses of trematodes (and see Zarowiecki et al. 2007).

Two haplogroups were identified in the COXI and COXIII mtDNA sequences for both *P. truncatum* and *M. albidus*, but we would expect greater genetic diversity if more specimens or longer amplicons were available (increased amplicon length correlates with increased number of haplogroups: LM (log transformed data); $F = 13.12$, $df = 31$, $p < 0.01$).

Certain trematodes appear to have much higher genetic diversity than others. In particular, more global species such as *Fasciola* sp. and *Schistosoma* spp. carry a vast number of haplotypes per examined mitochondrial DNA gene (see Table 5A and references therein; Semyenova et al. 2006). Such species are under strong selection pressure from drug treatment and so are perhaps incomparable with other helminths. Additionally, because of their economic importance, species such as *F. hepatica* have been extensively studied; this is likely to contribute to an apparent high genetic diversity because the number of individuals sampled relates positively to genetic diversity.

Table 5A Genetic diversity of trematodes, based on published information (studies highlighted using an ISI Web of Knowledge literature search for the terms: Trematod* AND Genetic diversity).

Parasite species (N where available)	Genetic Region	Number of base pairs	Number of haplotypes	Geographic Region	Reference
Fasciola hepatica	CO1		4	Iran	Moazeni et al. 2012
Opisthorchis viverrini	CO1		5	Thailand	Sithithaworn et al. 2012
Opisthorchis viverrini	ITS2		1	Thailand	Sithithaworn et al. 2012
Schistosoma japonicum (169)	Cytb-ND4L-ND4 ND1 ND4 (mtDNA NADH dehydrogenase subunits 1 and 4)	793-794 767 1463-1466	96 (combined)	Mainland China	Zhao et al. 2012
Coitocaecum parvum (120)	CO1	781	18	New Zealand	Blasco-Costa et al. 2012
Stegadexamene anguillae (124)	CO1	702	23	New Zealand	Blasco-Costa et al. 2012
Fasciola gigantica (60)	CO1	443	8	Mauritania	Amor et al. 2011a
Fasciola gigantica (60)	ITS1	435	1	Mauritania	Amor et al. 2011a
Fasciola gigantica (60)	ITS2	346	1	Mauritania	Amor et al. 2011a
Fasciola hepatica (22)	ITS1 and 2 combined	435 and 346	3	Iran	Amor et al. 2011b
Fascioloides magna (324)	CO1 and *nad1* combined	384 and 405	8 and 15	Europe and North America	Kralova-Hromadova et al. 2011
Trichobilharzia szidati/T. ocellata (39)	CO1	1125	7	Russia and Europe	Korsunenko et al. 2012
Clinostomum spp. (22)	CO1	557	21	Oklahoma, USA	Bonett et al. 2011
Fasciola hepatica (47)	ND1	549	14	Egypt	Amor et al. 2011a
Fasciola gigantica(42)	ND1	549	19	Egypt	Amer et al. 2011
Fasciola hepatica (47)	CO1	452	13	Egypt	Amer et al. 2011

Parasite species (N where available)	Genetic Region	Number of base pairs	Number of haplotypes	Geographic Region	Reference
Fasciola gigantica (42)	CO1	452	7	Egypt	Amer et al. 2011
Diplostomum pseudospathaceum (139)	ITS1		1	Finland	Louhi et al. 2010
Opisthorchis viverrini (86)	NAD1	645	19	Thailand, LaoPDR, Cambodia	Thaenkham et al. 2010
Crassicutus cichlasomae	CO1	290-355	86 (1-8 per locality)	Middle-America	Razo-Mendivil et al. 2010
Maritrema novaezealandensis (269)	CO1	706	141	South Island New Zealand	Keeney et al. 2009
Philophthalmus sp (246)	CO1	706	23	South Island New Zealand	Keeney et al. 2009
Cryptocotyle lingua (98)	CO1	655	34	North America	Blakeslee et al. 2008
Cryptocotyle lingua (98)	CO1	655	75	Europe	Blakeslee et al. 2008
Opisthorchis viverrini (3)	18S		1	Korea, China, Laos	Park 2007
Opisthorchis viverrini (3)	ITS2		1	Korea, China, Laos	Park 2007
Opisthorchis viverrini (3)	CO1		1	Korea, China, Laos	Park 2007
Fasciola hepatica (113)	*nad1*	316	13	Eastern Europe, Western Asia	Semyenova et al. 2006
Fasciola hepatica (107)	CO1	429	10	Eastern Europe, Western Asia	Semyenova et al. 2006
**Cercaria batillariae* (447)	CO1	800	70	Japan	Miura et al. 2005
**Cercaria batillariae* (447)	ITS2	800	1	Japan	Miura et al. 2005
Philophthalmid sp. (323)	CO1	400	13	Japan	Miura et al. 2005
Philophthalmid sp. (323)	ITS2	400	1	Japan	Miura et al. 2005

Parasite species (N where available)	Genetic Region	Number of base pairs	Number of haplotypes	Geographic Region	Reference
Deropegus aspina A (66)	ND1	636	19	Pacific Northwest	Criscione and Blouin 2004
Deropegus aspina B (89)	ND1	636	22	Pacific Northwest	Criscione and Blouin 2004
Nanophyetus salmincola (91)	ND1	639	75	Pacific Northwest	Criscione and Blouin 2004
Plagioporus shawi (92)	ND1	636	28	Pacific Northwest	Criscione and Blouin 2004
Clonorchis sinensis	18S	1030	3	Korea and China	Lee and Huh 2004
Clonorchis sinensis	ITS1	762	3	Korea and China	Lee and Huh 2004
Clonorchis sinensis	ITS2	451	1	Korea and China	Lee and Huh 2004
Clonorchis sinensis	CO1	393	2	Korea and China	Lee and Huh 2004
Opisthorchis viverrini	CO1	417	5	Northeast Thailand	Ando et al. 2001
Opisthorchis viverrini	ITS2	296	1	Northeast Thailand	Ando et al. 2001
Pseudamphistomum truncatum (16, 22)	*CO1* *CO3*	*387* *370*	*2* *1*	*Britain and continental Europe*	*(current study)*
Metorchis albidus (15) *(14)*	*CO1* *CO3*	*434* *394*	*2* *2*	*Britain and continental Europe*	*(current study)*

*Genus name not given.

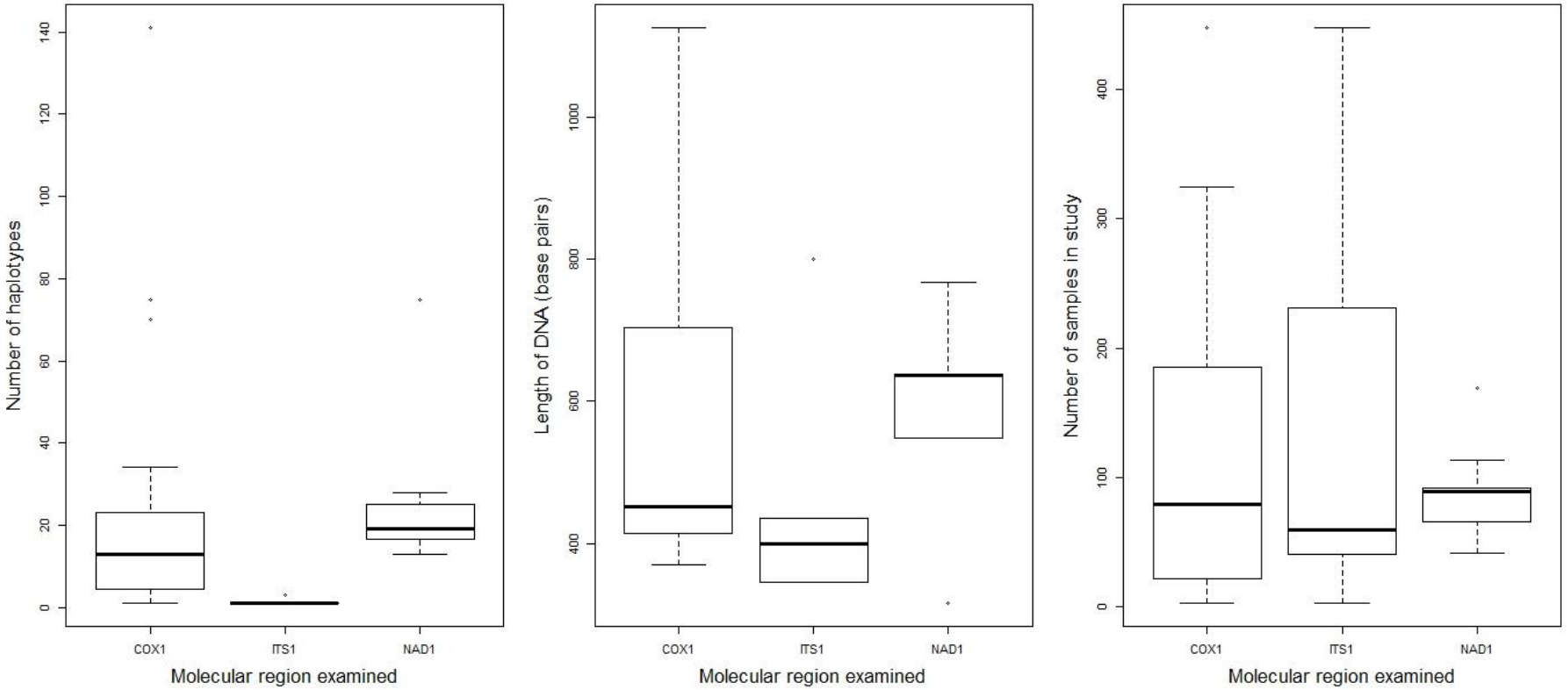

Figure 5A Comparison of the number of haplotypes identified, the length of the DNA amplicon studied and the number of samples included per study for different DNA regions used for trematode phylogenies from the literature (see Table 5A for data).

5A References

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6. Spatial and seasonal factors are key determinants in the aggregation of helminth populations in their definitive hosts: *Pseudamphistomum truncatum* in otters *Lutra lutra*

6.1 Abstract

Parasites are typically aggregated within their host populations. The most infected hosts are cited frequently as targets for optimal disease control. Yet a heavily infected individual is not necessarily highly infectious and so does not automatically contribute a higher proportion of parasitic infective stages than a host with fewer parasites. Here, *Pseudamphistomum truncatum* (Opisthorchiidae) infection of a definitive host, the otter (*Lutra lutra*), was used as a model system. Whether variation in parasite abundance, aggregation and egg production (fecundity, as a proxy of host infectiousness) can be explained by abiotic (season and region) or biotic (host age, sex and body condition) factors was investigated. Parasite abundance was affected most strongly by the biotic factors age and body condition such that adults and otters in good condition had heavier infections than sub-adults or those in poor condition, whilst there were no significant differences in parasite abundance among the host sexes, seasons or regions. Conversely, parasite aggregation was affected most strongly by the abiotic factors season and region, which was supported by all four independent measures of parasite aggregation (the corrected moment estimate *k*, Taylor's Power Law, The Index of Discrepancy *D*, and Boulinier's *J*). *P. truncatum* was highly aggregated within otters, with aggregation stronger in the Midlands and Wales than in the Southwest region of Britain. Overall, more parasites were found in fewer hosts in the summer, which coincides with the summer peak in parasite fecundity. Combined, these data suggest that (i) few otters carry the majority of *P. truncatum* parasites and that there are more infective stages (eggs) produced during summer; and (ii) abiotic factors are most influential when describing parasite aggregation whilst biotic factors have a greater role in defining parasite abundance. Together, parasite abundance, aggregation and fecundity can help predict which hosts make a large contribution to the spread of parasitic infective stages so that the functionally important factors within a given population can be identified to allow us to focus treatment strategies more effectively.

(*A version of this chapter has benn submitted for publication*: Sherrard-Smith, E., Perkins, S.E., Chadwick, E.A., Cable)

6.2 Introduction

Patterns of parasite intensity vary widely across a host population (Anderson and May 1991, Scott and Smith 1994) and generally parasite distributions tend to be aggregated, a pattern best defined by a negative binomial distribution (Shaw and Dobson 1995, Shaw et al. 1998, Woolhouse et al. 1997, Galvani and May 2005). The most infected individuals are frequently cited as key individuals to target for optimal disease control (e.g. Woolhouse et al. 1997, Perkins et al. 2003; Lloyd-Smith et al. 2005, Matthews et al. 2006). Yet high parasite intensities do not necessarily indicate that an individual has a correspondingly high infectiousness, or transmission potential. Parasite transmission is defined as the probability of contacting an infectious particle/individual and acquiring that infection. As such, the transmission potential of a host can, in part, be quantified by parasite fecundity, as this measures the number of potential infective stages (Shaw and Dobson 1995, Shaw et al. 1998). Arguably hosts that are simultaneously the most infected and harbour parasites that are highly fecund are likely to contribute strongly to the transmission potential of a parasitic disease. There is, however, inherent variation in the reproductive potential of a parasite, affected by both parasite and host age, sex, body condition and host immunity (Kaitala et al. 1997, Luong et al. 2010, Koehler and Poulin 2012), which may in turn be affected by environmental factors. Identifying the host and environmental factors that are associated with heavily infected hosts and/or highly fecund parasites will allow us to focus our treatment strategies more specifically. This is vital to prolong the efficacy of drugs, particularly in light of antihelmintic resistance to chemotherapeutic strategies (Laurenson et al. 2013).

Patterns of parasite abundance, aggregation and fecundity have been used frequently to consider the contribution an individual host can make to the spread of disease, although typically these variables are considered in isolation of one another (e.g. Madhavi 1979, Rolfe et al. 1991, Woolhouse et al. 1997, Perkins et al. 2003; Ferrari et al. 2004, Newey et al. 2005). The mean parasite abundance does not define parasite aggregation because parasitism can truncate the negative binomial distribution as a result of parasite induced mortality of the most infected individuals (Poulin 1993, Gregory and Woolhouse 1993). A high parasite abundance could lead to a high potential for carrying more infective stages if all other factors are equal, but increasing abundance of parasites can also result in elevated density-dependent competition or increased activation of host defence mechanisms (Shostak and Scott 1993) so

reducing survival or fecundity of each parasite (Jaenike 1996). To determine the nature of this trade-off parasite abundance, aggregation and fecundity need to be considered together. By examining all three variables in combination with ecological data it should be possible to identify the factors associated with hosts that are simultaneously the most infected and infectious. Once these factors, responsible for determining parasite abundance, aggregation and fecundity, are identified, this information can be used to optimise the efficacy of parasite treatment by using targeted control on specific hosts in specific regions and/or time periods (Nielsen 2012, Laurenson et al. 2013).

In the current study, parasite abundance is defined as the number of individuals of a particular parasite species in or on a single host, regardless of whether or not that host is infected with the parasite (following Bush et al. 1997). An individual with a high abundance could be considered more functionally important with regards to parasite transmission potential than one with a low abundance (or no parasites), such that treatment of heavily infected individuals could have a disproportionally high reduction of the parasite population (see Woolhouse et al. 1997, Perkins et al. 2003, Ferrari et al. 2004, Skorping and Jensen 2004, Magalhaes et al. 2012). Equally, parasite aggregation is a measure of the extent to which parasites are scattered unevenly among available hosts (Poulin 1996*a*, Shaw and Dobson 1995, Shaw et al. 1998). If a pattern exists where the majority of parasites are found within a minority of the hosts then there is potential to isolate and treat these heavily infected few (see Dobson et al. 1992, Hudson et al. 1992, Poulin and Morand 2000, Newey et al. 2005, Heylen and Matthysen 2011).

Parasite fecundity is the reproductive potential of a parasite and can be measured, for helminths, using *in utero* egg counts (Stear et al. 1995, Richards and Lewis 2001) or faecal egg counts. The latter represents the volume of infectious particles that are released into the environment (Madhavi 1979, Rolfe et al. 1991). However, eggs are often shed in the faeces sporadically (Tinsley 1983) causing issues for quantification of shed particles, if samples are taken infrequently (see Wilson et al. 2001). Instead, *in utero* egg counts for parasites with an elongated uterus may provide a more robust proxy than faecal egg counts for quantifying parasite transmission potential, particularly where hosts are elusive or faecal samples difficult to collect (e.g. defecation occurs in water).

In this study, parasite abundance and aggregation across a host population, and parasite fecundity using *in utero* egg counts of *Pseudamphistomum truncatum* (Trematoda, Opisthorchiidae) infections in otters, *Lutra lutra* (see Sherrard-Smith et al. 2009) was quantified. *Pseudamphistomum truncatum* is a mammalian biliary parasite with a three host life cycle that is trophically transmitted to otters via the consumption of a second intermediate host, cyprinid fish. The parasite matures in the otter and, typical of digeneans, accumulates egg capsules in an elongated uterus. Eggs are deposited into the environment with faeces and ingested by snails which subsequently release cercariae that can encyst on the cyprinid fish. This system is used to answer two questions: i) Are patterns of parasite abundance and aggregation related to seasonality and geographic location and/or host age, sex and body condition? ii) Is parasite fecundity higher in specific groups of hosts; in other words, is it possible to identify individuals that have the potential to disseminate significantly more parasite infectious stages than others? A helminth population from a long-term survey of wild animal cadavers is used as a model system. To our knowledge this is the first time that parasite abundance, aggregation and fecundity have been considered together to identify the factors that might contribute disproportionately to parasite transmission potential.

6.3 Materials and methods

6.3.1 Sample collection

Road-killed otters, *Lutra lutra* (N = 516 of which 72 were infected) were collected from across England and Wales as part of a national monitoring scheme (Cardiff University Otter Project CUOP, see Chadwick et al. 2011). Gall bladders were removed and examined for the presence of biliary parasites. *Pseudamphistomum truncatum* were identified morphologically (following Yamaguti 1971). The location of each otter based on Environment Agency (EA) River Catchment regions (see Sherrard-Smith et al. 2009), month and year of host death, age-class (adult or sub-adult), sex, length and body mass were recorded. A body condition index (BC) for otters was calculated based on otter length and mass (Kruuk et al. 1987). Body condition, previously referred to as K (see Kruuk et al. 1987, Sherrard-Smith et al. 2009), is here renamed BC, to distinguish it from the negative binomial dispersion parameter *k*, used in

this study to measure parasite aggregation. Body condition (BC) is measured by the following equation:

Equation 6.1: $$BC = \text{weight(kg)} / [a \times \text{length}^{n}]$$

where a = 5.02 and n = 2.33 for females, and a = 5.87 and n = 2.39 for males (following Kruuk et al. 1987). The hosts were split into 2 groups allowing a comparison to be made between hosts in good condition (BC ≥1) and those hosts in poor condition (BC < 1). Host animals were also categorised by age class as 'adult' or 'sub-adult', with sub-adults defined as males with a baculum (penis bone) length of less than 60 mm and females that were not yet reproductively active (see Sherrard-Smith and Chadwick 2010). Seasons were defined as spring: March, April and May; summer: June, July and August; autumn: September, October and November; and winter: December, January and February.

To assess whether there were distinct differences in the level of parasitic infection between abiotic or biotic factors, or whether the number of parasites correlated with the host condition index, General Linearized Models (GLM, with negative binomial error distributions) were fitted separately for each abiotic or biotic factor to the parasite abundance data. To compare the degree of aggregation of parasites within the population, differences between the abiotic factors, season of host death (winter, spring, summer or autumn) and geographic location (where sample sizes were large enough to allow comparisons; Wales, Midlands or Southwest England), in addition to biotic factors, age class (sub-adult or adult), sex (male or female) and condition (good or poor) (see Table 6.1 for sample sizes), were explored using 4 different metrics of aggregation.

6.3.2 Aggregation parameters

Several indices have been developed to explore the degree of parasite aggregation within a host population including the corrected moment estimate *k*, Taylor's Power Law, Poulin's Index of Discrepancy (*D*), and Boulinier's *J*, which can be used to identify groups of infected individuals. Comparing aggregation between populations is generally challenging for different reasons depending on the given aggregation index. The corrected moment estimate of *k* is the most commonly used measure of parasite aggregation and it is most widely accepted (Wilson et al. 2001), particularly because on comparison of various indices (variance to mean ratio, coefficient of variation, moment estimate of *k* and corrected moment estimate of *k*), corrected moment estimate of *k* was found to vary least with mean parasite

load and sample size (Gregory and Woolhouse 1993). Regardless, there is still an element of co-variance with the mean when using the *k* parameter (see Gregory and Woolhouse 1993).

Taylor's Power Law relates the between sample variance to the overall mean abundance of a given sample of organisms and is most useful when a group of samples or populations are available for consideration (see Wilson et al. 2001). The Index of discrepancy (*D*) is more host-centric than *k* or Taylor's Power Law and less sensitive to the distribution of parasites (Poulin 1993). The *D* index can vary between 0 (no aggregation) and 1 (all parasites are theoretically within a single host), and so can be used to compare datasets with varying prevalence and mean parasite load. Equally, Boulinier's *J* considers the relative clustering of parasites, by calculating the likelihood of additional parasites occupying a host, if that host is already infected by any given parasite (Boulinier et al. 1996). As such, Boulinier's *J* quantifies parasite aggregation from an individual host perspective rather than a population level metric and has the advantage that, unlike the *k* parameter, is not biased for small sample sizes (Gregory and Woolhouse 1993, Boulinier et al. 1996). Consequently, comparing aggregation between sub-samples of hosts is problematic because no single measure of aggregation is entirely reliable and aggregation indices vary according to sample size and abundance. To achieve confidence in the patterns observed, for the first time four different methods are applied to quantify parasite aggregation across abiotic and biotic factors, and assess whether there is congruence across multiple measures.

6.3.2.1 The corrected moment estimate (k)

The corrected moment estimate (*k*) of the negative binomial distribution quantifies an increase in parasite aggregation, for a constant mean, with a diminishing *k* value, i.e. it is an inverse measure of aggregation. For example, if the parasite population is highly aggregated in the host population *k* tends toward a theoretical limit of zero (where all parasites are concentrated within a single host). Conversely, for the same given mean parasite abundance, as *k* increases so parasite aggregation decreases so that the distribution tends toward a Poisson (random) distribution followed by a positive binomial as *k* increases to infinity. The corrected moment estimate *k* is calculated using the mean parasite abundance *x*, variance σ^2, and sample size N of the given population (see Equation 6.2). The *k* was calculated for each sub-sample of the host population according to abiotic or biotic variables using the following equation:

Equation 6.2:

$$k = (x^2 - \sigma^2 / N) / (\sigma^2 - x)$$

The frequency distributions within host sub-groups were compared to examine differences in *k* using GLMs with negative binomial error distributions.

6.3.2.2 Taylor's Power Law

To examine differences in parasite aggregation within host sub-groups, first the departure of the variance of the number of parasites per host among categories from a random distribution was quantified, and then comparable populations were examined by using a bootstrapping technique to calculate Taylor's Power Law parameter *b* following Boag et al. (2001). Briefly, 50 parasite counts from each population were sampled at random (with replacement) to calculate the log (mean + 1) and log (variance + 1). This was replicated 50 times giving an estimate of *b* (the gradient of the linear regression of log (variance + 1) onto log (mean + 1)). This process was repeated 100 times to calculate SE for the intercept (*a*) and *b*. Where possible, statistical comparisons between groups (e.g. spring, summer, autumn and winter) were performed using GLMs (Table 6.2). In some cases, the large number of zeros in the analysis led to *b* estimates of 0 and statistical comparisons were not possible using this method. A simple 2-sample Kolmogorov-Smirnov test was then applied to test whether samples had the same distributions.

