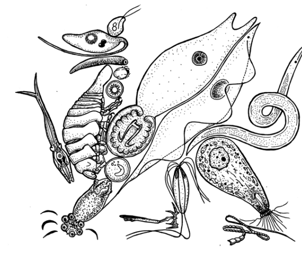

The Effect of Climate on Host-Parasite Interactions

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A thesis submitted for the degree of Doctor of Philosophy (PhD) in the Organisms and Environment Division, Cardiff University

June 2015

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"If there is one thing the history of evolution has taught us it's that life will not be contained. Life breaks free, it expands to new territories and crashes through barriers, painfully, maybe even dangerously, but…life finds a way" Ian Malcolm, Jurassic Park

Thesis abstract

Ecological systems are responding to changes in climate, and due to their ubiquitous nature, parasites will not be exempt from such changes. Understanding the effect that global climate change will have on parasites is of utmost importance, because there will be climate-driven implications for them and their hosts. This thesis addresses fundamental questions about the effect that a changing global climate will have on host-parasite interactions. First, a meta-analysis investigating general trends of parasites exposed to climatic perturbations found that warmer temperatures increased parasite abundance, did not affect fecundity and accelerated development time (Chapter 2). Field experiments were conducted in the Italian Alps, focussing on the yellow-necked mouse (*Apodemus flavicollis*) and its parasites as a model system. Low altitudinal sites (500 m) were significantly warmer and less humid than high altitudinal sites (1400 m), and as such, the change in altitude was used as a proxy for climate change. The effect of climate, host factors and parasite infection on one parasite life-history trait, *in utero* fecundity, was examined, and female hosts at low altitude harboured the least fecund parasites, which was posed to be driven by male-biased sex ratios in this cohort (Chapter 3). The effect of coinfecting parasites on the egg shedding-infection load relationship was further investigated, and this relationship was altered in coinfected hosts (Chapter 4). Interactions between coinfecting ectoparasites and helminths were quantified, and were found to be driven by host biology and climatic conditions (Chapter 5). Finally, infection with tick-borne endosymbionts and/or pathogens on tick behaviour was examined, and ticks infected with endosymbionts emigrated from dead hosts faster than uninfected individuals (Chapter 6). This thesis highlights the importance of considering the effect of host biology and coinfecting parasites in combination with climate when investigating the effect that future climatic changes may have on host-parasite interactions.

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Chapter 1

General introduction: Climate change and host-parasite interactions

1.1 Chapter overview

In this introductory chapter, work that has previously been conducted to investigate the effect that climate has on host-parasite interactions is described. This review is followed by a discussion on how host biotic factors and infection with multiple parasites (coinfection) interact. Finally, the field sites and the host-parasite study system; the yellow-necked mouse (*Apodemus flavicollis*) and its parasite community, which was used for the empirical work in this thesis, are described.

1.2 Climate change, ecological systems and their parasites

Between 1880 and 2012, global surface temperatures increased by 0.7-1.1°C and are predicted to continue rising, such that by 2100, they are likely to have increased by 1.1-4.8°C (IPCC, 2013). Warming is unlikely to be symmetrical, however, as minimum temperatures are increasing faster than maximum temperatures, and mid- and high-latitudes are experiencing longer freeze-free periods (Easterling *et al.*, 1997; Walther *et al.*, 2002). Patterns of precipitation are also predicted to change, although how this will be manifested is hard to predict; some areas will experience a decrease in precipitation, whilst others an increase (IPCC, 2013). As a result of these predicted and forecasted shifts, climate change is one of the biggest threats facing biodiversity (Karvonen *et al.*, 2010), but predicting the effects on ecological systems is challenging. Species will either have to adapt to the new climatic conditions, or shift their distributions to maximize fitness and ultimately avoid extinction (Thomas *et al.*, 2004; Pounds *et al.*, 2006). There is already evidence of species adapting to climate changes, by altering phenological events; for example, insect emergence and the arrival of birds at breeding grounds are occurring, on average, 8.5 days earlier in the present day than was recorded in the former part of the 20th century as a result of recent warming (Root *et al.*, 2003; Jenni and Kéry, 2003; Carvalho *et al.*, 2010). Climate change has also resulted in ecological shifts in distributions: Hickling *et al.* (2006) found 16 taxonomic groups (275 species) that occur in terrestrial and/or freshwater habitats have shifted the northerly edge of their range by 31-60 km in the UK over the past 40 years, and 227 of these species have also migrated to a higher altitude by approximately 25 m. For the majority of taxa, climate warming will result in a shift in their distributions, but for those species whose current niches are already at the extremes (e.g. polar and alpine fauna), climate change is likely to have the largest impact on their future viability by causing a

reduction in suitable habitat, potentially leading to extinctions (Thomas *et al.*, 2004; Lafferty, 2009a).