*6.3.2.3 The Index of Discrepancy (*D*)*

Poulin's Index of Discrepancy (*D*) quantifies the degree of inequality between the observed distribution and a hypothetical distribution where parasites are distributed equally among hosts. Here, zero represents perfect equality and 1 implies all the parasites are aggregated within a single host. To quantify *D* hosts were ranked from the most to the least infected individuals (including those without infection). It was then possible to calculate the proportion of parasites associated for any given percentage of infected hosts and *D* was calculated using:

Equation 6.3:

$$D = 1 - \frac{2\sum_{i=1}^{N}(\sum_{j=1}^{i} \mathrm{xj})}{\mathrm{x} * N(N+1)}$$

where N = total host population, X is the number of parasites in host *j*, and x is the mean number of parasites (see Poulin 1993).

To examine whether parasites are distributed differently between abiotic (season and region) or biotic (age, sex and condition) factors using *D*, a GLM with a binomial error distribution was fitted to the number of parasites per given proportion of hosts (0.01-0.99), bound to the total number of parasites in the associated factor (Yw). Each factor was considered independently so allowing comparison of the regression lines that describe each category (factors: season or geographic region, age, sex or condition).

6.3.2.4 Boulinier's 'J'

An alternative method to examine parasite aggregation within and among hierarchical scales was proposed by Boulinier et al. (1996). This method was developed to look at spatial scale impacts on parasite aggregation. The aggregation index, J_j (Equation 6.4), measures the increase in the expected number of other parasites on a host for any given parasite.

Equation 6.4:

$$J_j = 1/n_j \frac{\sum_{i=1}^{n_j} x_{ij}(x_{ij}-1)}{\left\{X_j\left(X_j - 1 + \left(\frac{n_j-1}{n_j}\right)^2\right)\right\}} - 1$$

Where n_j is the number of hosts in a given population j; x_{ij} is the corresponding number of parasites in individual, i, from population j; and X_j is the mean abundance for the total population j.

The Boulinier index (J_j) was calculated for each sub-sample of hosts according to season, region, host age, sex and body condition. To examine whether the *J* index indicated significantly different aggregation patterns between comparative sub-groups (e.g. males vs females) a bootstrapping method was devised to generate confidence intervals for a given population: 100 independent data sets with negative binomial distributions were simulated,

parameterised by the respective mean, *k* and host population sizes of each sub-sample (e.g. female hosts). J_j was calculated for each of these 100 generated data sets and then the J_j data were ranked from smallest to largest so that the 3rd and 98th values could represent the lower and upper 95% confidence intervals respectively. *A posteriori* testing in ANOVA was then applied to the simulated *Jj* data; where the confidence intervals overlapped with the *Jj* for the real data then it was concluded that there was no significant difference between parasite aggregations among sub-groups. Conversely, where confidence intervals did not overlap the *Jj* for a given sub-sample, then it was concluded that this was indicative of a significant difference between sub-groups of the host population.

Here, the degree of parasite aggregation among sub-groups of a host population was compared using the four different measures of parasite aggregation. The analysis of *Jj* is promising because this measure of aggregation does not vary with small sample sizes (a limitation of *k*). The parameter *D* has been criticised because the measure of aggregation depends on sample size (Ploeger 1994). Poulin (1996*b*) argues that *D* measures aggregation directly, as opposed to the indirect estimates provided by Taylor's Power Law and *k*, and quantifies the uneven use of hosts. *D* is based on both the number of uninfected hosts as well as the distribution of parasites among the infected host population. This measures the extent to which parasites use available hosts unevenly so that many hosts are uninfected whilst crowding occurs in a few – the definition of parasite aggregation according to Poulin (1996*b*). However, an aggregation estimate based on a small sample size and regardless of the method used, is likely to underestimate parasite aggregation because heavy infections are rare and therefore most likely to be observed only when sample sizes are large (Poulin 1993, 1996*b*). The *J* approach asks a slightly different question to *k*, Taylor's Power Law and *D*. For any given parasite, at the scale of aggregation among hosts, *J* asks: what is the expected increase in the number of additional parasites on a given host relative to a case where parasites are distributed randomly across the host population? It is appropriate therefore to consider a range of estimates of parasite aggregation to provide a comprehensive insight of a given system.

6.3.3 Parasite fecundity

Parasite fecundity can be measured by the number of reproductive units (eggs) within the uterus (Richards and Lewis 2001) and high fecundity can indicate individuals that are potentially most infectious. In the current study, the state of decomposition of hosts

(opportunistically collected road-kill) prevented the use of all 72 infected hosts for *in utero* analysis of the parasites. As such, to examine the parasite fecundity across different abiotic and biotic factors 35 infected hosts with a total of 255 *P. truncatum* were used. Of these, a subset 119 parasites from 19 hosts were flat-fixed and measured (length, width and area) before storage in 70% ethanol prior to egg counts, allowing us to test whether parasite fecundity is confounded by parasite size. A photographic method was developed to count *in utero* fecundity. Each parasite was teased apart in 2ml of distilled water within an adapted microscope slide with a 2mm high rim surrounding a $15mm^2$ central arena. The slide was scanned at x400 magnification and c. 500 images were taken of each slide to cover the $15mm^2$ area in fine detail. These images were knitted together without overlap of adjacent images. These images were then screened manually, counting all eggs touching or crossing the top and left edge and ignoring those on the bottom and right edge of each image, to avoid counting eggs twice. It was then examined how mean parasite fecundity varied with abiotic and biotic variables (mixed-effects GLM with Gaussian error distribution and identity link function). All statistical analyses were conducted using the package R, version 12.1 (R Development Core Team, 2010).

6.4 Results

6.4.1 Parasite abundance

Pseudamphistomum truncatum infects 13% of UK otters (N = 72 infected out of 516 otters, mean abundance 3.9±0.97, mean intensity = 28.3±6.24, range = 1-302). Parasite abundance differed between sub-adults and adults (age) such that adults had higher infections (GLM, negative binomial error distribution: $\chi^2_{1,515} = 1.995$, SE = 0.5141, $p < 0.05$). Similarly, otters in good condition had higher infections than those in poor condition $\chi^2_{1,515} = 2.012$, SE = 0.5298, $p < 0.05$). There were no significant differences in parasite abundance between seasons, regions or host sexes ($p > 0.1$ in all cases) confirming Sherrard-Smith et al. (2013).

6.4.2 Parasite aggregation

Analysis of the aggregation index *k* found no statistically significant seasonal differences (GLM negative binomial error distribution, $p > 0.1$) but parasites were more aggregated in the Midlands region compared to the Southwest ($\chi^2_{2,25} = 1.669$, SE = 0.773, $p = 0.095$) and Wales ($\chi^2_{2,25} = 1.669$, SE = 0.773, $p = 0.091$), but only at the 90% confidence level. There was no significant difference in *k* between host ages or sexes (GLM negative binomial error

distribution, $p > 0.1$ for all cases, Table 6.1). Parasites were significantly more aggregated in poor condition hosts compared to those in good condition (GLM negative binomial error distribution: $\chi^2_{1,514} = 1.875$, SE = 0.479, $p = 0.044$).

Analysis of Taylor's Power Law showed parasites from hosts in summer were more aggregated than those in either winter ($p < 0.01$, see Table 6.2) or spring ($p < 0.05$). Equally, parasites were less aggregated in the Southwest population compared to either Wales ($p < 0.001$) or the Midlands ($p < 0.001$). Parasites were not aggregated differently between host age or sex classes (Table 6.2) but parasites were more aggregated in hosts in poor condition compared to those in good condition ($p < 0.001$).

Table 6.1 Summary statistics describing the fitted negative binomial distributions for the host-parasite interaction between *Pseudamphistomum truncatum* and otters, *Lutra lutra*: Biotic or abiotic sub-groups of the host population are differentiated by season, region, host age class, sex and host condition. Bold type-face refers to the most aggregated population among factors and significant differences among sub-groups are identified.

Population sub-group		Prevalence (%)	Mean Abundance	Variance	N. of hosts	N. of infected hosts	Measures of parasite aggregation			
							k parameter	Index of Discrepancy *D*	Variance-to-Mean Ratio (VMR)	*Jj* (CI)
Season:	Summer	15.9	5.6	1332.1	69	11	**0.0089**	**0.96**[2,3,4***]	4.185	**42.210 (18.9 – 101.8)**
	Autumn	12.6	2.2	198.3	167	21	0.0187	0.95[3,4***]	**6.695**	40.372 (18.1 – 82.2)[1]
	Winter	13.8	5.1	575.3	167	23	0.0390	0.94	3.917	22.140 (11.9 – 47.2)[12,***]
	Spring	15.0	3.9	257.0	113	17	0.0506	0.94	4.089	16.701 (6.8 – 42.7) [1,2***]
Region:	Wales	17.4	4.5	702.8	144	25	0.0224	0.94[3***]	**4.341**	**33.931 (14.1 – 71.2)**
	Midlands	16.2	4.1	365.9	37	6	**0.0199**[1,3·]	**0.94**[a]	4.158	20.860 (7.2 – 39.6)[1***]
	Southwest England	34.0	11.5	1194.5	106	36	0.1019	0.89	2.903	8.9052 (4.6 – 16.2)[1,2***]
Age	Adults	17.5	5.6	780.3	285	50	0.0377	**0.96**	3.846	24.220 (14.5 – 52.0)
	Sub-adults	10.6	2.1	122.1	207	22	**0.0307**	0.95	**6.615**	**28.077 (14.1 – 65.1)**[**]
Sex	Females	12.5	4.5	745.5	232	29	**0.0225**	**0.96**[***]	4.423	**37.144 (16.1 – 71.6)**
	Males	15.1	3.5	269.9	284	43	0.0433	0.95	**4.437**	21.323 (12.3 – 43.7)[***]
Condition	Poor condition	13.0	1.7	60.2	192	25	0.0415	**0.96**[***]	**8.122**	21.370 (11.3 – 46.8)
	Good condition	14.5	5.3	729.3	324	47	**0.0358**[*]	0.95	3.949	**25.644 (14.7 – 43.1)**[***]

Total population	13.9	3.9	516	72

Significance of statistical tests represented by · p < 0.1, * p<0.05, **p<0.01,*** p<0.001. For factors with more than 2 sub-groups, numbers are used to indicate the sub-group that differs from the current comparison (e.g. significant difference for *Jj* between summer and autumn populations p < 0.1, summer and winter p < 0.001). a Sample size too small for comparison.

Table 6.2 Comparison of the degree of aggregation across populations using Taylor's Power Law where appropriate and where necessary the non-parametric 2-sample Kolmogorov-Smirnov test is used to test whether two samples are drawn from the same distribution

Factor	Sub-population	Taylor's Power Law *b* (CI)	*a*	Statistical difference
Season:	Summer	0.272 (0 – 3.17)	0.037 (0 – 0.52)	F = 7.58, df = 3, p < 0.001
	Spring	0.093 (0 – 2.40)	0.046 (0 – 1.10)	Summer vs winter t = 2.586, p < 0.01
	Autumn	0.017 (0 – 0.00)	0.001 (0 – 0.00)	
	Winter	0.035 (0 – 0.00)	0.020 (0 – 0.00)	Summer vs spring t = 2.174, p < 0.05
Region:	Southwest	1.972 (1.69 – 2.23)	1.955 (1.27 – 2.72)	F = 1340, df = 2, p < 0.001
	Wales	0.031 (0 – 0.00)	0.003 (0 – 0.00)	Southwest vs Wales p < 0.001
	Midlands*	0.051 (0 – 0.00)	0.000 (0 – 0.00)	Southwest vs Midlands p < 0.001
Age:	Adults	0.003 (0 – 0.00)	0.000 (0 – 0.00)	Non-parametric K-S test:
	Sub-adults	0.004 (0 – 0.00)	0.000 (0 – 0.00)	D = 0.070, p = 0.605
Sex:	Females	0.050 (0 – 0.00)	0.009 (0 – 0)	Non-parametric K-S test:
	Males	0.023 (0 – 0.00)	0.007 (0 – 0)	D = 0.026, p = 0.999
Condition:	Good	0.273 (0 – 2.82)	0.104 (0 – 1.22)	F = 21.85, df = 1, p < 0.001
	Poor	0.119 (0 – 3.39)	0.002 (0 – 0.00)	

*The Midlands region n = 37, therefore the parasite counts was reduced to 25 for this population

Using the *D* index analysis, parasites were significantly more aggregated during the summer (*D* = 0.96) than during other seasons (GLM: $\chi^2_{3,83} = 9.765$, $p < 0.001$) whilst parasites were less aggregated across the otter population in winter (*D* = 0.94) and spring (*D* = 0.94) than during autumn (*D* = 0.95) (GLM: autumn vs. winter $\chi^2_{3,83} = 4.803$, $p < 0.001$; autumn vs. spring $chi^2_{3,83} = 7.184$, $p < 0.001$). Only 6 otters out of 46 were infected in the Midlands region (see Table 6.1) preventing detailed analysis of any patterns that might arise between this region and others. Parasites in otters from the Southwest were, however, less aggregated than those in otters from Wales (GLM: $\chi^2_{2,62} = 1.404$, $p < 0.001$). There were no differences between host age classes (GLM: $\chi^2_{1,41} = 0.798$, $p = 0.425$) but parasites were more aggregated in female otters than males (GLM: $\chi^2_{1,41} = 7.529$, $p < 0.001$) and more aggregated in poor condition otters than those in good condition (GLM: $\chi^2_{1,41} = 3.803$, $p < 0.001$).

The J_j index identified significant differences among all abiotic and biotic factors. Parasites in otters from summer (J_j = 42.2; CI = 18.9 – 101.8) and autumn (J_j = 40.4; CI = 18.1 – 82.2)

were more aggregated than those in winter (J_j = 22.1; CI = 11.9 – 47.2) and spring (J_j = 16.7; CI = 6.8 – 42.7). The confidence intervals overlap least within the regional factor, indicating that parasites in otters from the Southwest (J_j = 8.9; CI = 4.6 – 16.2) were distributed more evenly among the host population than those in either Wales (J_j = 33.9; CI = 14.1 – 71.2) or the Midlands (J_j = 20.9; CI = 7.2 – 39.6) (Figure 6.1). Parasite aggregation was greater in sub-adults (J_j = 24.2; CI = 14.5 – 52.0), female otters (J_j = 37.1; CI = 16.1 – 71.6) and those in good condition (J_j = 25.6; CI = 14.7 – 43.1) than adults (J_j = 24.2; CI = 14.5 – 52.0), male otters (J_j = 21.3; CI = 12.3 – 43.7) or those in poor condition (J_j = 21.4; CI = 11.3 – 46.8) where a higher *Jj* value is indicative of greater aggregation among the host population (Table 6.1).

In summary, 3 out of 4 measures of aggregation found significant differences between the seasons; parasites aggregated in fewer hosts in summer (k = 0.008, D = 0.96, Jj = 42.210, Table 6.1) than in spring. Only Taylor's Power Law indicated aggregation was strongest in Autumn (Table 6.1). For all measures of aggregation, parasites in otters were more aggregated in Wales (k = 0.025, Taylor's Power Law b = 0.031, D = 0.94, Jj = 33.931) and the Midlands (k = 0.016, Taylor's Power Law b = 0.051, D = 0.94, Jj = 20.860) than in the Southwest populations (k = 0.097, Taylor's Power Law b = 1.972, D = 0.89, Jj = 8.905). The aggregation indices were similar for the host ages and sexes, all indicating that these factors do not have a significant impact on parasite aggregation (Tables 6.1 and 6.2).

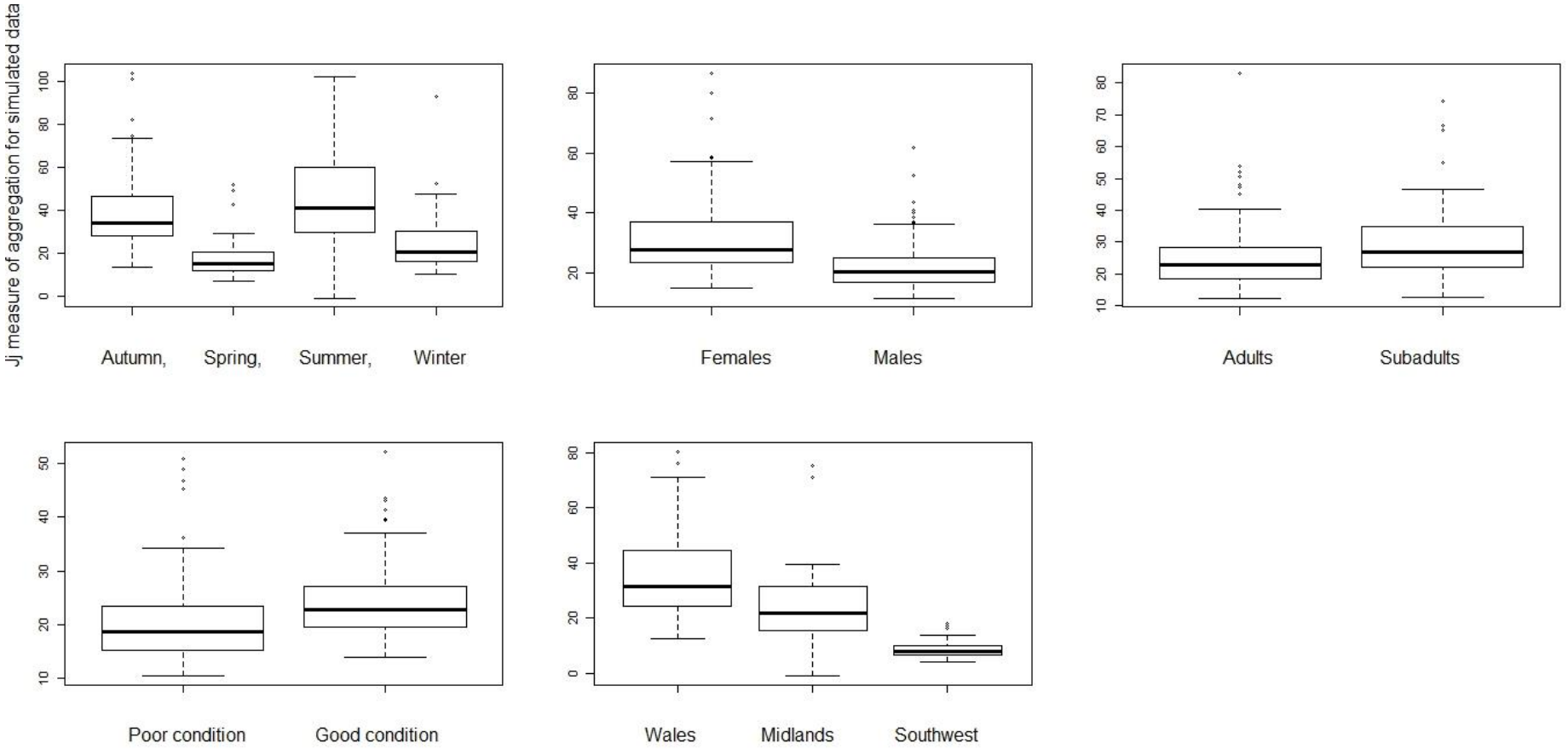

Figure 6.1 Comparison of the Boulinier et al. (1996)'s *Jj* measure of parasite aggregation for the 100 simulated populations parameterised on the mean, sample size and corrected moment estimate *k* for each sample respectively. A higher *Jj* indicates greater aggregation.

6.4.3 Parasite fecundity

Mean intensity for the 35 sampled hosts included in the fecundity analysis was 48.3 (range = 1-302, SD = 72.4). Fecundity ranged from 351 to 3391 eggs with a mean = 1435 (SD = 791.4) for N = 255 parasites. Initial analyses to examine whether parasite size is associated with parasite fecundity revealed a positive relationship between parasite size (parasite length and width) and the number of eggs produced (GLM of parasite length and width fitted to egg count for $F_{115,119} = 44.77$, $p < 0.005$) indicating larger parasites carry more eggs. Parasite fecundity, parasite length or width were not associated with season (ANOVA, $p > 0.1$) or host factors (age, sex and condition) indicating variation among these abiotic and biotic factors does not explain any observed differences in fecundity. Equally, mean parasite intensity per host did not correlate with mean parasite fecundity per host (LM: $F_{35} = 0.98$, $p = 0.329$) indicating there was no positive density dependent effect acting on parasite fecundity. Higher egg counts were associated with spring (mean egg count per parasite, 1627, SE ±154.2) and summer (1852, ±91.5) with lower counts in autumn (1422, ±80.6) and winter (1005, ±163.6), but the fecundity of parasites was only, statistically, significantly higher in summer compared to winter (GLM: $t_{31} = 2.091$, $p < 0.05$) (Figure 6.2) indicating individuals are potentially most infectious during summer. There was no statistical relationship between mean egg counts and host age, sex, or condition, or the intensity of the parasitic infection (GLM: $p > 0.1$).

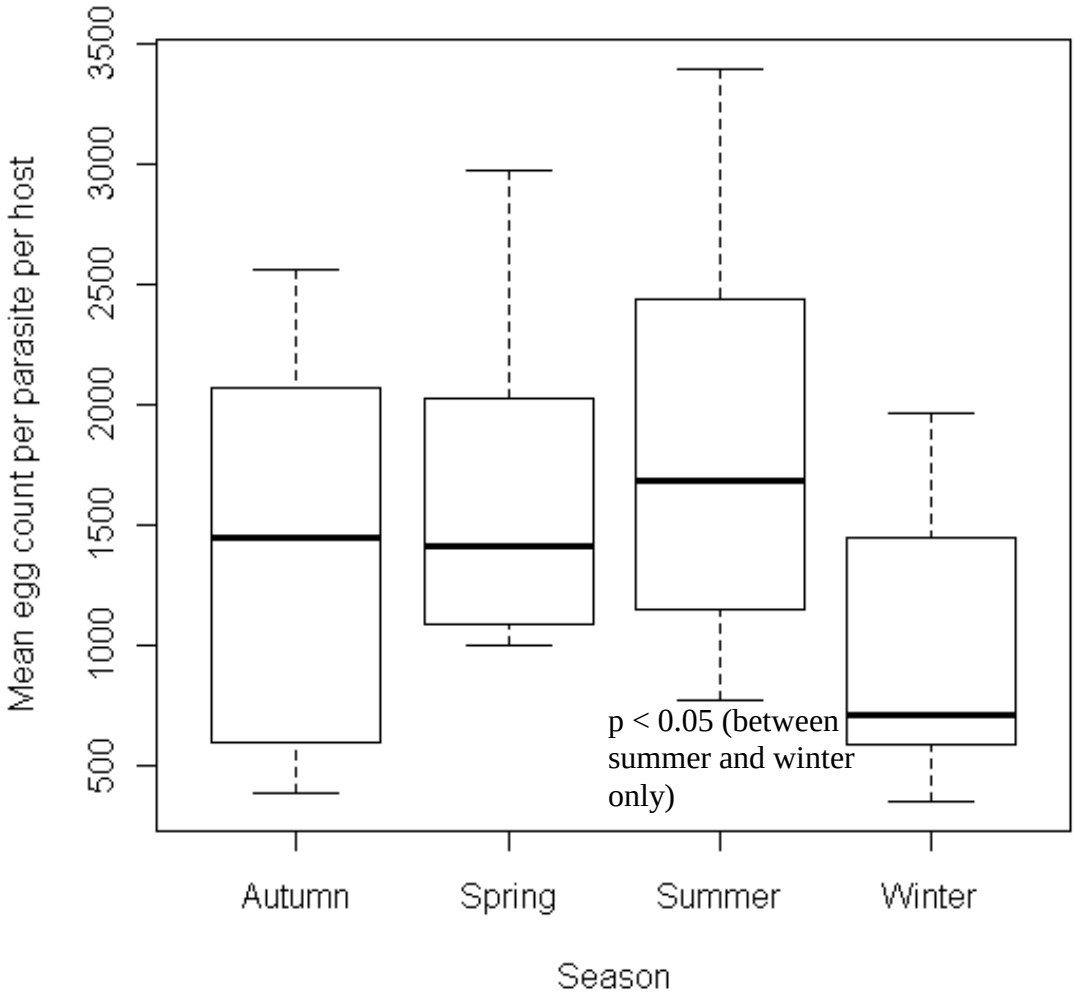

Figure 6.2 Differences in *Pseudamphistomum truncatum* fecundity with season; significant difference highlighted between summer and winter.