The persistence of species is dependent on their interactions with others that live in the same habitat (Stenseth and Mysterud, 2002). This represents one of the difficulties in predicting the response to changes in the climate, as warmer temperatures may alter the phenology of one species, which may have knock-on effects for another. Trophic mismatches in the appearance of animals and their food source has been reported in a range of systems, including American robins (*Turdus migratorius*) that arrive at their breeding grounds before the snow melt and thus the appearance of their food source in the Rocky Mountains (Inouye *et al.*, 2000). Similarly, the phenology of egg hatching of winter moths (*Operophtera brumata*) has advanced since the 1980's in the Netherlands (Visser and Holleman, 2001). Moths that hatch before or after the appearance of oak buds (*Quercus robur*) have reduced fitness as they will either have to starve or consume leaves that contain high levels of tannin, and reduces the fecundity of female moths (Feeny, 1970; Dewar and Watt, 1992; Buse and Good, 1996; van Dongen *et al.*, 1997). The interactions between hosts and their parasites (the term parasite is used here to refer to both parasites and pathogens) will not be exempt from such changes, as parasites are dependent on their hosts. Climate-induced alterations to either parasites or their hosts will, therefore, have implications for the health of the host.

The shifts in phenology and distribution observed for many species in relation to warmer temperatures has sparked considerable interest from infectious disease ecologists, as alterations to the ecology of species would also have profound changes to their parasite community (Patz *et al.*, 2000; Marcogliese, 2001, 2008; Epstein, 2001; Harvell *et al.*, 2002; Lafferty, 2009a). As the climate changes, some geographic areas may become less suitable habitats for both hosts and their parasites, forcing a shift in their distributions (Parmesan, 2006; Brooks and Hoberg, 2007). Changing host-parasite distributions can potentially lead to increased incidence of infectious diseases, or result in disease emergence in an area where it has not been previously observed. Distributional shifts in disease are already apparent in areas such as in the East African highlands. The occurrence of malaria was previously limited by cooler temperatures at higher altitudes, which were not suitable for the survival of either the malaria pathogen or the mosquito vector (Patz and Olson, 2006). Increases in temperature, however, have affected mosquito distribution and have been correlated with an increased incidence of malaria in the highlands (Afrane *et al.*, 2005, 2006; Pascual *et al.*, 2006; Chaves and

Koenraadt, 2010; Omumbo *et al.*, 2011; Stern *et al.*, 2011). In addition to the expansion of existing diseases, climate is purportedly accountable for the emergence and establishment of novel diseases; for example, during the summer of 1999, West Nile Virus (WNV) emerged in New York City (Lanciotti *et al.*, 1999). WNV is a flavivirus spread by mosquitoes (*Culex* spp.) in Africa and Asia, and is usually found in birds, with occasional spillover to humans. Although the route of entry to the USA is unclear, it is suggested that warmer-than-average temperatures, elevated humidity and heavy precipitation provided an ideal climate for the mosquito vector, which allowed the virus to spread to every state within 4 years (Dohm *et al.*, 2002; Soverow *et al.*, 2009; Randolph and Rogers, 2010).

Whilst climate change may allow both hosts and their parasites to expand their ranges, they may not be synchronized in doing so (Pickles *et al.*, 2013). For generalist parasites, if the common host species is not available (i.e. the host does not simultaneously expand its range), parasites may spillover to infect 'novel' hosts in order to survive. For example, the lung fluke *Haematoloechus floedae* was introduced without its American bullfrog host *Rana catesbeiana* to Costa Rica. Despite the absence of its established host, the parasite survived by infecting local frog species, *Rana taylori* and *R. furreri* (Brooks *et al.*, 2006). The American bullfrog has been successfully introduced into over 40 countries and is increasing its range in response to warmer temperatures (Nori *et al.*, 2011). As its distribution to novel areas increases, the American bullfrog has been increasingly implicated in the spread of the fungus *Batrachochytrium dendrobatidis*, which causes chytridiomycosis in amphibians, and is thought to be responsible for almost half of amphibian declines, as the disease currently results in 100% mortality (Pounds *et al.*, 2006; Hatcher and Dunn, 2011). Climate change is likely to facilitate invasions by non-native species that may be better adapted to the changing climate than native species, thus providing an additional competitive advantage over native species (Rahel *et al.*, 2008; Fey and Cottingham, 2011). Competition between invasive and native species may be further exacerbated by the introduction of novel parasites and pathogens from the invasive species to the native wildlife, which are unlikely to have resistance to the new parasites, and so impact the native host population. This is termed parasite-mediated competition (Tompkins *et al.*, 2003; Gozlan *et al.*, 2005).