6.5 Discussion

Identifying the abiotic and biotic factors that are associated with heavily infected hosts and/or highly fecund parasites could have profound implications for treatment strategies; a particularly important objective considering increasing anthelmintic resistance (Laurenson et al. 2013). Such knowledge of a host-parasite system could help to prolong the efficacy of drug treatments by ensuring a suitable percentage of parasites are eradicated whilst avoiding the need to blanket treat a host population. In the current study, parasite abundance, aggregation and fecundity were considered together, within a single system, with the aim of identifying key factors associated with the most infected hosts and those with the greatest transmission potential. Within the current helminth-otter system, biotic factors influenced parasite abundance such that adults and otters in good

body condition had higher mean worm abundance than sub-adults or poor conditioned otters. Conversely, both abiotic and, to a lesser extent, biotic factors explained aggregation and fecundity differences such that *Pseudamphistomum truncatum* worms were most highly aggregated in summer and female hosts, and worms produced and/or stored significantly more eggs during summer. There were significant regional differences in parasite aggregation with infections tending to be aggregated most strongly in otters from Wales and the Midlands and less strongly in otters from the Southwest. The different aggregation indices all highlighted seasonal and regional differences but the Boulinier's *J* metric was most sensitive and indicated differences among all factors examined here. The approach that was adopted in the current study, to assess parasite aggregation by examining the congruence between multiple measures, is robust because this allows much greater confidence in any patterns observed.

Differences in parasite aggregation have been observed previously between native and non-native hosts (Hodasi 1969), different causes of host death (Hudson et al. 1992), parasite body sizes (Poulin and Morand 2000), host body sizes (Poulin 2013), and seasons (Dronen 1978; Newey et al. 2005). Here, across the four different methods used to quantify parasite aggregation in the host population, the abiotic factors season and region had the greatest influence on the degree of parasite aggregation (Table 1). Three out of 4 measures of aggregation (*k*-parameter estimates, Index of Discrepancy *D* and *J*) found significant differences between the seasons indicating parasites aggregated in fewer hosts in the summer and were more over-dispersed in spring. Equally, for all measures of aggregation, parasites in otters from Wales and the Midlands were more aggregated than those in the Southwest otter populations.

In the current study, *P. truncatum* worms were most strongly aggregated across hosts in summer. Seasonal patterns in parasite aggregation have also been observed for *Trichostrongylus retortaeformis* in mountain hares (Newey et al. 2005). This helminth-lagomorph system exhibited increased parasite aggregation in winter, which was attributed to reduced parasite transmission and infection across winter months. The *P. truncatum* life cycle is more complex than that of *T. retortaeformis* because the former requires intermediate hosts. Currently, it is not known whether there is a peak season during which the majority of otters become infected because of the probable long life span of *P. truncatum* and opportunistic sampling of otters (road-kills). Understanding that parasites are most tightly aggregated in summer and over-dispersed during winter

and spring could indicate that blanket treatment strategies would impact more infected individuals if applied during winter, whilst targeted treatment strategies would eradicate higher proportions of the parasite population if applied successfully during summer.

In the current system, parasite aggregation differences were observed between regions of the UK where parasites were present at comparable levels (Midlands, Southwest and Wales Regions) so there is heterogeneity in host-parasite populations across the UK and factors that are driving aggregation within a region must act to different degrees. The regional differences observed may relate to host exposure; the probability of encountering a parasite, especially given that this parasite infects intermediate hosts which may have spatially heterogeneous distributions themselves. Further, the transmission route of infections has been shown to contribute to parasite aggregation patterns among regions (see Shaw and Dobson 1995) and could be important within the otter population if certain otters were consuming infected fish preferentially. The distribution of the first intermediate host (snails), will potentially create spatially distinct patterns of parasite aggregation among the second intermediate hosts (fish); patterns that may ultimately pass on to the definitive otter hosts. Spatial aggregation in helminths of otters may be enhanced by host-level density-dependent processes e.g. some endoparasites are limited by space within the host (Shaw and Dobson 1995). The observed regional differences also indicate that although treatment strategies can be applied to certain individuals during specified seasons, the most successful approach might differ for different geographic regions.

The aggregation indices were similar among host age, sex and condition classes indicating that the examined biotic factors are less important than abiotic factors in determining differences in the degree of *P. truncatum* aggregation in otters. However, both the Index of Discrepancy (*D*) and Boulinier's *J* identified differences (99% confidence level) in aggregation between the sexes suggesting aggregation was stronger in female otters compared to males. There are subtle differences in the ecology of otters between the sexes that may account for greater aggregation among females. From a behavioural perspective, the larger male otters tend to have larger geographic ranges so males have a greater exposure risk than more locally restricted females. However, parasites tend to have patchy distributions geographically so that females are only likely to become infected if resident at a parasite-rich location. As a result, across the population, fewer females will be infected but those that are infected may harbour

heavier infections perhaps resulting in the observed, slightly higher, parasite aggregation in females. Keymer and Anderson (1979) demonstrated that within an arena with uniform distribution of infective stages, naïve beetles became infected to different degrees possibly related to variation in host behaviour and/or immunity. It is very likely that host behaviour and immunity differences, as well as host and parasite genetics, also influence trematode aggregation across the otter populations.

Host age and condition (biotic factors), had a greater impact on parasite abundance than the abiotic factors examined (season and region) although host sex was not important. The predominantly road-killed otters used in the current study are typically aged between 1-2 years old (Sherrard-Smith and Chadwick 2010) and it is likely that the youngest and oldest proportion of the population may be under-represented. Older animals may be particularly important for parasite studies where infections are accumulated with age, such as the trophically transmitted trematodes considered in the current study. Accordingly, higher mean parasite counts were observed in adult otters compared to sub adults. If heavily infected older otters are under-represented then parasite aggregation may also be underestimated, reducing the degree of aggregation observed, and perhaps contributing to the observed absence of a difference between parasite aggregation in sub-adults and adults. This under-representation of older otters is a recognised limitation of the current study, and small sample sizes have been highlighted as an issue for previous research into parasite aggregation (see Wilson et al. 2001). There were no differences in aggregation between host age-classes in the current study. System-specific age-group differences have been observed in parasite aggregation (Quinnell et al. 1995) but are not always present (see Henricson 1977, Wilson et al. 2001, Heylen and Matthysen 2011). The parasite *Hypoderma bovis* has been previously observed to be most aggregated in younger cohorts of cattle (see Breyev and Minar 1976). This warble fly actively searches for its host and so differences in the attractiveness of various age-classes may dictate parasite aggregation. The fact that sub-adult and adult otters in the current system probably acquire parasites via the same transmission route may explain the lack of a difference in parasite aggregation patterns between age-classes.

In conclusion, the current study indicates that differences in parasite abundance are explained by biotic factors (host age class, sex and condition) whilst parasite aggregation patterns and parasite fecundity are explained by abiotic factors (season and region). Seasonality plays a fundamental role in the life cycle of many parasites such that peak

egg production of adult helminths coincides with ecologically relevant events, but these patterns are predominantly recorded through faecal egg counts (e.g. Madhavi 1979, Rolfe et al. 1991). In *P. truncatum*, although otters are present throughout the year, the observed summer peak in fecundity is synchronised with high abundance of the snail hosts (*Bithynia* species, Dunn 1978, Lam and Calow 1989; *Radix balthica*, unpublished data). The approach applied here (coupling parasite abundance with parasite aggregation patterns and parasite fecundity) is advantageous because: i) there is a capacity to understand underlying mechanisms controlling both those host individuals that are most heavily infected together with those that are most fecund and; ii) there are implications for disease risk assessment, treatment and disease management through recognition of key traits that are synonymous with the most heavily infected and highly infectious hosts allowing targeted control. Ultimately, this approach contributes to an understanding of the patterns that define parasite populations by highlighting key factors associated with parasite abundance, aggregation and fecundity in this helminth-otter system.

6.6 Acknowledgements

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7. New intermediate hosts identified for two trematodes established in otter (*Lutra lutra*) populations in the UK

7.1 Abstract

An understanding of parasite life cycles is essential for the prevention, control and management of diseases, but is particularly challenging for cryptic organisms with complex life cycles. We report here novel hosts for *Pseudamphistomum truncatum* and *Metorchis albidus* (Trematoda: Opisthorchiidae), identified recently in otter *Lutra lutra* populations in the UK. Morphological identification of adult worms is straightforward but earlier life stages are more cryptic, demanding both morphological and molecular approaches. The internal transcribed spacer sub-unit 2 (ITS2) ribosomal DNA was used to identify *P. truncatum* and *M. albidus* in their intermediate hosts. To our knowledge this is the first report of *Radix balthica* (Gastropoda, Lymnaeidae) as an intermediate host for *P. truncatum.* Metacercariae of *P. truncatum*, identified molecularly, were recovered from roach *Rutilus rutilus* and *M. albidus* metacercariae were detected in roach, rudd *Scardinius erythrophthalmus* and chub *Leuciscus cephalus* for the first time in UK fish. Roach and rudd are known hosts for these opisthorchiids in mainland Europe, but these are unique records for chub. We confirm the presence of adult *P. truncatum* in mink *Mustela vison*, an alternative definitive host. This work documents the complete life cycle of *P. truncatum* and reports novel intermediate hosts for *M. albidus* in UK wildlife. The confirmation of hosts at all life stages contributes to the wider assessment of these parasites in host populations.

7.2 Introduction

Parasites play an integral role in ecosystem functioning (Poulin 1999, Torchin et al. 2002, Poulin and Frederic 2008, Hatcher et al. 2012). There is, however, incomplete knowledge on existing parasitic species, their geographic and host ranges. This arises in part because of the cryptic nature of many parasitic species and, specifically, the challenge of detection and identification of larval stages (Poulin and Morand 2000, Cribb and Bray 2011). Yet larval digeneans contribute hugely to the total biomass of the aquatic environment (Lafferty et al. 2006) and can be important indicators of biodiversity (Hechinger et al. 2007).

Fish-borne trematodes are renowned in the eastern parts of the world as human zoonoses (Chai et al. 2005). The World Health Organisation estimates about 18 million people are infected globally with some sort of fish-borne trematode (WHO 2004) predominantly from the Families Heterophyidae and Echinostomatidae (Chai and Lee 1991, Rim et al. 1994, Sohn et al. 2009). Interest in trematodes has been reignited recently because of the rise in trematodiasis related zoonotic diseases in more western parts of the World (Robinson and Dalton 2009, Sripa et al. 2010). Fish can accumulate many different metacercariae (Paperna & Dzikowski 2006, Sohn et al. 2009), with related consequences for healthy aquacultural practice (Britton et al. 2011), such that comprehensive sampling would be required to fully comprehend trematode diversity (McVicar 1997). In Ireland, for example, 89% of roach *Rutilus rutilus* were infected with metacercariae of the opisthorchiid *Pseudamphistomum truncatum* (see Hawkins et al. 2010). Knowing which intermediate hosts are used by a particular trematode species is often a missing link in trematode life cycles. This limits our understanding of the role parasites play in the ecosystem (Lafferty et al. 2008). The non-fastidious use of gastropod and fish hosts by the sporocyst and metacercarial stages of many trematodes means comprehensive documentation of potential host species is rare. Yet the identification of many introduced, non-native parasites has been reported recently following metacercarial surveys (Williams et al. in press). Some species can have pronounced effects on the health and fitness of wild fish populations (Mitchell et al. 2005, Longshaw et al. 2010) and many are extremely small, located in cryptic sites within their host, heavily encysted and lack reproductive organs that serve as key identifying features. Molecular technologies can, however, support and confirm morphological identification (see Jousson et al. 1999,

Dzikowski et al. 2004, Pina et al. 2009, Skov et al. 2008, Marchiori et al. 2010, Al-Kandari et al. 2011, Born-Torrijos et al. 2012).

Trematodes from the Opisthorchiidae family use three hosts to complete their life cycle. The first intermediate hosts are freshwater snails from which free-living cercariae are released. The cercariae attach to cyprinid fish where encystment ensues until a mammal predates the infected fish (Dunn 1978). Excystation occurs within the mammal and about 4 weeks later, the parasite matures with the capacity for continuous release of encapsulated embryos (see Sukhdeo and Sukhdeo 2004). These eggs pass out with the mammals' faeces and the life cycle is completed once the egg capsule is ingested and a miracidia hatches within a snail. Trematode parasites can overcome seasonal fluctuations in abundance of their intermediate host through long-life expectancy in all hosts and continual egg production by the adult trematodes in the definitive host (Over 1982).

In the UK, two Opisthorchiidae parasites, *Pseudamphistomum truncatum* and *Metorchis albidus* have been identified recently in otters, *Lutra lutra* and, rarely, in American mink *Mustela vison* (see Simpson et al. 2005, Sherrard-Smith et al. 2009). There is some evidence that these parasites cause biliary damage to otters *Lutra lutra* (see Simpson et al. 2009) but little is known about the hosts used by early life stages of either digenean. The aim of this study was, therefore, to identify all life stage hosts of both digeneans in UK fauna.

7.3 Materials and Methods

7.3.1 The first intermediate host

Opportunistic collections and examination of freshwater snails from South Wales streams were used to identify the first intermediate host species of *Pseudamphistomum truncatum* and *Metorchis albidus.* A sample of up to 20 snails per species present were collected from 35 sites within the Usk and the Wye catchments (5 streams with 6-8 collection sites per stream separated by 500m; Figure 7.1). Collections were made once during each season in 2010 (February (winter), May (spring), August (summer) and November (autumn)). Sites were chosen for their accessibility and habitat suitability for freshwater snails.

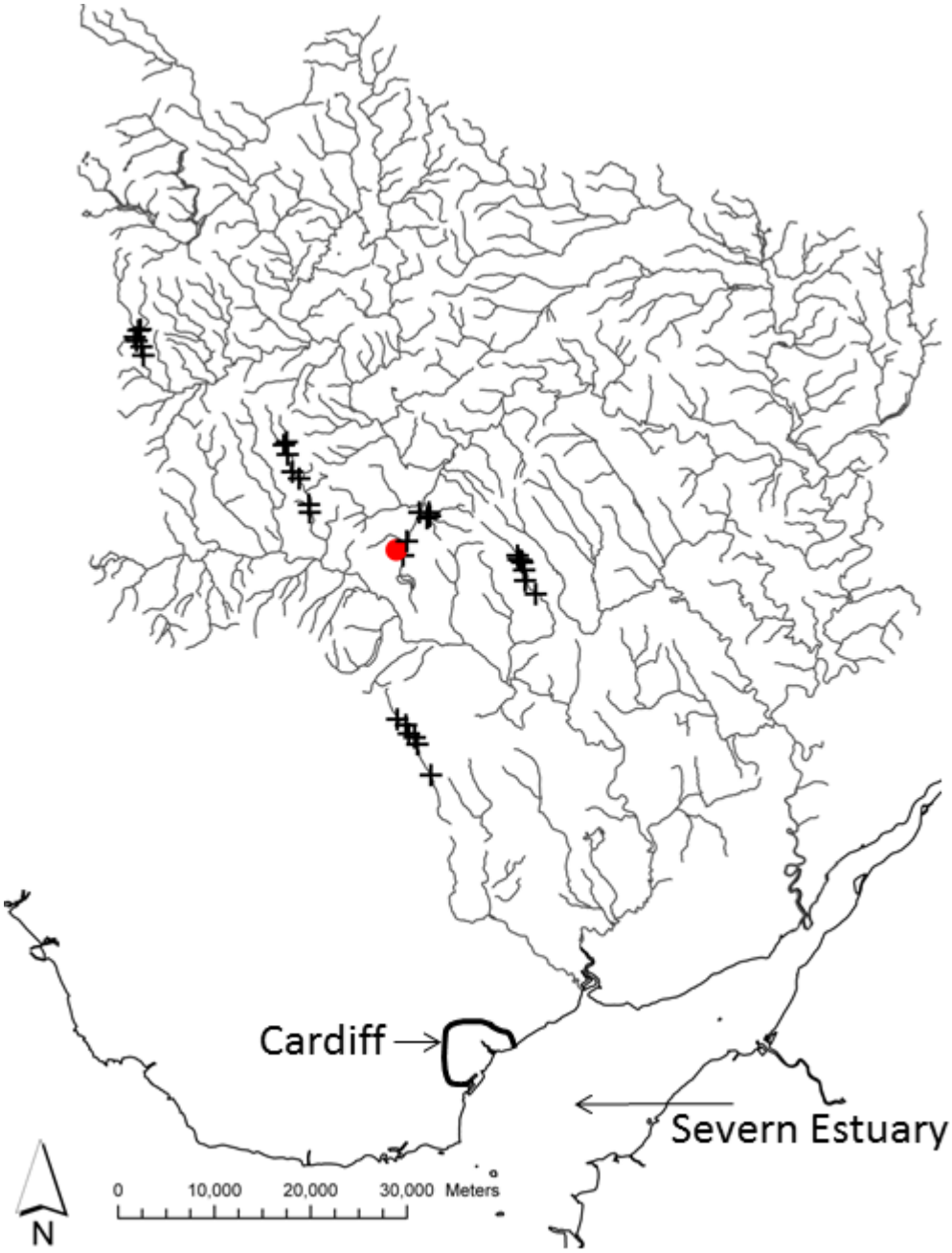

Figure 7.1 Map of the rivers sampled during this study, South Wales region. Locations of sampling spots are marked with a cross, the cercaria matching *Pseudamphistomum truncatum* is marked (circle).

Only larger snails are reported to carry significant digenean burdens (Degueurce et al. 1999) since the prevalence of most trematode species (e.g. *Fasciola,* see Deguerce et al. 1999; or *Diplostomum,* see Voutilainin et al. 2009) increases with snail size (Karvonen et al. 2006) and therefore where possible, larger snails were chosen for examination. Only 4 species (*Radix balthica* Lymnaeidae, *Potamopyrgus antipodarum* Hydrobiidae, *Physa fontinalis* Physidae and the limpet *Ancylus fluviatilis,* Planorbidae) were recovered at the collection sites but these were common. Snails were held in containers for 7 days at 9ºC

during which daily exposure to light was followed by examination of the water column for cercariae. After 7 days snails were exposed individually to heat (c.25ºC) and bright light for 4 hours to encourage cercarial shedding. Any cercariae released were collected and stored in 90% molecular grade ethanol for subsequent molecular analysis.

7.3.2 The second intermediate hosts

Fish health checks and disease investigations have been conducted by the Environment Agency (EA) and its predecessor (National Rivers Authority) since the early 1970's. Over this time, many thousands of fish have been examined throughout England and Wales as part of the EA's remit to maintain, improve and develop freshwater fisheries. This includes health checks, mortality investigations, non-native fish species monitoring and reports of new parasite findings and disease outbreaks. Between 2007 and 2012, 895 fish populations were submitted for fish health examination at EA Brampton. Each independent survey screened a sample of approximately 30 fish from each population, totally > 20,000 specimens representing 32 species over 5 years. The majority of samples submitted for examination were from lakes, reservoirs and ponds (estimated at approximately 90%); however, riverine samples were also examined annually. Fish stocking activities placed a bias on the location of waters sampled each year (see Table 7.1) and surveys include salmonids, particularly those used for sport, as well as cyprinids. Notably, the proportion of cyprinids sampled from each region varies greatly; for example, very few cyprinids were screened from the Southwest England, a hotspot for otter infections with *P. truncatum* (see Sherrard-Smith et al. 2009).

Table 7.1 Locations in England and Wales where fish (32 species) were sampled for the current study (between 2007 and 2012). Environment Agency Regions are defined for England and Wales using ecologically significant river Catchment boundaries (e.g. Sherrard-Smith et al. 2009). The proportion of fish examined for parasites, including digenean metacercariae, form each area is shown.

Environment Agency Region	**Proportion of fish samples examined (895 fish populations)**
Anglian	22.1% (c. 5900 fish)
Midlands	24.3% (c. 6500 fish)
North East & Yorkshire	8.8% (c. 2350 fish)
North West	20.5% (c. 5500 fish)
South East /Thames	8.7% (c. 2300 fish)
South West	4.8% (c. 1250 fish)
Southern	7.4% (c. 1900 fish)
Wales	3.4% (c. 900 fish)

Current protocols for routine health examinations of fish represent a compromise between time, cost and detail. Therefore, parasitological examinations involved both low and high power examinations of the skin, fins, eyes, heart, gills, lateral line, musculature, kidney, liver, spleen, intestinal tract, gall bladder and nares. Digenean cysts were initially detected using candling, the examination of fish tissues over a light box, dissected out and then stained appropriately for morphological identification where possible (see Bruno et al. 2006). Encysted digeneans were either mechanically removed with needles, or excysted through chemical treatments to replicate the intestinal tract of the definitive host (Fried 1994).

Two particular parasites (type 1 and 2) raised difficulties during morphological examination allowing only tentative identification. The first record of the Type 1 cyst occurred in September 2007 from a roach in Cambridgeshire, but species identification was dependent upon molecular confirmation. Type 2 was a relatively small (100-120µm dia.) and thick, 3-layered cyst. These cysts, deposited on the fish in clumps, were difficult to excyst which limited the retrieval of good quality specimens for morphological study. The type 2 cysts were also cryptic with other digeneans (particularly *Bucephalus polymorphus* and *Paracoenogonimus ovatus*) and mixed infections within the fins were common. To investigate whether type 1 and type 2 metacercariae were *P. truncatum* and *M. albidus*, individual cysts of each morpho-type were fixed in ethanol for subsequent molecular analysis.