One unique feature of some species' distribution is migration between different locations at certain time periods of the year. Migrations have health benefits in allowing individuals to escape from parasites that have built up in the environment over time

(Loehle, 1995). Migratory journeys may be reduced or cease if abiotic conditions change at breeding grounds such that it renders them suitable for permanent habitation (i.e. species become year-round residents; Harvell *et al.*, 2009). Reductions in migratory journeys could, therefore, increase parasite load and diversity within these species. Empirically, this pattern has been observed in monarch butterflies (*Danaus plexippus*) infected with a protozoan parasite (*Ophryocystis elektroscirrha*). The prevalence of this parasite is highest in populations that are non-migratory, and lowest in populations that migrate from eastern North America into Mexico (Altizer *et al.*, 2000). Not only does cessation of migration in monarch butterflies have the potential to increase parasitism rates, but climate-induced reductions in the distance of the migratory journey has produced greater parasite burdens (Altizer *et al.*, 2000; Harvell *et al.*, 2009).

To understand how species may respond to changes in climate, mathematical modelling can often be employed to map the current distribution of a species, and to predict how the species may adapt to changes in the climate, which are predicted using general circulation models (Johns *et al.*, 1997; Pearson and Dawson, 2003; Araujo *et al.*, 2005). A similar model system is CLIMEX, which compares the climate of a species' native region with other regions throughout the world, and can predict future distributions based on climatic scenarios (Yeates *et al.*, 1998; He *et al.*, 2012; Khormi and Kumar, 2014). When these data or approaches are not available, however, field experiments involving species living at high latitude and altitude can provide an early insight into the effect that climate change is likely to have on ecosystems more generally, as increases in temperature are expected to be most dramatic at these two extreme locations (Kutz *et al.*, 2013). Climate change is well-advanced in the Arctic and consequently, Arctic ecosystems are providing insight into climatic-driven biotic changes in host-parasite dynamics (Kutz *et al.*, 2013; Dobson *et al.*, 2015). Studies on the parasites of Arctic muskoxen, for example, have demonstrated that warmer temperatures result in the free-living stages of the parasites developing faster and producing higher worm burdens, resulting in higher host morbidity and mortality (Halvorsen and Skorping, 1982; Kutz *et al.*, 2005). There is also evidence of parasite invasion and range expansion in the Arctic, where warmer temperatures have aided parasite establishment in areas where the previous cooler climate prevented parasite development (Kutz *et al.*, 2013). Warming temperatures have led to range expansions for some deer and elk species, resulting in range overlaps with native muskoxen. Parasites from deer and elk can be introduced to muskoxen, and vice versa; so whilst parasites in the Arctic are developing faster under warmer temperatures, they may also increase contact rates between multiple species,

leading to spillover into species that have no natural resistance to novel infections, potentially producing wildlife epizootics (Dobson *et al.*, 2003; Kutz *et al.*, 2005; Dobson, 2009).

Whilst insights into the effects of climate change on host-parasite interactions have been observed at high latitudes in the Arctic, high altitudinal montane environments also provide a good model system for addressing climate change effects on host-parasite interactions (Jouda *et al.*, 2004; Gilbert, 2010; Zamora-Vilchis *et al.*, 2012). Specifically, host-parasite interactions at high altitudes, where the environment is cooler and drier, can be compared with those at lower altitudes, where the climate is warmer and wetter, and so represents a proxy of predicted future climate changes. Altitudinal gradients have previously been used to investigate the effect of climate on the morphology, phenology, growth, life-history traits and interspecific interactions of a range of systems, such as plants and insects (e.g. Hodkinson, 2005; Körner, 2007; Garibaldi *et al.*, 2011; Michalet *et al.*, 2014), and, therefore, can be used to study the effects of climate change on ecosystems when it is not possible to conduct long-term experiments (Pickett, 1989; Hodkinson, 2005). Altitudinal gradients have previously been used to investigate the effect of climate on the prevalence of tick-borne pathogens and avian blood parasites; these studies have found that the prevalence of tick-borne pathogens, along with the tick hosts, and avian blood parasites all decreased with altitude (Jouda *et al.*, 2004; Gilbert, 2010; Zamora-Vilchis *et al.*, 2012). The temperature-related range restriction of some vectors and diseases has provided a conservation refuge for some species. One well-known example is the cooler, high altitudinal sites providing a climate refuge to native birds from avian malaria (*Plasmodium relictum*) and its mosquito vector (*Culex quinquefasciatus*) in the Hawai’ian Islands. The emergence of avian malaria due to spillover from non-native bird species caused large declines in native bird species number and diversity; attributed to a lack of natural immunity and greater susceptibility to avian malaria than their introduced counterparts (van Riper III *et al.*, 1986; Atkinson *et al.*, 1995). Native birds were unable to survive at low altitudes where favourable climatic conditions encouraged the abundance of the mosquito vectors (van Riper III *et al.*, 1986). Loss of this refuge due to any shifts in climate that could increase the mosquitoes’ distribution would put the endemic native birds at serious risk of extinction. This example illustrates why understanding the response of parasites to temperature changes, therefore, is an important conservation planning tool.