7.3.3 Molecular identification of larvae

A total of 15 cercariae from 10 different snails and 5 isolated metacercariae from roach (type 1), rudd (two type 1 and one type 2) and tench (type 1) were identified to species level using the internal transcribed spacer sub-unit 2 (ITS2) region of ribosomal DNA (see Sherrard-Smith et al. *submitted*). In brief, DNA was extracted using a modification of the methodology described in Faria et al. (2011) whereby each specimen was exposed to 15µl TE containing 0.45% Tween 20 and 2µg Proteinase K for 3 h at 55°C and then 95°C for 10 minutes to denature the enzyme. For both cercariae and metacercariae amplifications, total PCR reaction volume was 10µl and comprised of 2µl of the DNA extract mixed with 10x PCR buffer II (Applied Biosystems), 50mM MgCl (Applied Biosystems), 2.5mM of each dNTP, 10pmol/µl of each primer (ITS2 rDNA: Ophet F1 5'-CTCGGCTCGTGTGTCGATGA-3' and Ophet R1 5'-

GCATGCARTTCAGCGGGTA-3' following Müller et al. 2007) and 5U Taq DNA polymerase (Invitrogen). The PCR ran at 95°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 53°C for 1 min and 72°C for 1 min, with a final extension of 72°C for 10 min (GenAmp PCR System 9700, Applied Biosystems). The PCR products were then sequenced in both directions using the same primers (Macrogen Inc.). Consensus sequences generated in Sequencher^{TM} (version 4.9) of 388 bp and 403 bp matched identical GenBank sequences *P. truncatum* (Accession number JF710315) and *M. albidus* (JF710316), respectively.

7.3.4 Alternative definitive host

In the UK, the American mink is invasive and is trapped to control population numbers (Bonesi and Palazon 2007). Trapped mink from Somerset (N = 21) and the Severn Catchment (N = 29) provided samples for the current study. To confirm that mink are a second viable host (see Simpson et al. 2005), mink gall bladders removed during post-mortem were examined for trematodes microscopically and identified using Yamaguti (1971).

7.4 Results

Here, we record for the first time the complete life cycle of *Pseudamphistomum truncatum* in the UK and in doing so identify new intermediate hosts for this digenean. Further, we document the first record of this parasite in British fish. In addition, novel second intermediate hosts for *Metorchis albidus* are reported.

7.4.1 The first intermediate hosts: gastropods

Of the four freshwater snail species screened in the current study, the gastropod *Radix balthica* (total number of *R. balthica* across all sites = 122) was the only species found shedding cercariae (comprising 3 trematode species). Only a single *R. balthica* specimen was infected with *P. truncatum* cercariae (British NGR: SO122297) identified by ITS2 sequencing. The host snail was collected in May 2010 from the River Llynfi (see Figure 7.1). This particular snail was co-infected with a second type of cercariae, the ITS2 sequence of which most closely resembled species from the genus *Plagiorchis* (2% sequence difference to GenBank Accession Number AF151952.1 *P. elegans*, namely adenine deletions at positions 5 and 22, transition from cytosine to thymine at 154, guanine to adenine at 157, an adenine insertion at position 161, transition of guanine to

adenine at 170 and a thymine insertion at position 301). Analysis of the ITS2 isolated an identical sequence (*Plagiorchis* sp.) from four *R. balthica* individuals from the River Honddu (SO009397) in May 2010. A third cercarial type was isolated from a different *R. balthica* individual, collected from the River Honddu (British NGR: SO009397) in May 2010 and this was most similar to the *Diplostomum* genus (2% sequence difference to *Diplostomum baeri* GenBank Accession Number AY123042.1, sequence differences are adenine deletions at position 5 and 22, transitions of adenine to guanine at position 162, guanine to adenine at 239 and transversions of guanine to thymine at 359, and adenine to cytosine at 374).

Potamopyrgus antipodarum (N=68), the only invasive snail species encountered, and *Physa fontinalis* (14) did not release any cercariae. The limpet *Ancylus fluviatilis* (N = 250) was by far the most common species but no cercariae were recovered from this gastropod. We did not isolate any *M. albidus* cercariae during the current study.

7.4.2 The second intermediate hosts: cyprinid fish

Molecular analysis revealed the 'type 1' metacercariae to be *Pseudamphistomum truncatum*. Some uncertainty surrounds the exact morphological characteristics of this parasite and work is underway to confirm this from fresh material and the examination of paracarmin stained specimens. As such, the following descriptions are generalised and must be viewed with some caution until identification through both molecular and morphological approaches have been resolved.

Suspected larval stages of *P. truncatum* did not appear to be encysted within the host tissues. It is possible that an extremely thin cyst wall was present. Parasites were easily removed by applying light pressure to host tissues using forceps or a scalpel, allowing individual and visually active worms to emerge from the surrounding fish tissue. The specimens measured approximately 0.7-0.9 mm in length and possessed a large intestinal caecum (Figure 7.2). Due to the difficulties of identifying larval stages of digeneans, molecular methods are currently the only way to identify these cryptic cysts.

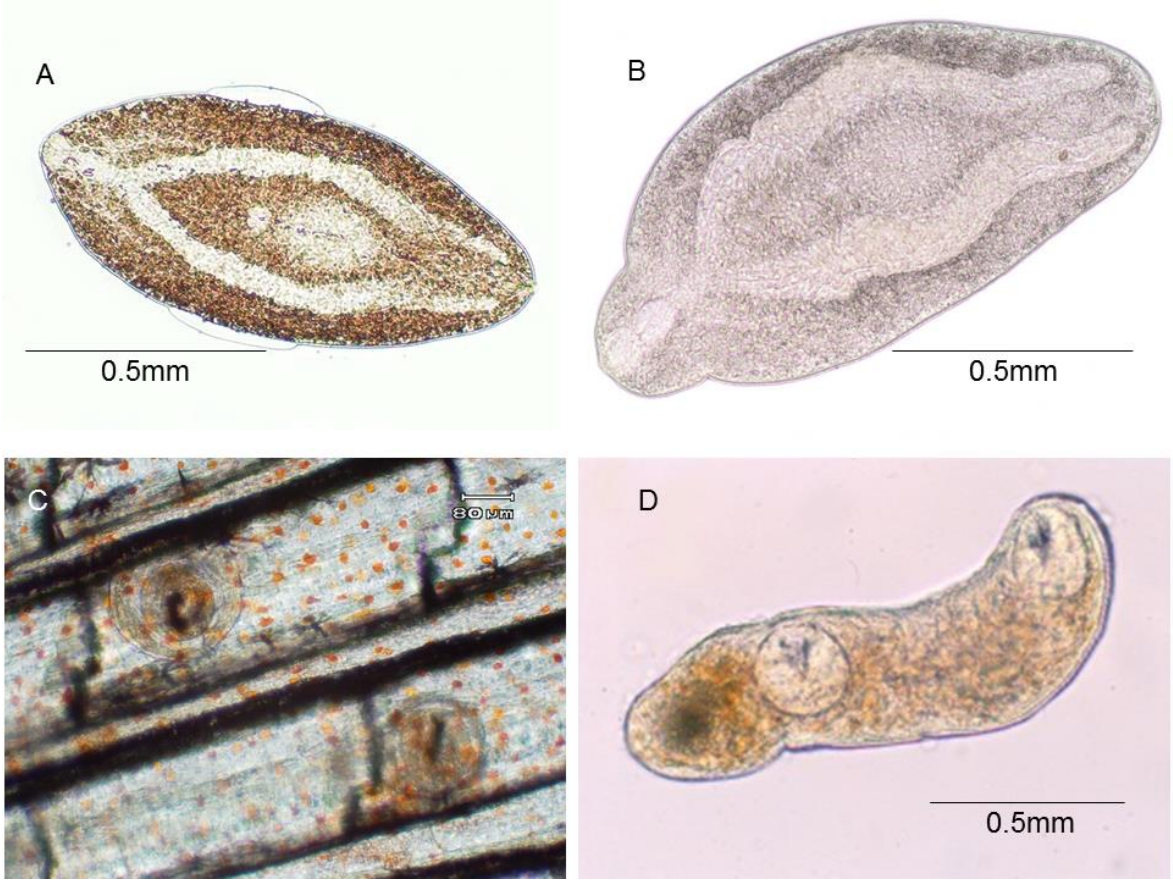

Figure 7.2 Life stages of Opisthorchiidae parasites from UK hosts; Images of: A, suspected *Pseudamphistomum truncatum* metacercaria (cyst); B, suspected *P. truncatum* excysted from roach *Rutilus rutilus*; C, *Metorchis albidus* cyst from rudd *Scardinius erythrophthalmus*; D, excysted *M. albidus* metacercaria.

The geographical locations where *P. truncatum*-like cysts were recorded between 2007 and 2012, included Norfolk (Number of examined fish = 10, prevalence 30%), Cheshire (N = 17, prevalence 5.9%), Cambridgeshire (N = 4, prevalence 25%) and Oxfordshire (N = 31, prevalence 6.5% see Table 7.2). In each case, low intensities were recorded ranging from 1-8 parasites per infected fish (roach and tench, Figure 7.3). Other fish species examined in these localities included Bream (*Abramis brama*), Bitterling (*Rhodeus amarus*), Rudd (*Scardinus erythropthalmus*) and Bleak (*Alburnus alburnus*) but no *P. truncatum*-like cysts were found.

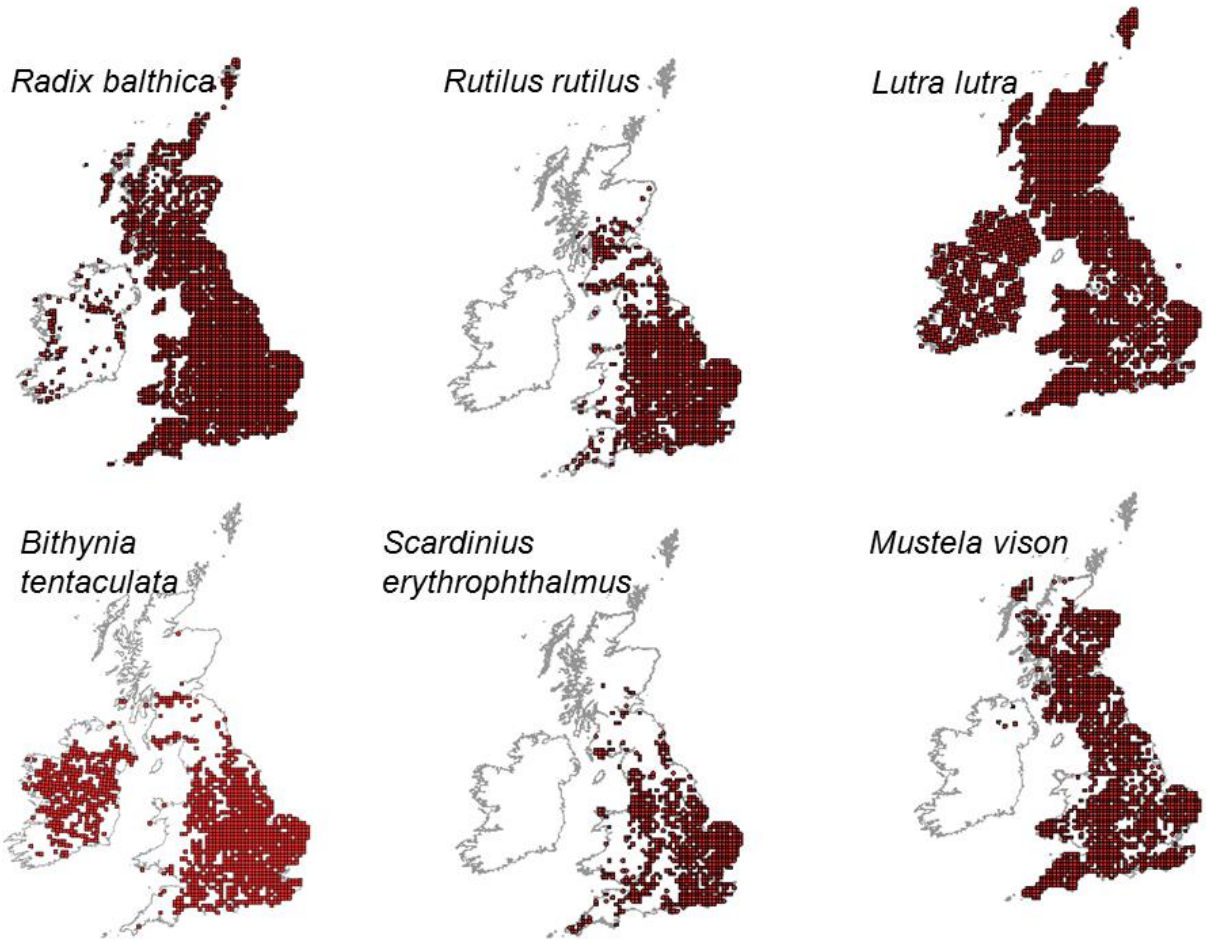

Figure 7.3 The current distribution of fauna identified as hosts for either *Pseudamphistomum truncatum* or *Metorchis albidus*: i) *Radix balthica*; ii) *Bithynia tentaculata*; iii) *Rutilus rutilus* roach; iv) *Scardinius erythrophthalmus* rudd; v) *Lutra lutra* otter and; vi) *Mustela vison* American mink as defined and provided by the National Biodiversity Network (NBN) Gateway, copyright © Crown Copyright. All rights reserved NERC 100017897 2004.

Molecular analysis identified the 'type 2' cysts as *M. albidus* from roach and chub (*Leuciscus cepahlus*) in Leicestershire (N = 20, prevalence 10%), Wessex (N = 30, prevalence 3%) and Yorkshire (N = 30, prevalence 15%, see Table 7.2). In contrast to the *P. truncatum*-like metacercariae, *M. albidus* has thick, multi-layered cysts (Figures 7.2). However, this cyst characteristic and positioning of the parasites' suckers (oral and ventral suckers of equal size, the latter positioned in the posterior half of the parasite; Figure 7.2) are not reliable or consistent features to morphologically distinguish the species. Specimens of the metacercarial larval stage of both parasites have been deposited in the Natural History Museum, London (Accession Numbers pending). For

both *P. truncatum* and *M. albidus*, the morphology of metacercariae bears no resemblance to that of the adult worms (see Scholz 2008).

In all cases within England and Wales, *P. truncatum* has co-occurred with *Hysteromorpha triloba*. *P. truncatum* was also found as a concurrent infection with *Bucephalus polymorphus, Rhipidocotyle campanula* and the myxozoan parasite *Myxobolus* sp. (see Table 7.2). Overall, the trematodes comprised the greatest proportion of the parasites infecting the studied fish: 20 parasite taxa were recorded, of which 40% were trematodes, compared to 15% cestodes, 10% protozoa and rare cases of monogeneans and nematodes (see Table 7.2).

7.4.3 The definitive hosts: piscivorous mammals

In Somerset where mink are abundant (Figure 7.3), prevalence of *P. truncatum* in mink was high (33%, 7 in 21 hosts) and comparable to that in the more piscivorous otters from the Southwest region (47.6%, 20 in 42 hosts, Sherrard-Smith et al. *unpublished*). In the Severn Catchment prevalence of *P. truncatum* was much lower in mink (3.4%, 1 case in 29) than that for otters (17.4%, 8 of 46, data from Sherrard-Smith et al. 2009).

7.5 Discussion

To our knowledge, this is the first time the intermediate hosts for *Pseudamphistomum truncatum* and *Metorchis albidus* have been detected within the UK. Specifically, this is the first report of *R. balthica* as a host species for *P. truncatum,* or any Opisthorchiidae trematode. Previously, *Bithynia* species were considered the host genus of both *M. albidus* (see Dunn 1978, Serbina and Iurlova 2002) and *P. truncatum* (see Dunn 1978, Skov et al. 2008). *M. albidus* is reported in *Bithynia inflata* and *B. tentaculata* (Prosobranchia, Bithyniidae) (Genov 1984) and *Codiella troscheli* (Bithyniidae) (Serbina and Iurlova 2002).

Table 7.2 Location, prevalence and co-infection details of suspected *Pseudamphistomum truncatum* and *Metorchis albidus* cysts found in freshwater fish from England over a 5 year period (2007-2012).

Parasite	Location	Host species (N)	Prevalence	Host habitat	Range	Co-infections
Pseudamphistomum truncatum	Norfolk	Tench (10)	3/10 (30%)	Gills & Muscle	1-8	**Trematoda**: *Hysteromorpha triloba,* Echinostomatidae **Apicomplexa**: Coccidians **Cestoda**: *Paradilpis scolecina*
	Cheshire	Roach (17)	1/17 (5.9%)	Muscle	1	**Trematoda**: Echinostomatidae **Arthropoda**: *Argulus foliaceus* **Protozoa**: *Ichthyobodo necator,* Trichodinids
	Cambridgeshire	Roach (4)	1/4 (25%)	Muscle	1	**Trematoda**: *Paraceonogonimus* sp., *Rhipidocotyle* sp., *Diplostomum* sp., *Tylodelphys* sp., Echinostomatidae, *Posthodiplostomum cuticola* **Ciliophora**: Trichodinids **Cnidaria**: *Myxobolus* sp. **Monogenea**: *Dactylogyrus* sp. **Nematoda**: *Anguillicola crassus*
	Oxfordshire	Roach (31)	2/31 (6.5%)	Muscle	1-2	**Trematoda**: *Hysteromorpha triloba,* Echinostomatidae, *Diplostomum* sp., Bucephalidae, **Cestoda**: *Ligula intestinalis,* **Monogenea**: *Dactylogyrus* sp., *Caryophyllaedes fennica* **Protozoa**: *Myxobolus* sp., *Dermocystidium* sp.
Metorchis albidus	Leicestershire	Rudd (20)	2/20 (10%)	Fins	1-5	Multiple co-infections
	Wessex	Roach (30)	1/30 (3%)	Fins	NR	**Trematoda**: *Hysteromorpha triloba, Diplostomum* sp. **Monogenea**: *Dactylogyrus* sp. **Protozoa**: *Myxidium* sp.
	Yorkshire	Chub (30)	5/30 (17%)	Fins, Skin	NR	NR

&Muscle
NR (not recorded).

There are approximately 33 species of freshwater snails and limpets in the River Usk and Wye and their associated tributaries (NBN Gateway Data: GA0003381425, GA0003381232, GA0003381361, GA0003381431, GA0003381342, *available online*). The four snails recovered during current sampling (*Radix balthica, Potamopyrgus antipodarum, Physa fontinalis, Ancylus fluviatilis*) are common to the South Wales tributaries. Cercariae, including those identified as *P. truncatum*, were recovered from only *Radix balthica.* In addition to *P. truncatum,* snails of this species were infected with a *Plagiorchis* sp. and *Diplostomum* sp. *R. balthica* is also host to other trematodes including *Fasciola hepatica* (see Caron et al. 2007) so is clearly an important host for digenean diversity.

It was not possible to identify the *Plagiorchis* or *Diplostomum* specimens, recovered in the current study from *R. balthica*, to species level. Both parasites, however, were a 98% match with other species from their respective genera (GenBank: Blast search February 2013). Within the Trematoda, an ITS2 sequence difference of 2% is almost always indicative of a distinct species (see Table 7.3, Morgan and Blair 1995; Nolan and Cribb 2005). To date, ITS2 sequences of 5 *Plagiorchis* species are held in GenBank (*P. elegans, P. koreanus, P. vespertilionis, P. muelleri* and *P. maculosus* on 07 February 2013). There are reports of *P. koreanus* (see Lord et al. 2012) and *P. muris* (see Rogan et al. 2007) in Britain. The only other type of cercariae recovered produced ITS2 sequences that most closely resembled those from the *Diplostomum* genus. Although there are 20 molecularly characterised *Diplostomum* species in central Europe (Georgieva et al. 2013), with a few specifically referenced within Britain (*Diplostomum phoxini, D. spathaceum, D. gasterostei* e.g. Dezfuli et al. 2007; Morley et al. 2005; Kennedy 2001 respectively), only 7 *Diplostomum* species ITS2 sequences are recorded on GenBank (*D. pseudospathaceum, D. spathaceum, D. mergi, D. baeri, D. huronense, D. indistinctum* and *D. mashonense*, February 2013). Clearly, further species identification is required to document the extensive diversity of both genera comprehensively.

Table 7.3 Similarity of trematode ITS2 sequences across different species and genera: 2 - 10% differences between species from the same genus (for example, *Diplostomum* sp. and *Plagiorchis* sp.), 2 - >25% difference between species from different Genera (*Pseudamphistomum truncatum* and *Metorchis albidus*, Opisthorchiidae are comparatively close, 2% difference).

	Diplostomum baeri	*Diplostomum pseudospathaceum*	*Diplostomum spathaceum*	*Metorchis albidus*	*Plagiorchis elegans*	*Plagiorchis maculosus*	*Plagiorchis muelleri*	*Plagiorchis vespertilionis*
	JX986857.1	JX986853.1	JX986847.1	JF710316	JX522536.1	AF316152.1	AF151947.1	AF151951.1
Diplostomum baeri								
D. pseudospathaceum	98% (868/889)							
D.spatheceum	97% (864/891)	99% (1028/1041)						
Metorchis albidus	<75%	<75%	<75%					
Plagiorchis elegans	<75%	<75%	<75%	<75%				
P. maculosus	<75%	<75%	<75%	<75%	97% (745/765)			
P. muelleri	<75%	<75%	<75%	<75%	90% (1060/1175)	94% (724/773)		
P. vespertilionis	<75%	<75%	<75%	<75%	91% (1057/1167)	94% (728/774)	97% (1229/1266)	
Pseudamphistomum truncatum	<75%	<75%	<75%	98% (381/388)	86% (263/307)	85% (263/309)	85% (259/303)	<75%

The discovery of *Pseudamphistomum truncatum* cysts on roach represent the first record of this parasite from any fish in the UK (see Kennedy 1976, Kirk 2000). Roach is an established host for *P. truncatum* in mainland Europe (Skov et al. 2008). Other fish species may also host this digenean in the UK as bream, rudd and bleak are also reported as intermediate hosts in mainland Europe (see Schuurmans 1931, Meneguz 2000). During the current study, it proved difficult to identify these cysts from morphological examinations alone. Even good quality, stained and mounted specimens of larval digenean parasites can be extremely difficult to identify with certainty. Consequently, current information of this parasite is restricted to confirmation from molecular approaches. The staining process required to confirm identification morphologically, deleteriously affects DNA and rendered molecular methods unusable. Therefore parallel confirmation of a single *P. truncatum* cyst, both molecularly and morphologically, is challenging. If the morphological results are accurate however; within England and Wales, low prevalence of *P. truncatum* on fish was observed across the four regions (Norfolk, Cheshire, Cambridgeshire and Oxfordshire) where the digenean was isolated and reflected the low prevalence of *P. truncatum* on definitive hosts in these locations (2 out of 23 otters infected from Norfolk, 0/12 Cambridgeshire, 0/8 Oxfordshire, no otters from Cheshire; using data from Sherrard-Smith et al. 2009). We would predict much higher prevalence in hotspot areas (Somerset and South Wales) where definitive hosts are most heavily infected (see Simpson et al. 2005, Sherrard-Smith et al. 2009) particularly because high prevalence, 89%, has been recorded for *P. truncatum* in roach from hotspots in Ireland (Hawkins et al. 2010). The Irish study used a digest to isolate cysts from the fish instead of the dissection method used in the current study; the disadvantage of the former is that it is not possible to assess host habitat preferences for helminths. In the current study, *P. truncatum* cysts, so far recovered as isolated cysts in fish musculature, could have been missed during dissections because their, currently unknown, principle host habitat is not dissected during routine screening. Other digenean cysts such as those from the Bucephalidae family were common however and these are very similar in size and appearance indicating the dissection method is robust. Therefore, it is unlikely that *P. truncatum* cysts were overlooked from screened tissues (skin, fins, eyes, heart, gills, lateral line, musculature, kidney, liver, spleen, intestinal tract, gall bladder and nares).