Parasites may be sensitive to fluctuations in the climate in their own right, particularly if they feed on hosts for relatively short times (e.g. ectoparasites, such as the mosquito) or spend a high proportion of their life-cycle external to the host (e.g. many helminth species). The number of days taken for parasites to develop (also known as degree-days) into infectious eggs or from eggs to infectious larval stages in the environment first requires a critical temperature to be exceeded, and further temperature increases may accelerate parasite development (Gibson, 1981; Arene, 1986; Griffin, 1988). Excessive temperature increases, however, can result in desiccation and be detrimental to survival (Gibson, 1981; Boag and Thomas, 1985; van Dijk and Morgan, 2008). The development and survival of some larvae is dependent on some level of moisture; though excessive moisture in the form of heavy precipitation can wash away eggs and larvae (Stromberg, 1997; Altizer *et al.*, 2006). Ultra-violet light may also impact upon free-living parasite stages, and persistent exposure has been shown to reduce the number of infective larvae (van Dijk *et al.*, 2009). Temperature increases as a result of climate change could alter the activity patterns of parasites by lengthening the time periods when they are active, resulting in increased disease risk to hosts (Aho *et al.*, 1982; Harvell *et al.*, 2002). For some parasites, however, activity may decrease; ticks waiting on vegetation to attach to a host (questing) descend into the leaf litter to rehydrate if temperatures increase beyond a critical level (Rudolph and Knulle, 1979; Gaede and Knulle, 1997; Kahl and Alidousti, 1997; Perret *et al.*, 2004). Increased temperatures could also have phenological effects leading to earlier seasonal emergence or extended seasons of parasites and/or increased number of generations per year of parasites (Marcogliese, 2001; Kutz *et al.*, 2005; Hakalahti *et al.*, 2006). Increased temperatures, however, will not favour all diseases; for example, *B. dendrobatidus,* which causes chytridiomycosis in amphibians, requires cool, moist conditions, and warmer temperatures may reduce disease spread (Daszak *et al.*, 1999), unless disease spread changes host distribution, as seen with the American bullfrog (Nori *et al.*, 2011).

In free-living systems, climate is just one factor that can influence parasite life-history traits, and laboratory experiments have provided insights into the direct effect that changes in temperature and humidity may have. Often it is only feasible to test a single environmental variable in laboratory experiments even though other factors including biodiversity (Ostfeld and Keesing, 2000), host immunity, habitat fragmentation (Afrane *et al.*, 2012), socio-economic factors (Sumilo *et al.*, 2007) and pollution (Khan and Thulin, 1991) are likely to play an important role. Field studies are, therefore, essential for identifying how natural systems respond to changing abiotic and biotic conditions.

For example, laboratory experiments suggested that increases in temperature and humidity significantly increase the number of ticks that develop (Heath, 1979), and in Sweden in 1994, an increase in the incidence of tick-borne encephalitis (TBE) was linked to warmer-than-average preceding winters (Lindgren and Gustafson, 2001). Similar increases in temperature and TBE incidence have also been reported in the Baltics, but this increase in incidence was thought to be due to socio-economic factors rather than climate: the temperature increases occurred simultaneously with the end of Soviet rule, which led to changes in land use, and potentially exposed more people to ticks than previously (Sumilo *et al.*, 2007). As such, experiments that combine laboratory and field studies that consider a range of biotic and abiotic factors are needed.

1.3 Host biology and parasitism

Whilst abiotic factors clearly have an affect on the life-histories of parasites, biotic factors also play an important role. In nature, hosts are not equally infected with parasites; instead they are aggregated within a host population, whereby the majority of the population harbour a low parasite burden, whilst the minority are infected with high parasite numbers (Shaw and Dobson, 1995; Woolhouse *et al.*, 1997; Shaw *et al.*, 1998; Perkins *et al.*, 2003). The heterogeneity in parasite burdens that exists between different hosts can be associated with the biology of the host. Comparative studies, for example, have found that male hosts tend to be the most infected sex (Poulin, 1996; Zuk and McKean, 1996), but this only tends to hold true when sexual size dimorphism is male-biased (Moore and Wilson, 2002). Larger animals consume more food than smaller hosts and are, therefore, likely to have greater exposure to ingested parasites, and/or because they provide a larger surface area for ectoparasites (Zuk and McKean, 1996; Perkins *et al.*, 2003; Harrison *et al.*, 2010). Hosts may experience greater exposure to parasites if their home ranges cover large distances (Brei and Fish, 2003). Mechanistically, sex-biased infection loads may be due to the presence of testosterone, which acts as an immune-suppressant, causing male hosts to be more susceptible to infection (Folstad and Karter, 1992; Poulin, 1996; Zuk and McKean, 1996). Testosterone can also change host behaviour, which influences parasite transmission. For example, testosterone levels in male red grouse (*Lagopus lagopus scoticus*) have been experimentally manipulated, and males with the highest levels of testosterone had larger territories, were more aggressive, more bigamous and demonstrated higher

copulation frequency, as well as being infected with greater numbers of parasites than control individuals (Moss *et al.*, 1994; Chandler *et al.*, 1994; Alatalo *et al.*, 1996; Mougeot *et al.*, 2004; Seivwright *et al.*, 2005; Redpath *et al.*, 2006). Testosterone-induced changes in the behaviour can also increase contact rates between individuals, potentially resulting in increased parasite transmission (Grear *et al.*, 2009). Conversely, pregnant or lactating females may experience higher parasite burdens, which may be due to hormones reacting with the immune response, making them more prone to infection (Crofton, 2009; Grzybek *et al.*, 2015). The age of the host also plays a role in generating heterogeneity because exposure to parasites will change over an individual's lifetime (Behnke *et al.*, 1999). For some infections, parasite intensity is greatest in young individuals when transmission rates are high, but this is then followed by an acquired immunity-induced reduction, which results in fewer parasites in older individuals (Cattadori *et al.*, 2005). Alternatively, parasite intensity accumulates over time, and/or immune responsiveness is reduced in older individuals (immunosenescence) culminating in older individuals harbouring the greatest parasite burden (Wilson *et al.*, 2002; Roberts and Hughes, 2014). Whilst climate affects parasite intensity and host biology also plays a role, it is likely that there may be some interaction between these factors. To date, the three-way interaction between climate, parasite intensity and host biology has not been investigated.

1.4 The effect of coinfection on parasitism

The majority of studies on the variation within host-parasite distributions and the effects of climate have been conducted with just one host and one parasite species. This methodology persisted for many years, despite a rich research area within medical biology examining the interactions between multiple, simultaneously infecting parasites; termed coinfection (e.g. Nadelman *et al.*, 1997; Karanja *et al.*, 1997, 1998; Staples *et al.*, 1999). Similar to humans, within wild populations, the majority of individuals are simultaneously infected with more than one parasite, referred to as an 'infracommunity' (Poulin and Morand, 2004) and more recently, as coinfection. It is, however, only within the last 10 years that the interactions between coinfecting parasites within the infracommunity have been examined within ecological systems (e.g. Lello *et al.*, 2004; Telfer *et al.*, 2010). Interactions between coinfecting species can be synergistic, whereby the presence of one parasite species changes the host environment making it more suitable for additional species and increases the fitness of

the second species (Behnke *et al.*, 2001a; Graham, 2008; Ferrari *et al.*, 2009). For example, mice infected with higher than average burdens of the nematode *Heligmosomoides polygyrus* were more likely to be infected with a greater range of species than uninfected individuals (Behnke *et al.*, 2009). Interactions can also be antagonistic, where the presence of one species has a negative impact upon the fitness of another species (Behnke *et al.*, 2001a; Ferrari *et al.*, 2009). Experimental removal of *H. polygyrus* resulted in tick intensity significantly increasing compared with individuals where *H. polygyrus* was not removed (Ferrari *et al.*, 2009). There is much variation in the outcome of parasite interactions in ecological systems, with some finding interactions between parasites (Lello *et al.*, 2004; Telfer *et al.*, 2010), whilst others have found none, or a 'null' effect (Haukisalmi and Henttonen, 1993a; Behnke *et al.*, 2005; Behnke, 2008). The mechanisms underlying parasite interactions may include a number of factors including competition for resources, crowding and immune-mediated effects (Behnke *et al.*, 2001a; Cox, 2001; Lello *et al.*, 2004; Pedersen and Fenton, 2007; Telfer *et al.*, 2010).

Coinfection clearly alters the interactions between parasites, and increasing or decreasing the presence or intensity of one parasite species as a result of abiotic factors will, therefore, have implication for the whole parasite community. There has been very little work carried out investigating abiotic effects and coinfection, but synthesis of multiple studies provides tantalising evidence that there could be an effect. For example, two parasites infecting rabbits, *Trichostrongylus retortaeformis* and *Graphidium strigosum,* were exposed to increased temperatures, and whilst both parasites demonstrated accelerated egg development, increased hatching rate and increased larval survival, *T. retortaeformis* was more responsive than *G. strigosum* (see Hernandez *et al.*, 2013). These two helminth species have previously been shown to interact with one another and with other coinfecting parasites of the rabbit (see Lello *et al.*, 2004, 2008). As such, any changes to either of these parasites as a result of climatic perturbations may affect the parasite community as a whole. Empirical systems provide the most revealing approach of determining and identifying the interactions that occur between abiotic and biotic factors, whilst simultaneously considering the effect of coinfection (Graham *et al.*, 2007).