The current study is the first record of *M. albidus* metacercariae from fish hosts within the UK; the earliest record dating back to 2003. Here, the digenean was recovered from

cyprinids (rudd, roach and chub) during screening in England (Leicestershire, Wessex and Yorkshire). The prevalence of fish infections was low in all areas and, similarly to *P. truncatum*, this may reflect low prevalence of the parasite in definitive hosts in the same locations (0 out of 4 otters infected from Leicestershire, 2/116 Wessex, 3/39 Yorkshire; using data from Sherrard-Smith et al. 2009). To our knowledge, this study is the first to define rudd, roach and chub as specific hosts for *M. albidus*, although there are reports of either 'freshwater fish' or 'cyprinids' as a general host type (see Dunn 1978). The importance of both digenean infections (in light of the many other larval digeneans that already infect freshwater fish) is yet to be established.

Neither *P. truncatum* nor *M. albidus* cysts were single species infections on the examined fish hosts demonstrating co-infection is the norm in British freshwater fish. Larval digenean infections dominated (40% of the parasite species present were trematodes) in the fish samples investigated. In addition, the *P. truncatum* infected *R. balthica* snail from the River Llynfi was co-infected with *Plagiorchis* species. Cort et al. (1937) concluded that co-infection of freshwater snails is generally random with respect to species assemblages but certain species occurred as co-infections less often than expected (including both *Diplostomum flexicaudum* and *Plagiorchis muris*) indicating some barrier to co-infection for these species at least. There is a suggestion that a dominance hierarchy acts on co-infection events for digeneans (Esch et al. 2001, Lim and Heyneman 1972, Wright 1973) and species complexes have been explained by both direct antagonism (e.g. Lim and Heyneman 1972, Yoshino 1975) and chance (random) events (e.g. Koie 1969, Rohde 1981). Yet in many cases, co-infections or interspecific competition has been deemed unimportant for digenean infra-community structure (see Fernandez and Esch 1991, Curtis 1997, Esch et al. 2001). The comparatively high diversity and abundance of digeneans in UK freshwaters ratifies the general consensus that such parasites are a vital component of ecosystem functioning, ecosystem health and food web dynamics (e.g. Lafferty et al. 2006, 2008).

For *P. truncatum* and *M. albidus*, we cannot comment on the spatial distribution of the larval stages of these parasites in Britain. As yet there is very little overlap where both intermediate and definitive hosts have been comprehensively screened from the same region. Southwest England would be ideal for such a study, where both digeneans are common in otters, but to date, fish-screening in this region has focused on salmonids.

Based on data from the otter population (Sherrard-Smith et al. 2009), there are hotspots of *P. truncatum* in Wessex, Dorset and South Wales but the parasite is present at very low prevalence across the remainder of the UK. Otter populations are currently increasing following crashes during the 1960s (Strachan and Jefferies 1996, Jones and Jones 2004, Crawford 2010). If low prevalence across much of Britain is a true reflection of the parasite distributions then increases may be anticipated with the recovery of the otter population. This would mirror the significant increase in abundance of the digenean *Hysteromorpha* sp. following the recovery of its definitive host (the cormorant, *Phalacrocorax carbo*) populations across the UK (Newson et al. 2007).

The American mink, a second viable definitive host for both *P. truncatum* and *M. albidus*, were imported to the UK in 1929 and had established feral populations in Devon by the late 1950s (see Chanin and Linn 1980, Halliwell and MacDonald 1996). It is generally recognised that mink utilise a more terrestrial habitat in the UK to avoid competition with the larger otters (Bonesi et al. 2004) but the current study supports their status as piscivorous hosts for *P. truncatum* (see Simpson et al. 2005). Their presence and population expansion may contribute further to an increase of infections in fish. It is most likely that the mink has acquired infection since arriving in the UK. To our knowledge *P. truncatum* has not been reported in North America. There is, however, a report of *M. albidus* in dogs from California (Freeman and Ackert 1937) although both *M. albidus* and *P. truncatum* are now thought to be native to Britain (Chapter 5).

Here, we show that *R. balthica* (a first intermediate host), rudd, tench, chub and roach (second intermediate hosts), and mink and otters (definitive hosts) are infected by *P. truncatum* and *M. albidus*. The host range indicates that there is potential for both *P. truncatum* and *M. albidus* populations to expand further across the UK. The establishment success of trematodes is generally accredited to their ability to maintain a presence in the environment through prolonged life stages within each host, rapid production of infective stages and dormant phases during periods that would be otherwise inhospitable to free living stages (see Price 1980). By contributing to the increased discovery of cryptic digenea (see Cribb and Bray 2011), the current study supports the evaluation of the vital interaction between digenean parasites and ecosystems (e.g. Lafferty et al. 2006, 2008).

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8. Host population dynamics of *Pseudamphistomum truncatum*: high sensitivity of the second intermediate host

8.1 Abstract

The success of generalist parasites with complex life cycles may relate to the rapidity with which they are able to infect, and then be maintained by, novel hosts. Yet despite major ramification for disease transmission, the author does not know of any empirical data for helminths available on the proportion of parasite eggs that are successfully cycled through the system and this has a major impact on parasite abundance. The trematode, *Pseudamphistomum truncatum* (Opisthorchiida) was recently identified in the UK otter population. Aspects of *P. truncatum*'s life cycle are well understood but it is unknown how host dynamics affect this parasite's life-history parameters. Here, a simple three host (snail, fish and mammal) population dynamic model for trematodes provides a first step towards identifying those parameters that have a large impact on potentially disease-causing trematode systems. All models must be used with caution because they can only make suggestions about the dynamics of a particular system but may highlight those aspects of the host-parasite relationship that require further empirical study. The model is used to identify the parameters that i) define the proportion of parasite eggs successfully transmitted; and those that are most influential in regulating ii) the intermediate and iii) definitive host infection dynamics. The simplicity of the model should make it suitable for other systems. The current model, parameterised with empirical data from UK populations suggests that at most 10% of eggs are successfully transmitted into the snail population. The proportion of second intermediate hosts, fish, that become infected with helminths is very sensitive to changes in model parameters, more so than snails and definitive hosts. A decreasing birth rate of otters results in a higher proportion of the otter population carrying infections but this could be an artefact of the current logistic assumption for otter population dynamics. This study presents a simple, but flexible, three-host population dynamics model for trematodes and is a rare example of a model of this type that can be parameterised by empirical data.

8.2 Introduction

The optimisation of control and management policies requires an understanding of the factors that regulate parasite distributions and the dynamic stability of natural populations (see Anderson and May 1982, Townsend et al. 2009). Where generalist parasites with complex life cycles are considered, empirical data is often rare. Theoretical epidemiological models have analysed many aspects of host-parasite interactions, acting as a substitute for empirical data. The pioneering works using models considered malaria transmission (Ross 1911, Kermack and McKendrick 1927a,b,c and childhood infectious diseases (Hope Simpson 1948, 1952). Subsequently, these models were adapted for macroparasitic infection with a particular focus on schistosomes (Anderson and May 1978, 1979, 1982, Woolhouse 1991, 1992, 1994, Chan et al. 1995, Carabin et al. 2000, Hisakane et al. 2008, Zhao and Milner 2008, Basáňez et al. 2012, Boatin et al. 2012). These models can be of great value in understanding how host populations may be affected by environmental perturbations, in the absence of the considerable resources required to investigate a system empirically (see Appendix 8.1).

Pseudamphistomum truncatum (Trematoda, Opisthorchiida) is native to Europe (Ivanov and Semenova 2000, Harris and Yalden 2008) and has been identified recently in freshwater ecosystems within the UK (Simpson et al. 2005, Sherrard-Smith et al. 2009). The parasite can damage the otter gall bladder particularly when infections reach high intensities (Simpson et al. 2005, Sherrard-Smith et al. 2009). The life cycle of *P. truncatum* is complex. Parasite eggs are consumed by gastropod intermediate hosts (reviewed in Sukhdeo and Sukhdeo 2004), which include *Bithynia* sp. (Dunn 1978) and *Radix balthica* (Chapter 7). Cercariae emerge from these snail hosts and encyst on the second intermediate host: for example roach, *Rutilus rutilus* (see Skov et al. 2008, Hawkins et al. 2010). The parasites are trophically transmitted to the definitive host with consumption of infected fish and excystation is followed by migration to the gall bladder where the worms mature (Sukhdeo and Sukhdeo 2004). To date, in the UK *P. truncatum* has been isolated from two species of definitive host; the Eurasian otter, *Lutra lutra* (see Simpson et al. 2005, 2009, Sherrard-Smith et al. 2009) and American mink, *Mustela vison* (see Simpson et al. 2009). Elsewhere in Europe, the parasite has been reported in red fox, *Vulpes vulpes* (see Saeed et al. 2006), mink, otters, dogs, *Canis familiaris* (see Simpson et al. 2005), American muskrat, *Fiber zibethicus* (see Ivanov and Semenova, 2000) and domestic cats, *Felix domesticus* (see Nielsen and Guidal, 1974). The intermediate hosts

are recognised as the gastropod snails *Bithynia sp.* and *Radix balthica* and the cyprinid fish (see Chapter 7).

Currently, there are two major hotspots for *P. truncatum* in the UK: the Usk and Wye Catchments in South Wales, and Somerset in Southwest England. What can be inferred from these locations could be applied elsewhere were the parasite to increase its spatial range. Empirical data is not available for the complete life cycle on alternative definitive or intermediate hosts so a theoretical model might allow us to estimate natural population dynamics. For instance, although a relatively small proportion of snails become infected with trematode eggs (e.g. Boerlage et al. 2013), we know nothing about the success rate of eggs in the environment once they are released with the definitive host faeces, and such data would be very difficult to measure *in situ.* Transmission stages are likely to experience extremely high mortality rates because of their small size, limited mobility, survival time and infectivity (Jennings and Calow 1975, Poulin 1996, Koehler et al. 2013). Factors that affect mortality rates play a key role in the selective pressures acting on parasite populations. Here, our aim is to use a susceptible-infected (SI) deterministic model to i) explore the transmission of parasite eggs within the system. Equally, certain aspects of the parasite life cycle will affect the ultimate prevalence within the definitive host, the otter, a species cited as near-threatened on the International Union for Conservation of Nature red list (IUCN *data available online*). This chapter also asks how the prevalence of trematodes in ii) the intermediate hosts and iii) the definitive host will vary with predation rates and seasonal fluctuations, using a simple deterministic model parameterised with empirical data from *P. truncatum* and its three life-stage hosts.

8.3 Materials and Methods

Here, a simple deterministic model for opisthorchiid infection in host populations is described, as a first step toward a better understanding of three host trematode dynamics. The model framework is extremely flexible because of its simplicity but we present the output of the model that best-fits the empirical evidence currently available on *P. truncatum*.

Parameters for the model are based on data from the River Wye and Usk; an area where available data for *P. truncatum* has been documented most comprehensively (e.g. Sherrard-Smith et al. *submitted,* Figure 8.1). The Wye is a large catchment with a

drainage area of approximately 4,180km^2 (Lewis et al. 2007). The main river runs c.250km, from an altitude of 677m on Plynlimon in Mid Wales to the Severn Estuary near Chepstow (Lewis et al. 2007). The Usk is one of the largest rivers in Wales; the main channel is c.120km in length (114.266km from OS data), the drainage area is 1,358km^2 (Larsen et al. 2009). The length of the river system including tributaries is 566.56km (OS data). The Usk runs from an altitude of c.500m on Mynydd Du and flows east across the Northern edge of the Brecon Beacons then heads south before reaching the Severn Estuary (Larsen et al. 2009).

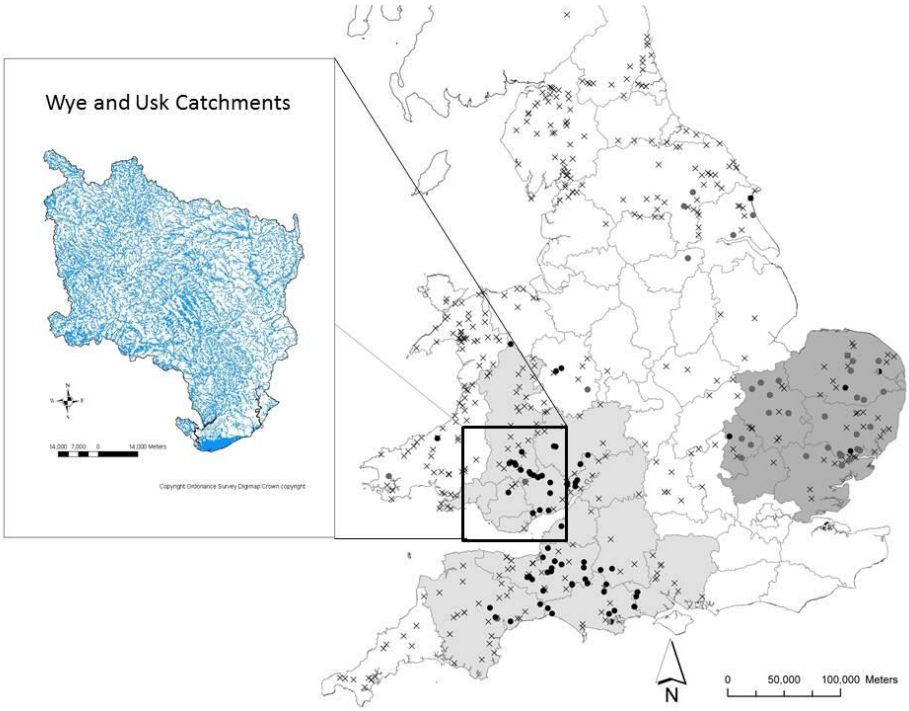

Figure 8.1 Distribution map of digeneans (*Pseudamphistomum truncatum* as black circles, *Metorchis albidus* as grey circles and uninfected otters marked as crosses) in otters across England and Wales. Insert (courtesy of Dr Isabelle Durance) shows the Wye and Usk Catchment still water pools, streams and water courses used to calculate the maximum area of freshwater representing suitable habitat for freshwater snails and fish. Black lines represent the Catchment boundary.

8.3.1 Model development

The passage of *P. truncatum* parasites through their life cycle is described using a deterministic compartmental model (Figure 8.2). This model users differential equations to define key aspects of host populations; these include the birth (or hatch) rate, death rate and carrying capacity of each population. An additional differential equation is incorporated to define the eggs lost, and those consumed by snails to complete the parasite life cycle. As an important first step, this model makes a key simplifying

assumption that host population dynamics are independent of infection dynamics. This is a necessary assumption for this first step toward modelling three-host systems, and appears justified in the case of the definitive host (the impact of the parasite on otters is small when intensities are low, see Sherrard-Smith et al. 2009). We acknowledge, however, that the impact on fish or snail hosts is yet to be established.

Each host species is assumed to experience logistic growth within the model to reach a fixed carrying capacity. Although across the UK otters are an expanding population (see Mason and MacDonald 2004), this is a reasonable assumption because otter populations in the South Wales and Somerset regions are now considered to be at carrying capacity (see MacDonald 1983, Mason and MacDonald 2004, Stanton et al. 2009) in part because of an increase in fighting injuries suggesting encounter rates are relatively high in these regions (e.g. Simpson 2006). Equally, fish are monitored comprehensively in these regions (EA unpublished data) indicating a relatively stable freshwater community. Infection dynamics are linked by predation rates between the three host species modelled using empirical data on *P. truncatum* in Somerset and South Wales (Sherrard-Smith et al. in press, Chapter 7) for parameterisation. Once infected, hosts are assumed to stay infected until death. This is a reasonable assumption for the intermediate hosts given the short life expectancy (1-2 years) of snails and the dormancy of digenean cysts in fish. The life span of *P. truncatum* in otters is unknown, but other trematode life expectancies can be variable ranging from 6-9 months for hemiurids (Margolis and Boyce 1969) to years for certain schistosomes (Loker 1983).

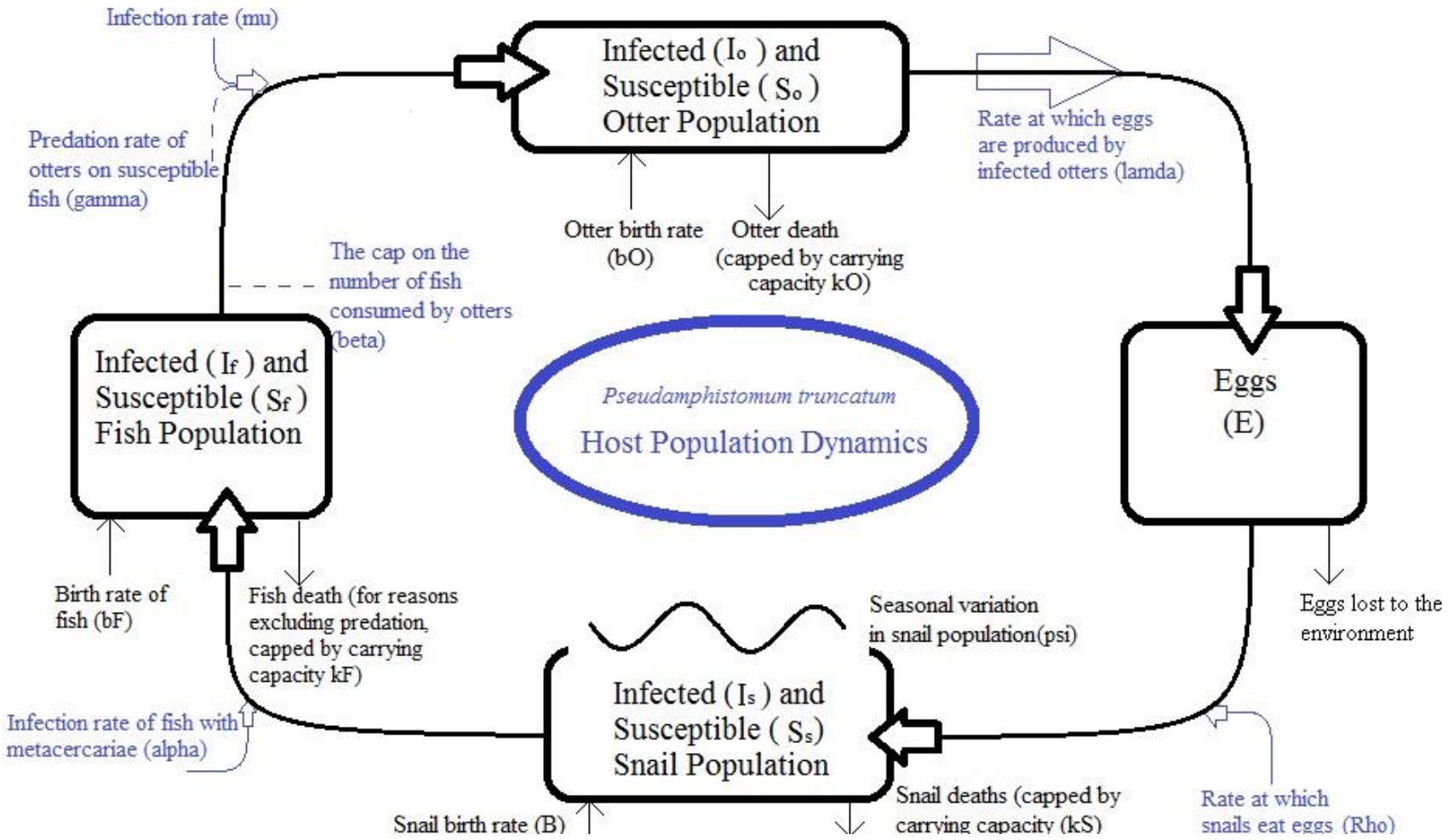

Figure 8.2 Illustration of the host population dynamics of *Pseudamphistomum truncatum*

8.3.2 Differential equations

Each pair of differential equations describes the interaction between susceptible (uninfected hosts) and infected individuals within a host population. In all equations K represents carrying capacity, b indicates birth (or hatch) rates, S($_x$) represents the susceptible host population (otter = o, snails = s, fish = f) number and I($_x$), those hosts already infected; such that S + I = Total host population:

8.3.2.1 Otter population dynamics

Equation 8.1:

$$\frac{dS_o}{dt} = b_o\,(S_o + I_o) - \left(\left(\frac{b_o}{K_o}\right)(S_o + I_o)\right)S_o - \mu\gamma S_o\left(\frac{I_f}{\beta + I_f}\right)$$

Equation 8.2:

$$\frac{dIo}{dt} = \mu\gamma S_o\left(\frac{I_f}{\beta + I_f}\right) - (\left(\frac{b_o}{K_o}\right)(S_0 + I_0))I_0$$

where otter birth-rate (b_o) influences the total population (susceptible otters, S_o + infected otters, I_o) and is limited by carrying capacity (K_o). Otters are lost from the susceptible population through consumption of infected fish (I_f) at a rate (μ). The predation rate of otters on fish occurs at a rate γ. There is a limit, however, on the number of infected fish the otters can consume ($I_f/(\beta+I_f)$) determined by β because other prey are present in the otter diet, or fish may die independently of predation (and without being scavenged). The summed susceptible and infected population is limited by carrying capacity and otter death.

The otter population inhabiting the Wye is considered highly stable (NBN Gateway Wales Otter Survey Database, Joint Nature Conservation Committee). The rather discontinuous distribution of otters in the UK is estimated at 10,395 individuals (NBN Gateway) with strongholds in Scotland, Wales and the Southwest of England. Otters are reported to cover 16-20km of linear river habitat daily to access sufficient resources in Wales (Anon 2002, Kruuk 2006). The total length of rivers defined by the Usk and Wye Catchments is c.816.56 km (see Lewis et al. 2007; Larson et al. 2009). Assuming each

otter uses 16-20 km (excluding cubs which are dependent on their mother) then the carrying capacity of otters in this area is estimated at c.40-50 individuals (K_o).