1.5 Investigating the effect of climate and host biology on host-parasite interactions in a free-living system

To investigate the interactions between climate and host-parasite interactions, fieldwork was carried out across three replicated mountain field sites (Monte Bondone, Monte Baldo and Panarotta) in the province of Trentino in the northeastern Italian Alps (Fig. 1.1). Yellow-necked mice (*Apodemus flavicollis*) are one of the most common species of small mammals that are found in the broadleaf woodlands in this region and are the focal species of this thesis. The parasite community of mice in this area has been well-studied (Ferrari, 2005; Kallio-Kokko *et al.*, 2006; Perkins *et al.*, 2006, 2008; Rosà *et al.*, 2007; Carpi *et al.*, 2009; Ferrari *et al.*, 2009; Rizzoli *et al.*, 2009). One of the most common parasites found in yellow-necked mice is the gastrointestinal nematode, *Heligmosomoides polygyrus* (see Ferrari, 2005). This species, and the related laboratory model *Heligmosomoides polygyrus bakeri,* is commonly used as a model species when investigating host-parasite interactions and immune responses (Behnke and Wakelin, 1977; Jenkins and Behnke, 1977; Behnke *et al.*, 1983; Quinnell *et al.*, 1991; Jackson *et al.*, 2009; Hewitson *et al.*, 2011; Maizels *et al.*, 2012; Reynolds *et al.*, 2012) and, as such, forms the focus of multiple experiments in this thesis.

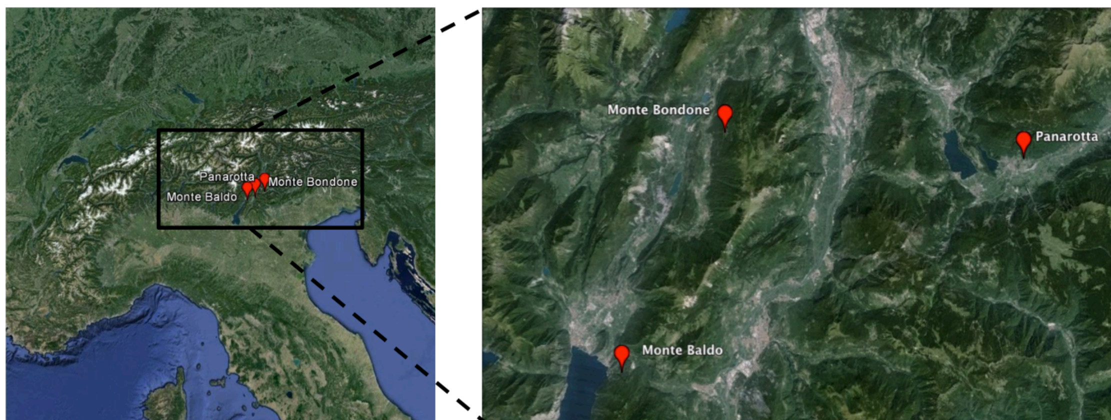

Figure 1.1 Map showing the location of three mountains (Monte Bondone, Monte Baldo and Panarotta) where field sites were located in the province of Trentino, in the north-eastern Italian Alps.

1.6 Thesis aims

This thesis aims to investigate the effect that climate may have on host-parasite interactions. It is composed of five experimental, self-contained chapters (Chapters 2-6), which are being prepared for publication. First, a broad meta-analysis was conducted, investigating the effects of a temperature perturbation on three life-history traits: abundance, fecundity and development time across multiple parasite taxa (Chapter 2).

Following on from this, a field experiment examined whether a key parasite life history trait, fecundity (defined as the number of eggs *in utero* per mm length of worm) was affected by changes in climate along an altitudinal gradient, and the effect of host factors and coinfecting parasites was also examined (Chapter 3). Next, the effect that coinfection may have on the infectiousness of a host, measured by the number of eggs shed in faeces of yellow-necked mice was investigated in Chapter 4. Coinfection was further investigated in Chapter 5; the parasite community of mice along an altitudinal gradient was quantified, and interactions between parasite species were determined, and specifically, the effect of host biology and changes in climate along an altitudinal gradient on parasite community interactions was examined. The last data chapter describes an experiment that investigated whether infection with tick-borne endosymbionts and/or pathogens altered tick behaviour (Chapter 6). Finally, Chapter 7 brings together the salient findings of the study, and highlights future research questions. All chapters (excluding Chapter 1 and Chapter 7) have been written as manuscripts for submission, which has inevitably led to some overlap, typically in the methods, between them.

Chapter 2

The effects of temperature perturbations on parasite life-history traits

2.1 Abstract

Parasite life-history traits are phenotypically plastic and are expected to be sensitive to changes in climate, which ultimately may affect parasite transmission. To quantify broadly the effects of temperature across host-parasite taxa, a review of the published literature was conducted on three key parasite life-history traits: abundance, fecundity and development time. A meta-analysis of 443 data points from 36 publications revealed temperature perturbations above or below adapted, or 'ambient' levels significantly increased parasite abundance, but only for temperature increases within the range + 0.1-5°C. Small effects sizes were found for the relationship between temperature and fecundity, with warmer temperatures (only above + 10°C) increasing parasite fecundity, whilst decreasing temperatures significantly reduced fecundity, but only within the range of - 0.1-5°C below ambient. Parasite development times significantly accelerated with increases in temperature, but were unaffected by reduced temperature. Specific host and parasite taxa were found to be driving these general patterns, such that increases in parasite abundance due to warmer temperatures were driven by parasites of ectothermic host taxa infected with platyhelminths. All host and parasite taxa examined, with the exception of amphibians, had parasites with accelerated development time. In summary, temperature increases within the range predicted for climate warming may increase parasite abundance, accelerate development times, but not affect parasite fecundity. Ultimately, these changes may not lead to increased infectious propagule pressure (i.e. increased transmission), but result in elevated parasite burdens due to an increase in the number of parasite generations, and higher parasite loads, which is expected to have negative effects on host health.

2.2 Introduction

Global mean temperatures are predicted to increase by 1.1-4.8°C, with parallel increases in precipitation and humidity before the year 2100 (IPCC, 2013). Extreme climatic events due to 'global weirding', the phenomena whereby weather patterns become more erratic and unpredictable, have been linked to the emergence of diseases (for example, cholera outbreaks after extreme rainfall events; de Magny *et al.*, 2008). Warmer temperatures have shifted the mosquito line in the mountains of Hawai'i from an altitude of 1500 m above sea level, to 1900 m in 1995, resulting in a doubling of avian malaria within a decade (Freed *et al.*, 2005). Similarly, the rapid spread of West Nile

virus (WNV) from New York City to every state in the USA within the space of 10 years was putatively facilitated by temperature (Lanciotti *et al.*, 1999; Randolph and Rogers, 2010). In the UK, increases in the prevalence of gastrointestinal parasites infecting sheep has also been linked with warmer temperatures (van Dijk *et al.*, 2008; Kenyon *et al.*, 2009). Undoubtedly, changes in the geographical ranges of hosts and their parasites have led to increased disease emergence events, with both positive and negative effects on biodiversity (Brooks and Hoberg, 2000; Daszak *et al.*, 2000; Fayer, 2000; Marcogliese, 2001; Harvell *et al.*, 2002; Lafferty, 2009a). Whilst there are an increasing number of reviews that assert that climate change will increase the global burden of diseases, within humans and ecological systems (Harvell *et al.*, 2002; Epstein, 2005; Patz *et al.*, 2005), there are still considerable knowledge gaps, especially with respect to empirical data (Rohr *et al.*, 2011). One such gap is to determine if there are broad effects of climate perturbations on parasite life-history traits; empirical data find both positive and negative effects on traits under warming scenarios (Lightner and Ulmer, 1974; Koprivnikar and Poulin, 2009; Paull and Johnson, 2011; Hernandez *et al.*, 2013).

Determining the effects of climate change on ecological systems often relies on long-term datasets recording phenological events, and distribution shifts (Gordon, 1948; Levine, 1963; Walther *et al.*, 2002). These datasets are rare, but when not available, quantitative comparative analyses can be used to reveal broad patterns of how biota respond to climate, for example, upwards shifting tree-lines (Harsch *et al.*, 2009), changes in Arctic plant community composition (Walker *et al.*, 2006) and reduced crop yields (Challinor *et al.*, 2014). Parasites, although intricately linked with their hosts, are sensitive to climatic fluctuations because many parasites' life cycles and transmission routes occur external to the host. Although not designed to test for the effect of climate change *per se*, multiple studies have examined the effects of changing temperatures on parasite life-history traits. Parasites typically have an optimum range at which they develop, and whilst increases in temperature beyond this optimum can reduce the abundance of some species (Koprivnikar and Poulin, 2009), it can increase in others (Lightner and Ulmer, 1974). Due to an expected trade-off between parasite abundance and fecundity (Stear *et al.*, 1995; Tompkins and Hudson, 1999; Richards and Lewis, 2001), the effect of temperature changes on these traits is unclear. For other life-history traits there appears to be similarity in the direction of parasite responses to climate. Within optimum temperature ranges, the development time of parasites is accelerated by

higher temperatures with, for example, eggs of the trematode *Ribeiroia ondatrae* hatching within 15 days at 26°C, compared to 73 days at 17°C (Paull and Johnson, 2011). Similarly with fecundity of two herbage-transmitted helminths of the European rabbit (*Oryctolagus cuniculus*), a greater proportion of eggs hatched following exposure to experimentally-induced higher temperatures (Hernandez *et al.*, 2013).