Otter birth-rates were initially estimated from the literature. The sex ratio of otters in the UK is approximately 1:1 (Heggeberget 1988, Sidorovich 1991). If the carrying capacity is assumed to be 50 otters, this corresponds to approximately 25 females. In the UK, otters have an average of 1.5 cubs per year (Chadwick and Sherrard-Smith 2010), with generally, just over 70% of mature females reproductively active at any one time (Hauer et al. 2002) and as such we estimated 30 otter births per year. Reproductive rate can be estimated as the proportional increase in otters to the population. In this case, an increase of 0.58 otters per week (30 otters/52 weeks) corresponds to a birth rate (b_o) of 0.02 (an increase of 0.58 otters per week to the total population of 50 otters). In the Usk and Wye Catchments prevalence of *P. truncatum* in otters is 43.5% (20 infected out of 46 otters).

8.3.2.2 Snail population dynamics

Equation 8.3:

$$\frac{dS_s}{dt} = b_s\left(1+\varphi\cos\left(\frac{2\pi\left(\frac{t}{52}\right)}{T}\right)\right)(S_s+I_s) - \left(\left(\frac{b_s\left(1+\varphi\cos\left(\frac{2\pi\left(\frac{t}{52}\right)}{T}\right)\right)}{K_s}\right)(S_s+I_s)\right)S_s - \rho S_s E$$

Equation 8.4:

$$\frac{dI_s}{dt} = \rho S_s E - \left(\left(\frac{b_s\left(1+\varphi\cos\left(\frac{2\pi\left(\frac{t}{52}\right)}{T}\right)\right)}{K_s}\right)(S_s+I_s)\right)I_s$$

where snail carrying capacity (K_s) is limited in a similar manner to the otter population. Seasonality is modelled by $1+\varphi\cos\left(\frac{2\pi\left(\frac{t}{52}\right)}{T}\right)$ where the parameter (φ) determines the

amplitude of the seasonal wave and, as φ increases the amplitude of this wave becomes more severe but set at 2, allows the snail population to cycle between 450,000 and 500,000 individuals throughout the year (Figure 8.3). This is incorporated here as an annual depletion followed by increase in the overall population (susceptible snails, S_s + infected snails, I_s) controlled by the parameter (φ). The rate at which susceptible snails become infected (ρ) depends on the number of parasite eggs (E) consumed. Once again there is no recovery once a snail becomes infected – a reasonable assumption for trematode systems. The unit of time, T, is 1 week.

Snail populations were examined at three tributaries of the Wye Catchment using methods adapted from Watson and Ormerod (2004). Briefly, approximately 1 km length of river was selected for each tributary (Sirhowy, Llynfi and Honddu Rivers) and 6-8 5m long transects were marked along each stretch. Sampling predominantly focused on the marginal zones (within 1m of the bank) on either side of the river and on the mid-channel zone. Snails were extremely rare in the mid-channel zone supporting previous literature indicating the slower moving marginal water is a preferred habitat for gastropods (Økland 1990). A 15 min active search was conducted at each site including stone turning, allowing collection of gastropods and density estimates per m^2 (see Table 8.1). *Radix balthica* was the only infected species located in these streams (Chapter 7). To calculate the carrying capacity of snails, an estimate of the effective area of suitable habitat available to snails was defined by plotting the marginal zones of all tributaries and streams within the Wye and Usk Catchments = 17,808,698 m (Figure 8.1, ArcMap GIS, version 9.2). A carrying capacity, K_s, was calculated by multiplying area with density per square metre = c.5,000,000.

Table 8.1 Summary of location, host snail species present, the density of those species and total area of the Usk and Wye region with corresponding prediction of the snail carrying capacity (K_s).

River/ Location	Density of *Radix balthica* (m^{-2})	Total length (m) suitable habitat for Usk &Wye Rivers	
Sirhowy	0.3	17,808,698 (from	
Honddu	0.15	Figure 8.1)	
Llynfi	0.4		**Total snails**
Mean density for	0.283		**estimation (K_s)**
the Usk &Wye			= **5,045,792**

There is little in the literature about fish and snail recruitment rates (which for simplicity we model as hatch rates) and so we explored a range of possible rates for both host populations. After parameter testing, both hatch rates were fixed to a rate of 0.1 (an influx of c. 500,000 snails per year, 100,000 fish per year). As stated, the simplicity of the model means these values are flexible but fixing at 0.1 ensured the proportion of infected otters fitted what is observed using empirical data for the Usk and Wye Catchment.

8.3.2.3 Fish population dynamics

Equation 8.5:

$$\frac{dS_f}{dt} = b_f(S_f + I_f) - \left(\left(\frac{b_f}{K_f}\right)(S_f + I_f)\right)S_f - \alpha S_f I_f - \gamma(S_o + I_o)\left(\frac{S_f}{\beta + S_f}\right)$$

Equation 8.6:

$$\frac{dI_f}{dt} = \alpha S_f I_f - \gamma(S_o - I_o)\left(\frac{I_f}{\beta + I_f}\right) - \left(\left(\frac{b_f}{K_f}\right)(S_f + I_f)\right)I_f$$

where carrying capacity (K_f) limits fish populations and hatch-rate (b_f). The transmission rate (α) determines the rate at which susceptible fish become infected. So, the population is at a dynamic equilibrium at a set carrying capacity. The rate of consumption (γ) dictates movement of susceptible fish (S_f) to the infected population (I_f) and the parasite population passing back to the otters is then limited by the function ($I_f/(\beta+I_f)$) or ($S_f/(\beta+S_f)$) for the infected and susceptible populations independently.

Fish population data were available to estimate density per square metre (see Table 8.2: Environment Agency public access data). To calculate fish carrying capacities, observed densities from the Environment Agency for each potential fish host (cyprinid species) was multiplied by freshwater area across the Usk and Wye Catchment 704,136m^2 (Table 8.2). This estimated carry capacity for all potential host fish (K_f) at c. 1,000,000.

Table 8.2 Summary of the location, host fish species present, the density of those species (data from Environment Agency, Wales) and total area of the Usk and Wye region to estimate the fish carrying capacity (K_f).

Cyprinid species in the Wye and Usk	Observed density of fish in the Usk (per m^2)	Observed density of fish in the Wye (per m^2)
Bream *Ambramis brama*	0	0
Bleak *Alburnus alburnus*	0	0.000516
Barbel *Barbus barbus*	0	0.001032
Carp *Cyprinus carpio*	0	0
Gudgeon (various species)	0	0
Chub *Squalius sp.*	0	0.009457
Dace *Leuciscus leuciscus*	0	0.019075
Minnow *Phoxinus phoxinus*	1.491275	30.0517
Roach *Rutilus rutilus*	0	
Rudd *Scardinius erythrophthalmus*	0	
Tench *Tinca tinca*	0	
Mean density for cyprinids in the Usk & Wye		1.435

8.3.2.4 Parasite eggs

Equation 8.7:

$$\frac{dE}{dt} = \lambda I_o - \omega E$$

describes the final life stage, the eggs (E). This is simply the rate at which eggs are released by infected otters (λ) limited by those lost to the environment at a defined rate (ω).

We estimated the number of parasite eggs produced per otter (λ) using the mean *in utero* egg count for *P. truncatum* of 1,435 eggs (Chapter 6), and the mean abundance of *P. truncatum* in the Wye and Usk Catchments of 3.9 worms per otter (Sherrard-Smith et al. *In press*). This would give a total of 5,597 eggs; therefore, assuming only some eggs will be released (see Tinsley 1983), an estimated 100 eggs released per infected host per week was chosen as a starting value for estimation of the parameter λ. The model was used to examine how this release of eggs would affect the cycling of the parasite through its host populations. There is no literature on the proportion of eggs that are lost to the environment; therefore we tested a range of values to examine this rate of loss (ω) and its impact on the system as a whole.

8.3.3 Parameter estimation

Otters are generalist predators but are primarily piscivorous (Jedrzejewska et al. 2001, Kruuk 2006). Numerous fish species contribute to otter diet (Kruuk and Moorhouse 1990) but the trematode *P. truncatum* uses cyprinids, in particular rudd, tench and roach (Dunn 1978, Simpson et al. 2005, Skov 2008). The β parameter was incorporated to cap the numbers of infected fish consumed by otters and considers the dilution effect of otters consuming alternative prey (see Copp and Roche 2003, Britton et al. 2006). We explored the sensitivity of the models output over a span of parameter (μ, γ, ρ α, β) ranges (see Table 8.3) to ensure infections in otters were maintained at around 40%. This allowed us to examine those parameters that had the greatest impact on the prevalence of *P. truncatum* in otters and intermediate hosts.

Seasonal fluctuations were incorporated to the snail population (parameter - φ). Peak periods of shedding have been identified in trematode populations (e.g. Karvonen et al. 2006). *P. truncatum* fecundity in the definitive host is highest in summer (Chapter 6) indicating seasonal likelihood of infections of snail populations are operating. There will be ensuing seasonality in infection of fish and the rapid turnover of fish population may exacerbate seasonal effects, but the long life expectancy of the encysted parasite would limit any seasonality in the otter population. Parameter ranges were explored by numerical simulation using the odesolve package within GNU General Public Licence R version 14.2 (R Development Core Team 2008).

8.4 Results

Examplar output of a 20 year simulation from the current model is shown in Figure 8.3. This simple model is built to fit the observed prevalence of *Pseudamphistomum truncatum* in the Usk and Wye Catchment (43.5%) and can be compared to the similar prevalence of the parasite in otters from Somerset (30.3%), and the combined Catchments within Wessex and Devon (39.2%) – these regions were used to confirm model parameterisation. We also see that the majority of fish in an infected location become infected very quickly. This fits with the high infection rates that are observed for other digeneans using fish as a second intermediate host (e.g. Chai et al. 2005, Guoqing et al. 2001, Kumchoo et al. 2005) and high infection rates of *P. truncatum* (89%) on roach from Ireland (Hawkins et al. 2010). Further, the relatively low infection rates of the snail population is supported by very low incidences of snail infections observed in South

Wales streams (see Chapter 7) although there is spatial heterogeneity in prevalence of infection in field populations of many other trematodes (e.g. Byers et al. 2008, Fredensborg and Poulin 2006).

Table 8.3 Summary of parameters and starting values for the three host trematode model. Where appropriate, parameters are based on the Usk and Wye Catchment Region as a representative ecological unit hosting the trematode *Pseudamphistomum truncatum* (Opisthorchidae).

Variables	**Description**		
S_o	Susceptible Otter		
I_o	Infected Otter		
S_s	Susceptible Snails		
I_s	Infected Snails		
S_f	Susceptible Fish		
I_f	Infected Fish		
E	Eggs free in environment		
Parameters	**Description**	**Starting values**	**Range tested**
b_o	Birth rate of otters	0.02	0.002 - 0.2
K_o	Carrying capacity of otters	50	
μ	Infection rate of otters	0.15	0.0025 - 0.25
Γ	Predation rate of otters on fish	0.1	0.01 - 1
B	Stabiliser capping number of fish that can be consumed per otter per unit time	5	1 - 20
b_s	Hatch rate of snails	0.1	0.01 - 0.5
Φ	Seasonal effect in snail population	2	
K_s	Carrying capacity of snails	5000000	
P	Consumption rate of snails eating eggs = Infection rate of snails becoming infected with eggs	0.00000001	1×10^{-9} - 1×10^{-4}
b_f	Hatch rate of fish	0.1	0.01 - 1
K_f	Carrying capacity of fish	1000000	
A	Infection rate of susceptible fish becoming infected with metacercariae	0.00001	1×10^{-7} - 0.001
Λ	Number of eggs produced by each infected otter	100	1 - 100000
Ω	Egg loss rate to the environment/consumed	0.5	0.01-0.99

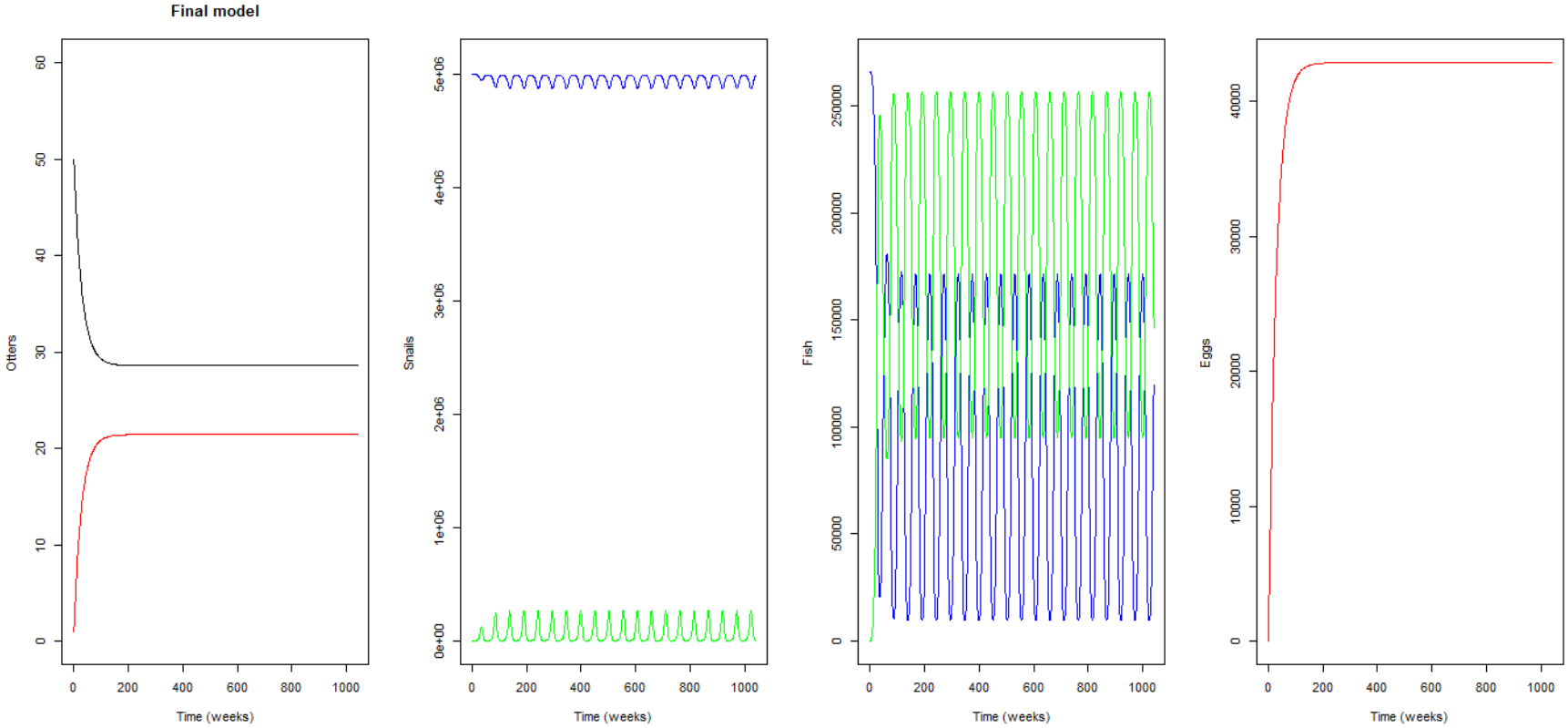

Figure 8.3 Susceptible-Infected (SI) deterministic model of host population dynamics in a three host trematode, *Pseudamphistomum truncatum* (Opisthorchiidae) system: Otter (susceptible = black line, infected = red line), Snail (susceptible = blue line, infected = green line) and Fish (susceptible = blue line, infected = green line). The number of eggs released is shown in the final graph (red line).

Aim 1: Estimation of the proportion of eggs that are successfully transmitted

There is no literature on the proportion of *P. truncatum* eggs that are transmitted successfully between vertebrate and invertebrate hosts. The current model is used to explore how the rate of parasite egg production by the otter population (λ) and the number of eggs that are lost in the environment (ω) (see Equation 8.7) may affect the proportion of hosts that become infected by this trematode.

Equation 8.7:

$$\frac{dE}{dt} = \lambda I_o - \omega \mathrm{E}$$

Each parameter in equation 8.7 was tested across a range of values that maintained a 40% infection rate within the otter population (this is a stable prevalence observed for the consecutive years 2007-2010 in Somerset and South Wales (see Sherrard-Smith et al. *submitted*)). According to this model, the otter population will not be affected by the number of eggs released or recovered. As the value of λ increases from 1 to 100,000, the proportion of snail and fish infected increases toward 1 (Figure 8.4). Conversely, as the value of ω increases from 0.01 to 0.99, the proportion of infected snails and fish decreases. This decrease is expected because an increasing proportion of eggs are lost to the environment. According to this model, varying the parameter ω does not affect the quantity of eggs released by the otters, in our case 100 eggs per infected otter per week (Figure 8.5). Further, the low levels of infection observed in snails (Chapter 7) and the high levels of infection expected in fish populations (Hawkins et al. 2010) should be maintained if $\omega \geq 0.5$ and λ is 100 or less (eggs released per infected otter per week). So, if the total number of eggs per infected otter is 5,597 eggs (see section 8.3.2.4: Parasite eggs, above) and there are 20 infected otters, a total of 111,940 eggs are present at a given time, T. If λ = 100, then 20,000 eggs are released and 50% (10,000 eggs) are lost to the environment. On this basis, this model suggests that 10,000/111,940 = c.9% of the eggs will be successfully transmitted from the otters to the snail populations.

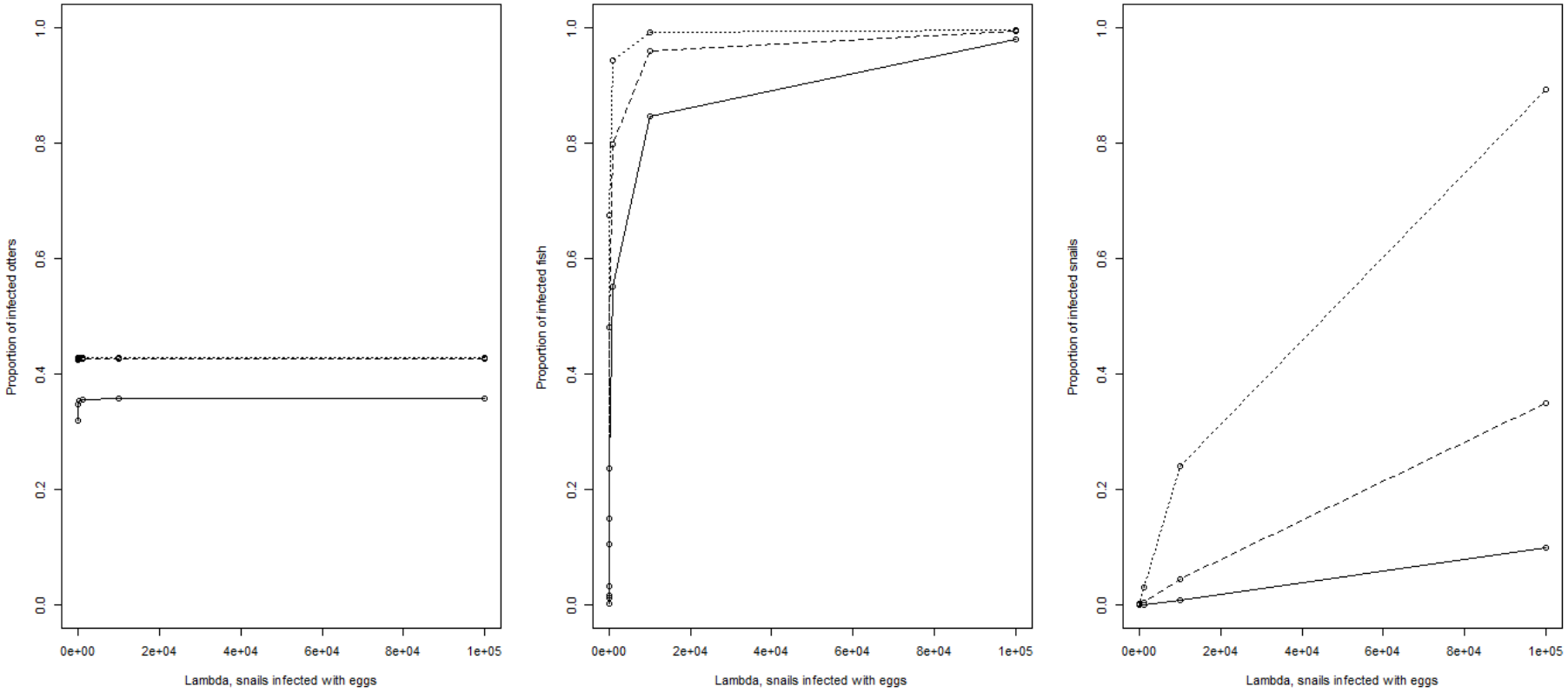

Figure 8.4: The change in the proportion of *Pseudamphistomum truncatum* infected otters, fish and snails with increasing λ at different time points (T = 50 solid line, T = 150 dashed line and T = 300 dotted line). The proportion of infected otters does not change regardless of the value of λ. Almost all fish are infected as soon as λ increases above 1000. There is a steady increase in snail infections with increasing λ.

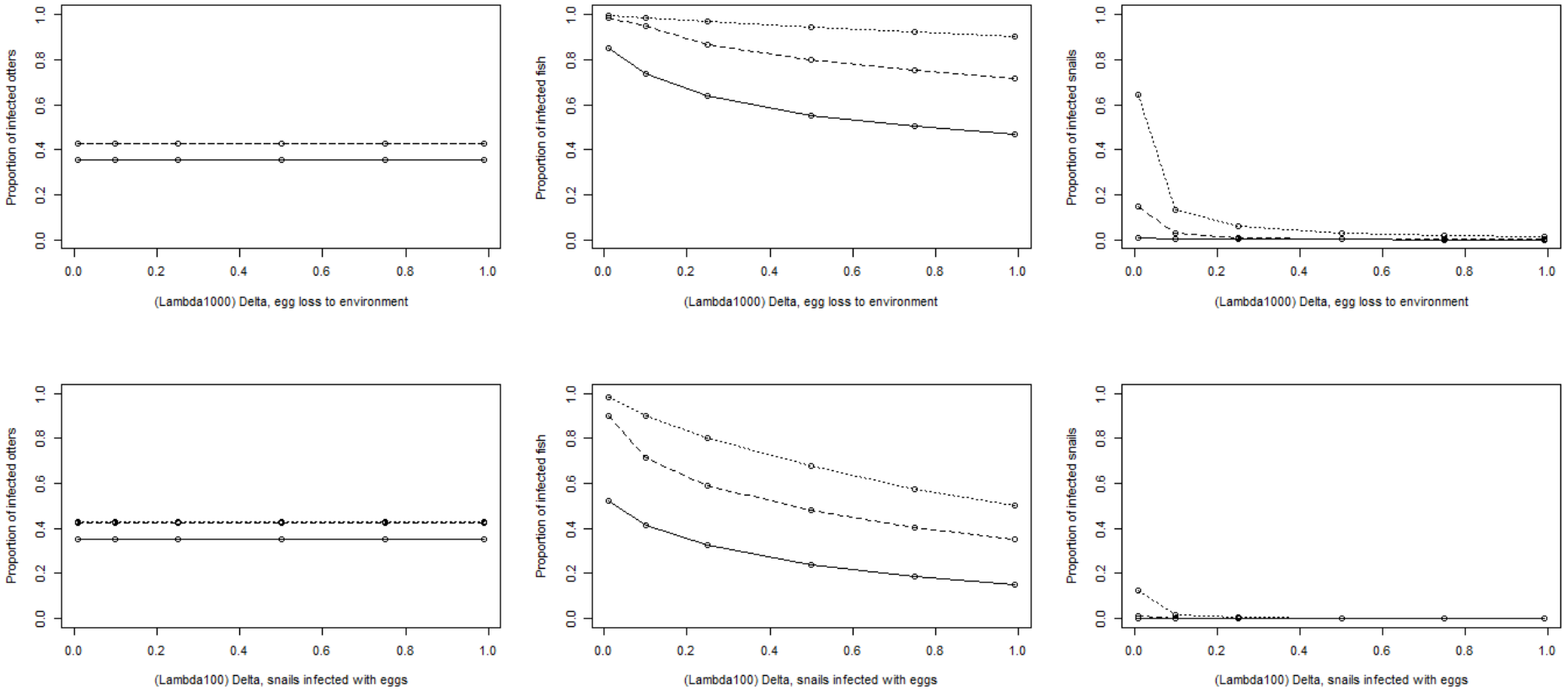

Figure 8.5: The change in the proportion of *Pseudamphistomum truncatum* infected otters, fish and snails with increasing ω at different time points (T = 50 solid line, T = 150 dashed line and T = 300 dotted line). The proportion of infected otters does not change regardless of the value of ω. As ω increases from 0 to 1, the proportion of snails and fish that are infected decreases regardless of whether λ = 1,000 (top row) or λ = 100 (bottom row).

Aim 2: How do model parameters impact prevalence of infection in the intermediate hosts?

The intermediate hosts, the snail and fish populations, are sensitive to changes in model parameters (see Table 8.4) and may help create the fixed prevalence in the otters in this model. The number of eggs released per infected otter (λ) did not ultimately alter the proportion of infected otters but did have a significant impact on snail and fish parasite prevalence (Figure 8.4). An increase in λ from 100 to 1000 eggs released per otter accounted for c.20% increase in infected snails at time point T = 300, while a further increase in λ to 10,000 eggs corresponded to an additional 60% increase in infected snails during the peak season of the year (Figure 8.4). In the fish populations a tenfold increase in λ from 10 to 100 had the largest impact on the proportion of fish infected and where λ > 100 almost all fish become infected (Figure 8.4). Similarly, an increase in the loss of eggs to the environment, ω, led to a decrease in the proportion of infected fish and snails (Figure 8.5).

The majority of parameters, in addition to λ and ω, affected the fish population (Figure 8.6). As the rate of infection in snails (ρ), fish (α) or otters (μ) increases, or predation rate of otters on fish (γ) increases, so does the proportion of fish that are infected. Conversely, when fish hatch rate (b_f) increases then the proportion of infected fish decreases. Equally, as the birth rate of otters increases (b_o), the proportion of infected fish decreases.

Snail hatch rate, b_s, influences the proportion of fish infected. The increase in snails increases the chance of infection for fish. The model indicates that at low b_s, the proportion of fish that become infected is higher and more consistent than at high b_s.

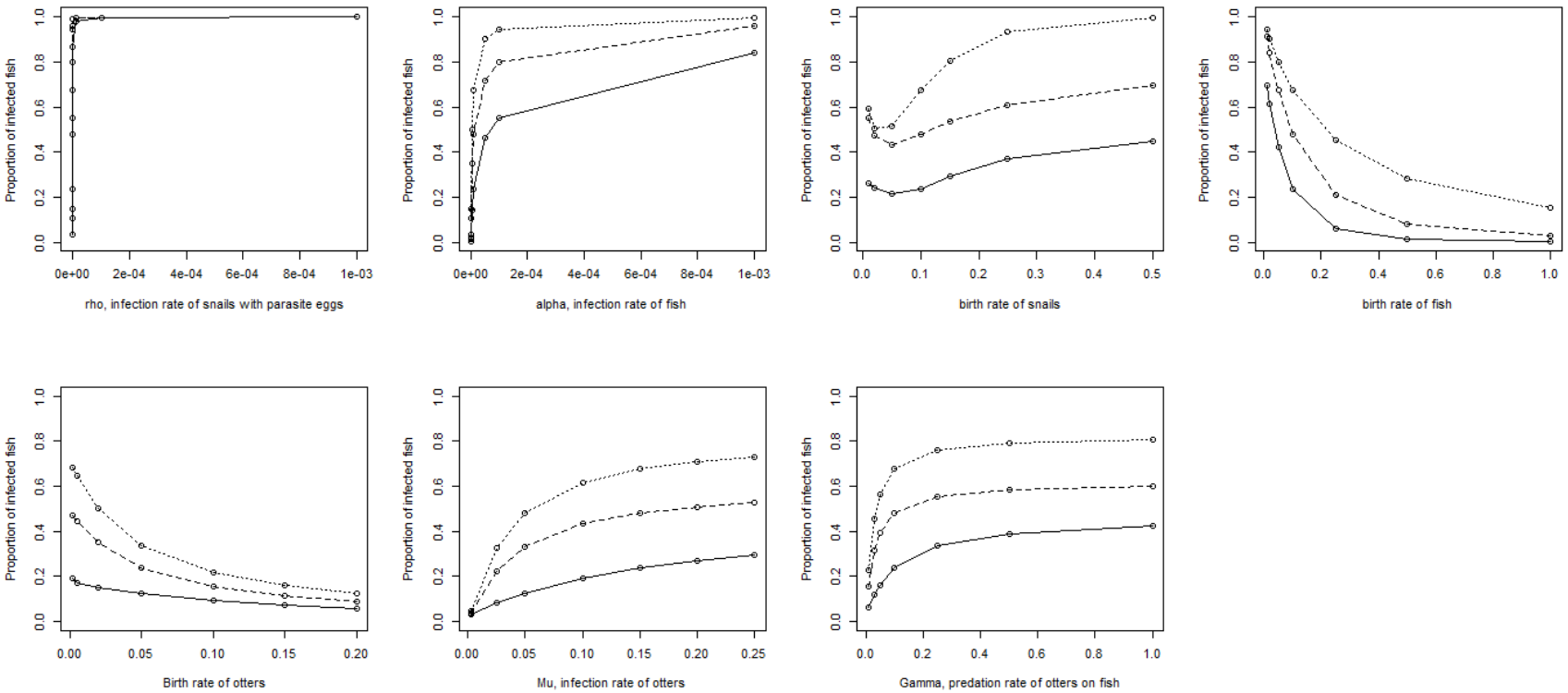

Figure 8.6: The change in parameters affects the proportion of fish infected with *Pseudamphistomum truncatum* at time T = 50 weeks after the first case (solid line), T = 150 (dashed line) and T = 300 (dotted line): Very small increases in the value of the infection rate of snails with parasite eggs (ρ) and the infection rate of fish (α) lead to a rapid increase in the proportion of fish infected tending toward 100%. As the hatch rate of snails (b_o), the infection rate of otters (μ) and the predation rate of otters on fish (γ) increase there is a relatively steady increase in the proportion of fish that are infected. Conversely, as fish hatch rate (b_f) and otter birth rate (b_o) increase, there is a decrease in the proportion of fish that are infected.

The infection rate of snails with parasite eggs, ρ, caused considerable shifts in the prevalence of *P. truncatum* in snails. A 2 fold increase in ρ (1 x 10^{-8} to1 x 10^{-6}) corresponded to a huge increase in infected snails (Figure 8.7). Even where snail hatch rate, b_s, was high there was no overall change in the proportion of infected fish or otters.

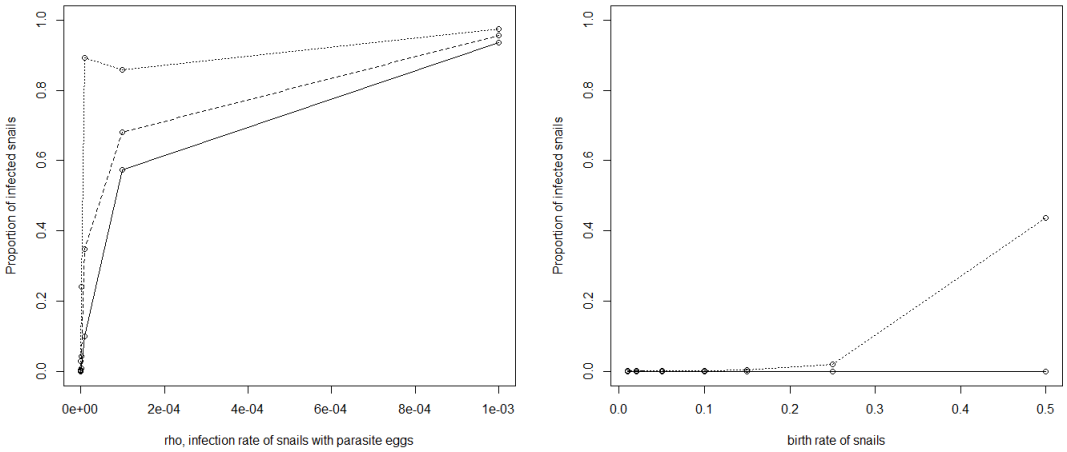

Figure 8.7: Changes to the snail population: as the infection rate of snails with parasite eggs (ρ) increases so does the proportion of snails that are infected with *Pseudamphistomum truncatum*. As the hatch rate of snails (b_o) increases so does the proportion of infected snails but only at time T = 300, prior to this (T = 50 and T = 150) there is very little effect of snail hatch rate on the proportion of infected snails.

Aim 3: How do model parameters impact prevalence of infection in the otter?

Parasite population dynamics in the otter were robust to large variations in model parameters (see Table 8.4). The only parameters to influence the proportion of infected otters were otter birth rate (b_o, as birth rate increases, proportionally fewer otters become infected), predation rate on susceptible fish (γ, as more fish are consumed, the proportion of infected otters increases), and otter infection rate (μ) (see Figure 8.8). As otter infection rate (μ) increases, there is an increase in the proportion of infected otters, with increasing overall egg release an intuitive consequence (Figure 8.8). Increasing predation rate of otters on fish (γ) acts to remove a subset of susceptible individuals so the resulting proportion of infected fish increases and this has a subsequent positive feedback on the number of infections accumulated in otters over time (Figures 8.5 and 8.8).

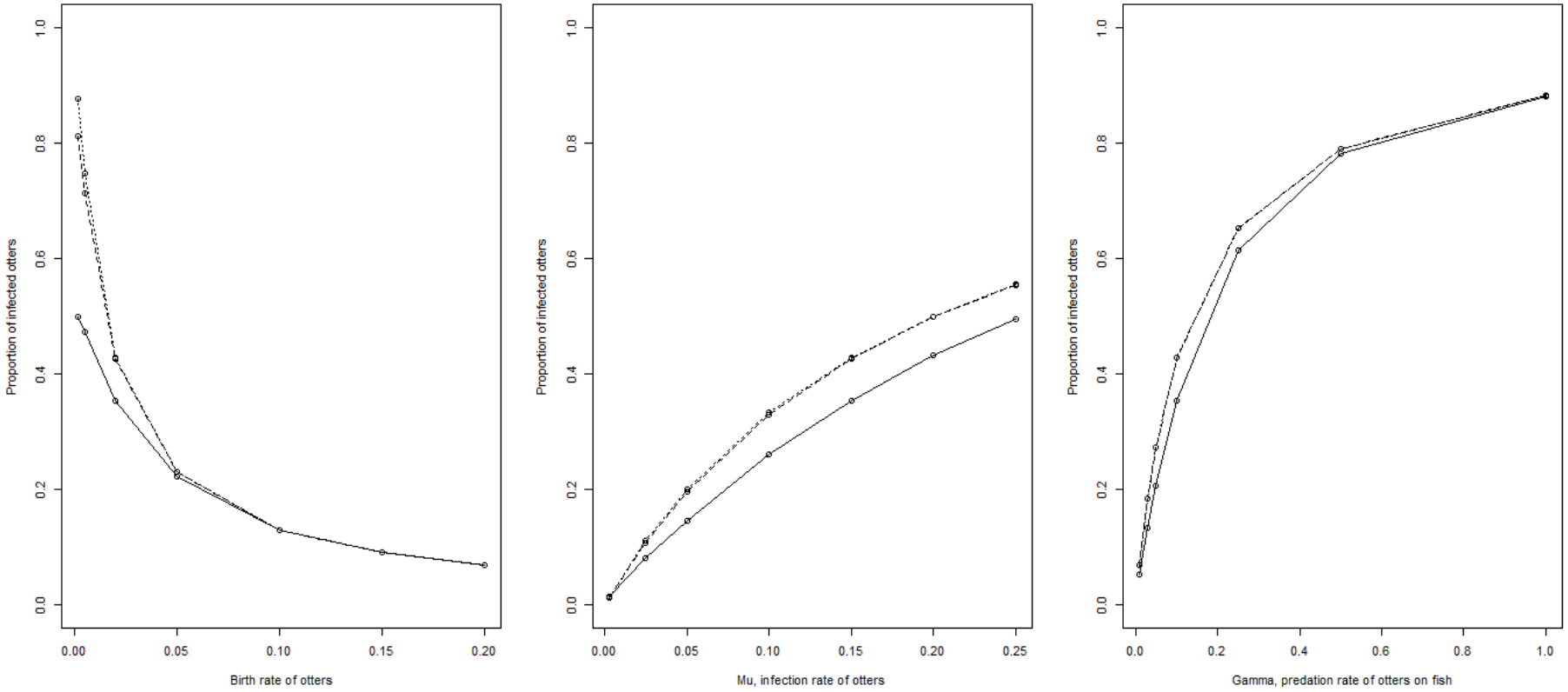

Figure 8.8: The impact of changing parameters on the proportion of *Pseudamphistomum truncatum* infected otters at time T = 50 weeks after the first case (solid line), T = 150 (dashed line) and T = 300 (dotted line): as otter birth rate (b_o) increases, the proportion of infected otters decreases; as the infection rate of otters, μ, increases so does the proportion of infected otters; as the predation rate of otters, γ, so does the proportion of infected otters.

8.5 Discussion

The population dynamics model presented here is built on empirical data of *Pseudamphistomum truncatum* infection rates in areas where the parasite is most abundant in the UK. This model appears to be an effective starting place for future study of the dynamics of three-host trematode systems. The recognition of emerging fish-borne trematodiasis means tools such as this may be effective in elucidating the underlying mechanisms that govern trematode dynamics.

Here, the population dynamics of *P. truncatum* as the parasite cycles through three host types are examined in a simple deterministic model. There is a lack of such models investigating three host trematode species, although multiple host use has been explored for instance where a particular life stage can infect more than a single host species (see Dobson 2004). The predicted model prevalence for *P. truncatum* in each host was robust to large variations in parameter ranges (see Table 8.3) and was fitted to the observed prevalence of *P. truncatum* in hotspot locations in the UK (Somerset Catchment, the combined Catchments within Wessex and Devon as well as the Usk and Wye Catchment).

Aim 1: Estimation of the proportion of parasite eggs that are successfully transmitted

The current model suggests that c.9% of parasite eggs contribute to infection in the snail population. As far as we are aware, there is no empirical quantitative data on the proportion of eggs that are successfully taken up by snails in wild populations. The infection rate of snails with parasite eggs, ρ, caused considerable shifts in the prevalence of *P. truncatum* in snails. Otters deposit spraint (faeces, the source of parasite eggs in the snail habitat) little and often across a variety of habitats perhaps contributing to few parasite eggs passing to the snail population. The 9% success of eggs passing into snails is probably an overestimate of what might be found in reality. Empirical studies suggest trematode infection rates are low in snail populations even when multiple helminth infections are considered (Brown et al. 1988 but see Jokela and Lively 1995).

The model does not incorporate negative impacts of trematodes on snails. Trematodes can have a sterilising effect (e.g. Combes 1982), they can favour snails of a certain age or size (Poulin 1999, Koehler et al. 2013) and there may be dominance hierarchies between trematode species within the intermediate host (Soldanova and Kostadinova 2011). Further, differences in genetic susceptibility between snails (King et al. 2010) will impact

the proportion of successfully transmitted eggs. Equally, survival of parasite egg stages depends on environmental factors including temperature (Tinsley et al. 2011). Currently, the effects of these factors are grouped together within a single rate of loss for this model.

Table 8.4 Summary of the sensitivity analysis effects on prevalence of a three host trematode system based on *Pseudamphistomum truncatum* (Opisthorchiidae) infecting snails, fish and otters in a c.5600km^2 region.

Parameters	Description	Effect
b_o	Birth rate of otters	Impacts all host populations; low b_o causes increased proportions of otters to be infected, elevated seasonality on snail populations and greater proportion of infections in fish
μ	Infection rate of otters	High μ results in higher proportions of infected otters. Overall proportion of either intermediate host remains relatively constant. Seasonal fluctuations increased in snail populations.
Γ	Predation rate of otters on fish	When Γ is low, seasonal fluctuations are less distinct, a low proportion of otters become infected.
b_s	Hatch rate of snails	The greater b_s, the greater the degree of seasonal fluctuations. No impact on otter or fish population dynamics.
P	Consumption rate of snails eating parasite eggs = Infection rate of snails becoming infected with eggs	Most sensitive parameter; below a rate of 1 x 10^{-8} almost no snails are infected yet trematodes continue to cycle through the system. Conversely, a rate of 1 x 10^{-5} produces unrealistic patterns. The model indicates snail infection rates lie between these extremes.
b_f	Hatch rate of fish	The greater b_f, the lower the overall proportion of fish infected. No impact on otter or snail population dynamics.
A	Infection rate of susceptible fish becoming infected with metacercariae	Generally, a high proportion of fish are infected regardless of infection rate. No impact on otter or snail population dynamics.
Λ	Number of eggs produced by each infected otter	The greater the value of λ, the greater the impact of seasonal fluctuations on the snail populations. No impact on the otter population.

Aim 2: How do model parameters impact prevalence of infection in the intermediate hosts?

The current model suggests that where the parasite is abundant almost all viable fish hosts will carry the parasite during peak seasons, supporting the observations that 89% of roach in Ireland were infected with *P. truncatum* metacercariae (Hawkins et al. 2010). Analysis of fish helminths tends to demonstrate high spatial variation explained by intermittent colonisation events (Esch et al. 1988). The current model indicates the proportion of fish that become infected with helminths is very sensitive to changes in the model parameters (see Table 8.4). Metacercariae identification is challenging, partly due to cryptic species, but also because of cyst size in comparison to that of the host making morphological screening of larger fish extremely time-consuming.

Changes in fish hatch rate impacts the parasite cycling through the fish population such that where hatch rate is high (b_f = 1) seasonal fluctuations in parasites are most pronounced but, on average, only about 20% of the fish population are infected (Figure 8.6). This does not fit empirical data (e.g. Hawkins et al. 2010) suggesting that lower hatch rates are more probable in wild populations. When hatch rates are high, naïve fish enter the population and death rate increases to maintain the fixed carrying capacity parameterised into the model. Therefore, the assumption of a fixed carrying capacity in this model perhaps contributes to the ultimate decrease in the proportion of infected fish. This simple model also indicates that the infection rate of fish (α) must be relatively low since α values above 0.001 result in all fish becoming infected. The management and re-stocking of water courses create an artificially high influx of fish and may decrease the proportion of infected fish in the system in the same manner that was seen with an increasing fish hatch rate (b_f). The fish hatch rate is an important parameter according to this model. Although trematode metacercariae are dormant in fish, the ecological impacts of these infections are not easy to predict because behavioural changes may be induced, with potential fitness consequences (Barber et al. 2000).

Aim 3: How do model parameters impact prevalence of infection in the otter?

A dynamic equilibrium where there is no further increase in the proportion of infected otters is achieved rapidly in this simulation model. The rapid colonisation by the trematodes of each host population is a consequence of the simplistic assumptions of the model but may be realistic; recent evidence suggests that trematodes can become

established incredibly fast in novel locations (Soldanova and Kostadinova 2011). Based on the current model predictions, the relatively stable prevalence of *P. truncatum* in otters between 2007 – 2010 (Sherrard-Smith et al. *submitted*) suggests that this parasite is well established in Somerset and South Wales.

Most concerning from a conservation perspective was the observation that a decreasing birth rate of otters (b_o) results in a higher proportion of the otter population carrying infections (Figure 8.8). In this simplistic model however, such a result could be an artefact of the logistic assumption for otter population dynamics. As otter birth rate decreases, otter deaths must also decrease to maintain the carrying capacity at the fixed value of 50. So, for a given time period T_1 to T_2, fewer otters are in the system. Yet when no other parameter is altered, otters are still able to become infected at the same rate regardless of a high or low birth rate. So for time T_1 to T_2 there may be an artificial increase in the proportion of otters that are infected. To explore this further, an additional parameter, ε, was included to decrease the carrying capacity of otters alongside a decreasing birth rate. This can effectively remove the direct link between otter birth rate and death rate in the model. Equation 8.1 becomes:

Equation 8.1a:

$$\frac{dS_o}{dt} = b_o\,(S_o + I_o) - \left(\left(\frac{b_o}{\boldsymbol{\varepsilon} K_o}\right)(S_o + I_o)\right)S_o - \mu\gamma S_o\left(\frac{I_f}{\beta + I_f}\right)$$

And Equation 8.2 becomes:

Equation 8.2a:

$$\frac{dIo}{dt} = \mu\gamma S_o\left(\frac{I_f}{\beta + I_f}\right) - \left(\left(\frac{b_o}{\boldsymbol{\varepsilon} K_o}\right)(S_0 + I_0)\right)I_0$$

But even so the trend for an increased prevalence of *P. truncatum* in otters with decreasing birth rates remained (Figure 8.9). In the past, otter populations across the UK crashed as a consequence of pollution and hunting pressures (see Jefferies and Mitchell-Jones 1993). This model suggests that when otter populations are under pressure from other stresses such as pollution, there may be indirect implications for the otter population

from parasites. Yet ultimately, the parasite population is relatively stable in the definitive host, according to the current model.

8.5.1 Model critique

This model is an important first step for helminth dynamics in three-host life strategies. Currently there are two major restrictions to this model; firstly, if the host population dynamics were affected by the parasite this could result in drastically different model outcomes. Secondly, *P. truncatum*, like most parasites, are aggregated amongst the otter population (Chapter 6) and parasite aggregation is also likely to occur in the snail and fish populations, but is left out of the current model. Equally, parasite aggregation could alter the cycling of the parasite through the host population; this could be built into an extension of the model, but more data on infection levels in intermediate hosts would be required.

This initial attempt to model the host dynamics of a three-host trematode system has highlighted a variety of system specific and more general questions that are yet to be answered. Considering the system specific questions first, the manner of egg release by specific trematodes is often unknown. Certain species continually release eggs whilst others are more seasonal or sporadic (Nollen 1983). Although the number of eggs released here is assumed to be constant, the number of eggs lost to the environment upon release from the definitive host is unknown and this affects the infection rate of the snail host.

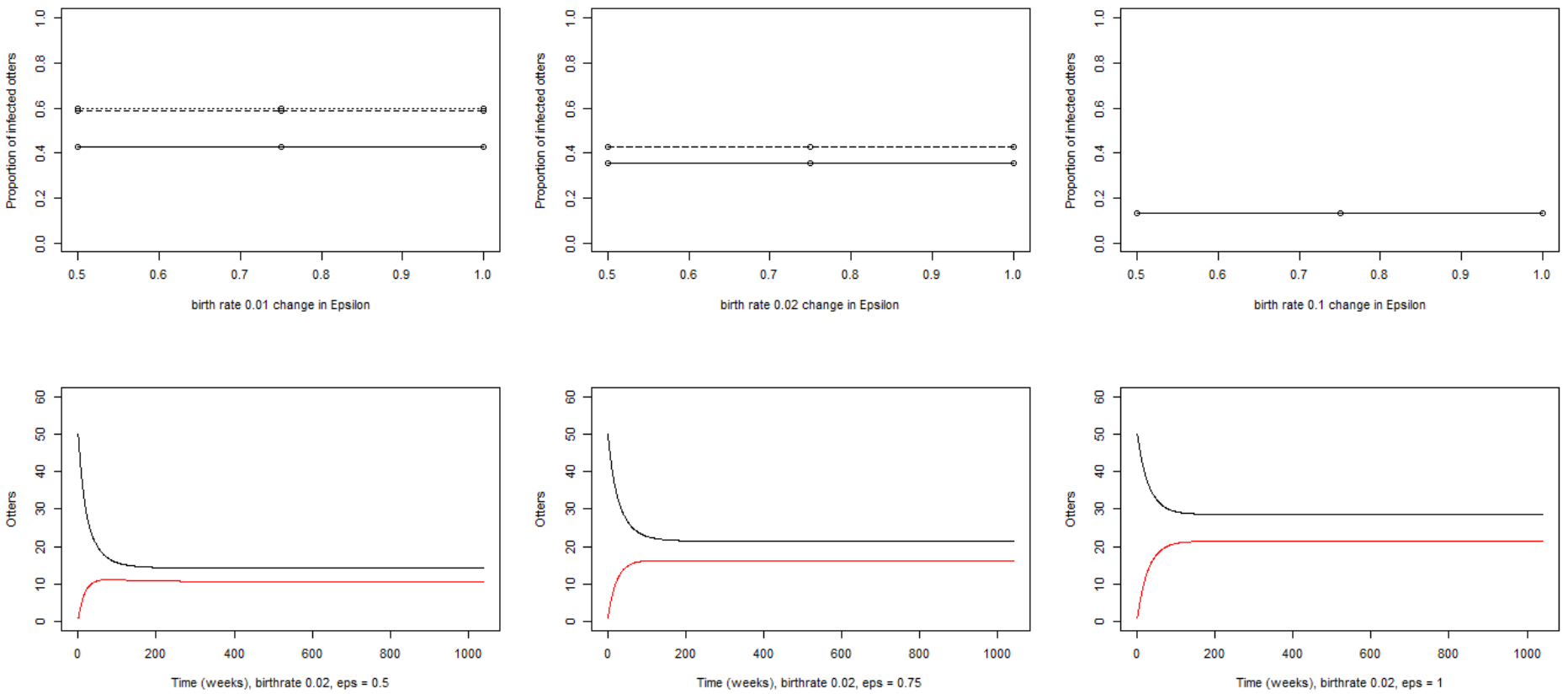

Figure 8.9 The response of the otter population to a decreasing carrying capacity. As otter birth rate (b_o) increases from 0.01 to 0.1 the proportion of infected otters decreases, whilst decreasing the carrying capacity has no additional effect on the proportion of infected otters (top set of graphs). At a fixed birth rate, the proportion of infected otters remains at c.40% regardless of carrying capacity (bottom set of graphs, black line = Susceptible otters; red line = infected otters).

The ability of trematodes to influence the overall carrying capacity for each host is unknown yet it is widely accepted that parasites, in general, have an integral role in host community structure, ecosystem properties and ecosystem functioning (Hatcher et al. 2012, Poulin 1999). What happens if one component host of the life cycle is increasing or decreasing in population size, i.e. what happens when carrying capacity is not fixed? Will trematodes cap host populations at a different level to that which could be achieved in the absence of such parasites? The limit on our understanding of potential hosts is also restricting because certain species may act to maintain the parasite population whilst others are dead-end hosts – this is beyond the scope of the current model. Further, anthropogenic impacts of such systems are ignored here, but influx of viable fish hosts, through the re-stocking of natural ecosystems, is likely to have a significant impact on the dynamics of such parasites. This is particularly important because the proportion of fish that become infected is high. Incorporating a stochastic element to this model is an intuitive next step for this research.

8.5.2 Conclusions

The current model, parameterised with empirical data where available, describes the host population dynamics of the trematode *P. truncatum.* Model output suggests that c.9% of parasite eggs are successfully transmitted into the snail population. As far as we are aware, there is no empirical data on the proportion of eggs that are successfully taken up by snails in wild populations. The proportion of fish that become infected with helminths is very sensitive to changes in the model parameters, more so than snails and definitive hosts. Both the snail and fish populations experience seasonality through an increase in the susceptible population and consequent reduction in the proportion of infected hosts following hatching. The proportion of fish that are infected is much greater than the comparative snail populations perhaps explaining the enhanced sensitivity of the fish populations in this model. The model predicts prevalence in the definitive hosts is invariant to large changes in parameter estimates particularly those describing intermediate host dynamics (both hatch and infection rates of snails and fish). The model also shows that decreasing the birth rate of otters results in a higher proportion of the otter population carrying infections but in this simplistic model such a result could be an artefact of the logistic assumption for otter population dynamics. Although this pattern remains when otter populations decrease as a result of decreasing carrying capacity, further investigation is warranted. This study presents a simple, but flexible, three-host population dynamics model for trematodes. The simplicity of the model

should make it suitable for application to other systems and facilitate the inclusion of additional functions to make the output more realistic.

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Appendix 8.1 A brief summary of models describing host-parasite population dynamics

Early helminth models examined the key host and parasite life history attributes (i.e. the time scale of infection, host life span and the degree of aggregation of parasite populations, the reproductive biology and the transmission potential of the helminth) and these were used to define host-parasite population and transmission dynamics (see Anderson and May 1982, Dobson and Roberts 1994). A wider range of macroparasitic infections have been studied more recently (see Basáñez et al. 2012, Adler and Kretzschmar 1992, Kretzschmar and Alder 1993). Initially models focused on single host-single parasite systems (for example see Anderson and May 1982, 1985, Berding et al. 1987, Dobson and Hudson 1992), but additional elements were incorporated to explore multiple parasite species infections (see Begon et al. 1992, Roberts and Dobson 1995, Gatto and De Leo 1998, Jackson et al. 2006). Further, host immunity (see Schweitzer and Anderson 1992a, Woolhouse 1996), seasonal patterns (Roberts and Grenfell 1991, Dobson and Hudson 1992, Fenton et al. 1998) and stochasticity (Marion et al. 1998, Cornell et al. 2004, Walker et al. 2010) have all been incorporated to produce more complex models (reviewed in Roberts 1995). Modelling the interaction of parasites with their hosts as the host ages (Chan et al. 1995, 1996, Michael et al. 1998, Bouloux et al. 1998) has helped develop an understanding of the most successful treatment strategies for some of the most devastating, global helminth infections, including Schistosomiasis (see Anderson and May 1979, 1982, 1985, Woolhouse 1991, 1992, 1996) and Filariasis (see Guyatt et al. 1993, Chan et al. 1998, Walker et al. 2010). Many studies have modelled the success and cost effectiveness of different treatments (Medley et al 1993, Guyatt et al. 1993, 1995) or other management strategies, such as livestock grazing (see Barnes and Dobson 1990, 1993).

Recently, mathematical modelling has been highlighted as a priority of the Disease Reference Group on Helminthiases (DRGH) (Boatin et al. 2012). Advances in statistical methods now facilitate a more accurate estimation of model parameters such as parasite life span (Plaisier et al. 1991, Fulford et al. 1995), variation in the host immune response (Riley et al. 2003) and parasite establishment rates (Duerr et al. 2006, Basáñez et al. 2002, 2012). Models have been used to investigate questions which are often impossible to address through empirical studies: for example, the optimum infection strategy of macroparasites under varying resource availabilities and host encounter rates (Fenton and Rands 2004). Further, models of host population dynamics allow us to predict how systems might change with time in relation to predictable shifts in environmental conditions (Russell et al. 2004, 2006). Combining the

model outcomes of such theoretical studies with available empirical data can maximise our understanding of host-parasite systems (Woolhouse 1991, 1992, 1994, Basáňez et al. 2012).

Population dynamics models have highlighted the inherent stability of host-helminth systems (Anderson and May 1982, Berding 1986, Roberts and Grenfell 1991, Dobson and Hudson 1992, Hudson and Dobson 1997, Michael et al. 1998). In contrast to viral and bacterial infections which have a faster evolutionary time, helminth systems are incredibly resistant to environmental perturbation events such as storms, droughts and anthropogenic management changing landscapes (Roberts and Grenfell 1991, Dobson and Hudson 1992). Re-infection and host acquired immunity against helminth infection can also operate (Anderson and May 1982, Schweitzer and Anderson 1992a, b). Further, helminths can drive the population dynamics of their respective hosts (Dobson and Hudson 1992, Hudson and Dobson 1997, Rosa et al. 2011).

Digeneans infect between 2 and 5 hosts during their life-cycle, interspersed with life stages that are not attached to a host, at which time the organism is directly exposed to external environmental conditions (Coombes 2001). The dynamics of trematodes that require three hosts in wild fauna may behave differently to the comprehensively studied two host trematodes, such as the schistosomes (see Anderson and May 1978, 1979, 1982, Woolhouse 1991, 1992, 1994, Chan et al. 1995, Carabin et al. 2000, Hisakane et al. 2008, Zhao and Milner 2008, Basáňez et al. 2012, Boatin et al. 2012). Models for such systems are lacking because sufficient data for model parameterisation is often unavailable (Barlow 1995). Yet understanding these helminth dynamics is essential for disease management; particularly since recent evidence suggests helminths influence microparasite dynamics through facilitating multi-species invasions of the host (Ezenwa et al. 2010, Ezenwa and Jolles 2011).

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9. General discussion

9.1 Synthesis

Parasitism is, perhaps, such a successful life strategy because of the flexibility it allows organisms to accommodate changing conditions. This dissertation has contributed to our knowledge of two major parasite groups, the opisthorchiidae trematodes and ixodid ticks, and their interaction with a mammalian host, the Eurasian otter. In general, otters appear to have a relatively low diversity of macro-parasites (Simpson 2007, Chadwick 2007). Parasite abundance tends to increase with host population density (Dobson 1990, Arneberg et al. 1998, Wilson et al. 2001); an observation that might contribute to the observed increased prevalence and abundance of biliary trematodes in South Wales and the Southwest of England (where otter populations are most dense) compared to Central England regions where otter populations are still increasing (Chapter 4).

The most common parasite taxa recorded from otters are the trophically transmitted helminths (e.g. Hansson 1968, Schuster et al. 1988, Jefferies et al. 1990, Shimalov and Shimalov 2000, Torres et al. 2004 but see Mendez-Hermida et al. 2007, Chadwick et al. 2013). Yet before the current work, the abiotic and biotic factors dictating the distribution of these parasites had been considered only rarely (e.g. Jefferies et al. 1990, Sherrard-Smith et al. 2009, Simpson et al. 2009). To date, the majority of work on opisthorchiasis focuses on the Asian populations of *Clonorchis sinensis* and *Opisthorchis viverrini* (for example Chai et al. 2005, Robinson and Dalton 2009, Sripa 2010) but opisthorchiasis is still considered one of Europe's neglected diseases (Hotez and Gurwith 2011). Helminth infections of the Eurasian otter (*Lutra lutra*) are common (e.g. Hansson 1968, Schuster et al. 1988, Jefferies et al. 1990, Shimalov and Shimalov 2000, Torres et al. 2004 but see Mendez-Hermida et al. 2007) but understudied, The present empirical studies on *Pseudamphistomum truncatum* and *Metorchis albidus* in wild hosts are the first to consider both the abiotic and biotic stressors that shape parasite distributions on otters, a large mammalian host (Chapters 4 and 6). Additional hosts for these digeneans have been identified: the snail *Radix balthica* (recorded for the first time as a host of *P. truncatum*), and tench and chubb (added to the known list of cyprinids that can host both *P. truncatum* and *M. Albidus*) (Chapter 7). These data have been used to parameterise a model describing the population dynamics of such parasites (Chapter 8).

There is a strong association between climate and both tick and trematode distributions in the otters (Chapters 3 and 4). In particular, increased temperatures are positively associated with the prevalence of *Ixodes hexagonus, P. truncatum* and *M. albidus*, whereas rainfall and groundfrost are negatively associated with the two digenean species. Positive phases of the North Atlantic Oscilllation are associated with warmer and wetter conditions coinciding with increased tick prevalence but these conditions may have opposing impacts on the digeneans making predictions on the future distributions more challenging.

Both digeneans have been recovered for the first time from British wildlife within the last 10 years (Simpson et al. 2005, Sherrard-Smith et al. 2009). Therefore it was hypothesised that both species were introduced recently to the UK. With the continual development of molecular methods we are likely to see an increase in species simply through the increased capabilities to identify cryptic species and we concluded that the trematodes *P. truncatum* and *M. albidus* in Britain are probably not recent introductions (Chapter 5). Across Europe mtDNA sequences were synonymous with British specimens and therefore it is probable that the native range of both parasites extends into Britain. This raises the question of why these digeneans were not discovered previously? The increase in survey efforts over recent years is likely to have contributed. Yet Somerset, one of the current hotspots for *P. truncatum* infections, has a relatively long record of wildlife investigation (see Simpson, Veterinary Investigation Centre), suggesting a recent increase in this region at least. Increasing host numbers are perhaps responsible, both through recovering otter populations following major declines during the 1960-70s, and the introduction and establishment of another definitive host, the American mink (*Mustela vison*). Alongside this, improved conditions within water courses as a result of conservation efforts and the removal of toxic pollutants (e.g. Kean and Chadwick 2013) may increase the abundance of intermediate hosts. Further, the warmer conditions (see Chapter 4) of recent years may have increased parasite numbers to detectable levels.

This dissertation provided a rare opportunity to collect and analyse large amounts of data on parasites of a wild mammal population. Consequently, it was possible to use the model to explore transmission potential. Heavily infected individuals are often targeted for treatment but will only be influential to parasite transmission if they simultaneously release a large number of infective stages. Targeting both the most infected and those hosts releasing high proportions of infective stages (see Chapter 6; Matthews et al. 2006, Chase-Topping et al.

2008, Luong et al. 2010, Lass et al. 2013) will enhance the efficacy and efficiency of targeted treatment (Chapter 6). By allowing us to focus our treatment strategies more specifically this work has implications for disease control and can thereby benefit conservation.

9.2 Road-kill sampling

During the 3 years of this PhD project, samples were collected from 586 otters, credit to the excellent infrastructure of the Cardiff University Otter Project that has established long-term and wide-scale surveillance of a wild mammal across England and Wales. Data from road-killed animals are invaluable and can provide an insight into the ecology and evolution of wild, elusive and protected species. Such data, however, carry with them inherent biases (see Wilson et al. 2001; Chapter 3). For instance, tick abundance is probably underestimated in the current study and all the data represent a relatively stochastic sub-sample of the otter population (see Sherrard-Smith and Chadwick 2010). As highlighted in Chapter 3, tick infestations could reflect differences in emigration patterns when abandoning a dead host (see 9.4 Future work), while tick emigration rates from dead hosts may interact with local microclimate. Throughout this thesis it is important to consider that road-kill samples might not fully represent the otter population. Age-analysis indicates that the otter population, based on predominantly road-kill data, consists of mostly 1-2 year old animals (Sherrard-Smith and Chadwick 2010) and so the youngest and oldest proportion of the population may be under-represented. European studies that include an increased proportion of non-road killed otters find a broader age distribution with individuals of 8+ years old (Heggberget 1991, Ansorge et al. 1997, Hauer et al. 2002). Older animals may be particularly important for parasite studies where infections are accumulated with age, such as the trophically transmitted trematodes considered here. Further, if heavily infected older otters are under-represented then parasite aggregation may be underestimated because heavy infections are not recorded (see Chapter 6). Overlooking most heavily infected hosts is cited as a potential reason for inconsistencies with the analysis of parasite aggregation when small (host number) sample sizes are considered (see Wilson et al. 2001) and is a limitation of this thesis.

While faecal screening can be used to examine endoparasite distributions through identification of eggs or larvae, they provide no information on the biotic associations that might act on parasite distributions. During this PhD, preliminary examinations not included in the thesis, showed digenean eggs were rarely found in faecal samples (taken from the rectal region of carcasses) even when taken from heavily infected otters. Road-kill autopsies

remain the only way to obtain large sample sizes of wild, protected mammals for individual level analysis allowing the rare opportunity for internal examination of specimens, an understanding of biotic associations and temporal and spatial monitoring of any given parasite.

9.3 Future directions

9.3.1 Comparisons between native and novel hosts

During the course of this PhD, in addition to the otter, a second major definitive host of *P. truncatum* and *M. albidus* was identified as the American mink, *Mustela vison.* The two mustelids (otters and mink) provide a rare opportunity to compare the parasitic fauna of a native and introduced host. Both species have similar life histories but mink were introduced in the early 20th Century whilst otters are native to the UK. Each mammal feeds on fish in addition to other fauna and the biliary parasites occupy the same habitat within the host – the gall bladder. Non-native introductions can cause severe negative implications for comparable native populations (see Hatcher et al. 2012). Yet to establish as a successful population in the first instance, the introduced species must overcome stresses from the novel environment. Local parasites are one such challenge and can have an impact on introduced hosts. Understanding how these new interactions operate can benefit control programs, management of introduced species and contribute to our knowledge of the evolutionary mechanisms acting on novel host-parasite associations. Consequently, future research could tackle the question: where an introduction has occurred, how does the interaction between a parasite and a native host compare to that with an introduced host? The few studies that have considered this question generally report a higher parasite species richness and abundance in the native host (e.g. Dunn and Dick 1998, Roche et al. 2010, Gendron et al. 2012, Lacerda et al. 2012, Ondračková et al. 2012) but this is not always the case (see Pasternak et al. 2007, Kestrup et al. 2011). The arrival of a novel host can alter the dynamics of established host and parasite interactions through the ability of parasites to use hosts as reservoirs (see Rudge et al. 2009). The contribution that the novel host population makes toward transmission of the parasite is therefore of fundamental importance. Parasite fecundity is an integral component determining the transmission potential of a host (Shaw and Dobson 1995, Shaw et al. 1998, Kaitala et al. 1997, Chylinski et al. 2009). As an extension of this thesis, mink samples have been collated and examined allowing comparison of parasite fecundity in a native and introduced host (Sherrard-Smith et al. *unpublished data*).

9.3.2 Intermediate hosts

In Chapter 7, the intermediate hosts for *P. truncatum* and *M. albidus* were considered to gain an understanding of the complete parasite life cycles. The small and opportunistic study on intermediate hosts could be strengthened by snail dissections and larger sample sizes, particularly through the spring / summer months when peak infections are apparent. To examine which host is influencing the parasite spatial distribution to the greatest degree, the genetic structure of the parasites and hosts at each life stage could be deciphered and then over-laid to examine whether the parasite co-evolves with a particular host. It would also be enlightening to ask about parasite abundance, parasite aggregation and parasite fecundity (see Chapter 6) within the snail and fish intermediate host populations to decipher whether these early lifestages are affected by the same biotic or abiotic factors that shape the distribution of adult *P. truncatum* populations. For a parasite with multiple life stages, disease control strategies will be most effective if all stages are considered and targeted appropriately.

9.3.3 Ectoparasitic emigrations and vector status

There is currently limited data on vector borne diseases in otters and other wild fauna of the UK. If the tick *Ixodes hexagonus* that is found on otters can act as a vector, then the wide ranging otters could be reservoir hosts for multiple diseases. An analysis of the microparasites of otter ticks and otter blood samples was performed by a student at the University of Salford in collaboration with Prof. Richard Birtles to examine whether otters play a role in the maintenance of notable microparasites such as *Borrelia* sp., the causative agent of Lyme disease. During the study however, microparasite infection was not found in the otter blood or the associated ticks. However, this may have been a false negative result, perhaps a consequence of the freeze-thaw process of the samples used, impeding the amplification of microparasitic DNA. Yet, if there really is no vector-borne microparasitic infection within the otter population, the result suggests that either otters or *I. hexagonus* may act as dead-end hosts for such zoonoses. Fresh samples from otters are required to examine this issue further and the following step could be to investigate microparasite presence in *I. hexagonus* from other hosts (e.g. hedgehogs, foxes, badgers) and investigate whether microparasites can be maintained in *I. hexagonus* in lab conditions.

9.3.4 Phylogenetic history and migration between the mainland continent and Britain

The phylogenies of both *Pseudamphistomum truncatum* and *Metorchis albidus* across were investigated (Chapter 5). Although mtDNA has been reported to provide sufficient data on

trematode phylogenies in previous studies (e.g. Zaroweicki et al. 2007), little could be learned about the relatedness of populations across Europe using the COXI and COXIII mtDNA regions. While a microsatellite approach could produce a greater source of information this was beyond the scope of the current PhD. Alternatively, it might be possible to elucidate migration history between the continent and Britain if regions of the mtDNA with greater variation were identified. One potential region is the control region D-loop, which tends to be highly polymorphic and may allow us to estimate how long ago the British and European populations were separated, and to examine the extent and direction of migration between the land masses since the populations first diverged (see Charruau et al. 2011). In doing so, a better understanding of the mechanisms that facilitate the movement of parasites such as *P. truncatum* and *M. albidus* could be achieved.

9.3.5 Population dynamics

Mathematical models can be a useful tool for evaluating an entire system by highlighting aspects that are yet to be explored. Equally models have the potential to test hypotheses for stochastic events such as climate warming, unusual weather events, hunting pressures and habitat fragmentation, that may disrupt the balanced dynamics of a given system (see Basáñez et al. 2012). Chapter 8 is an initial attempt to model the host dynamics of a three-host trematode system and highlights various unknown aspects of this system. The current model (Chapter 8) assumes fixed carrying capacities for each host population, but in reality host populations are dynamic. With dynamic host populations, the question could be posed whether trematodes cap host populations at a different level to that which could be achieved in the absence of such parasites? Our limited understanding of potential hosts is also restricting because certain species may act to maintain the parasite population whilst others are dead-end hosts – this was beyond the scope of the current model. Anthropogenic impacts of such systems have been equally discounted, but the influx of viable fish hosts, through the re-stocking of natural ecosystems, is likely to have a significant impact on the dynamics of such parasites. This is particularly important because the proportion of fish that become infected is high. Further, as climate changes there will be inevitable consequences for such systems. Understanding of how climate change can perturb these systems will help prevent, control and treat outbreaks of trematodiases in the future and incorporating a stochastic element to this model would be a potential next step for this research.

9.4 References

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