The heterogeneity in parasite responses could be borne from the physiological adaptations some parasite species have to survive extreme environmental conditions, within a generation. Nematode survival strategies, for example, can encompass a gradient of responses to adverse environmental conditions, from quiescence (a cessation of activity e.g. feeding, reproduction) to cryptobiosis (an absence of motility and metabolism, Demeure *et al.*, 1979; Wharton, 1986). These strategies can occur in response to temperature (e.g. Bertolani *et al.*, 2004), but can also be induced by water or oxygen deficiencies. In addition, the transmission mechanism of the parasite may provide a buffer from the environment; for example, *Syphacia obvelata*, common in wild rodents is suggested to be less sensitive to climatic fluctuations than other helminths on account of it being directly transmitted during grooming, where infective eggs are ingested from the fur around the anus (Froeschke *et al.*, 2010). Ectoparasites (ticks, fleas, lice, etc.) spend their entire lifecycle exposed to the ambient climate. One such ectoparasite, the tick *Ixodes ricinus,* has a geographical distribution that excludes it from above an altitude of approximately 1500 m, above which climatic conditions are not suitable for its survival (Daniel, 1993; Jouda *et al.*, 2004). This temperature-mediated distribution suggests that ectoparasites continuously exposed to the external environment as opposed to endoparasites, including helminths, with a free-living developmental stage may be expected to be greater affected by climatic variables (Lafferty, 2009b; Hernandez *et al.*, 2013). As such, a source of heterogeneity of parasite responses to climate may be broadly determined by parasite taxa.

Whilst the specific details of a parasite life cycle may allow for varying degrees of plasticity to environmental change, the temperature homeostasis of a host may also alter the propensity of a parasite to be sensitive to climate change (Lafferty, 2009b). Thus, the combination of host-parasite taxa is an important source of variation in the response to changing climate. Ectothermic hosts are reliant on external temperatures, whilst endotherms maintain a constant internal temperature and, therefore, may be particularly affected by any changes in climate because environmental conditions differentially influences the hosts physiological functions, such as the development rate, growth rate

and locomotion (Deutsch *et al.*, 2008; Jonsson and Jonsson, 2009). In vertebrates, the adaptive immune response can stop functioning altogether if the temperature drops below a critical temperature (Avtalion, 1969; Miller and Clem, 1984; Bly and Clem, 1992; Lillehaug *et al.*, 1993; Magnadóttir *et al.*, 1999; Watts *et al.*, 2001; Alcorn *et al.*, 2002; Magnadóttir, 2006). As a result, disease incidence can increase at low temperatures as antibody production is reduced or inhibited (Avtalion, 1969; Fryer *et al.*, 1976; Magnadóttir *et al.*, 1999; Alcorn *et al.*, 2002; Xu *et al.*, 2011). There is also evidence indicating that temperature increases above the optima can lead to suppressed immunity (Xu *et al.*, 2011), suggesting that any variation from an optimal temperature will result in hosts being more susceptible to parasites and disease.

To quantify broadly the effects of both increases and decreases in temperature on three aspects of parasite life-history traits: abundance, fecundity and development time the existing literature was analysed using a meta-analytical approach. In addition, we determined whether general patterns in life history traits were associated with certain host or parasite taxa.

2.3 Materials and methods

2.3.1 Meta-data compilation

A database of previously published studies was compiled to investigate the effect of climatic perturbations (experimentally-induced changes in temperature) on animal parasite life-history traits. Using the ISI Web of Knowledge, search terms consisted of combinations of the terms “parasite” and “pathogen”, plus “temperature” and “climate” for papers published between 1930 and February 2015. Publications focusing on parasitoids and parasites of plants were excluded from searches. Papers included in the dataset were those that provided a mean, standard deviation or errors, sample size and quantified the extent to which temperature was altered over the course of the study. A total of 220 studies were initially identified, of which 36 contained sufficient details to be included in the meta-analysis (see Appendix A.1, Table A.1.1). Several of the studies included multiple experiments, contributing a total of 443 data points to the meta-analysis.

Three parasite life-history traits were recorded; abundance, fecundity and development time. Abundance included the mean number of parasites per host, including hosts with

zero parasites (n = 141 data points from 10 studies). Fecundity was defined as a broad approximation of the reproductive output of the parasite, which included eggs counted from parasitic cultures *in utero* (n = 35 data points from seven studies), and/or the number of eggs that hatched from one life stage to the next (n = 23 data points from two studies). Studies that used faecal egg count data did not contain sufficient information on sample sizes or standard deviation/errors to be included within the analysis. Parasite development was included as the time taken to develop from one parasitic life stage to the next (n = 318 data points from 21 studies), of which 19 (n = 300) examined development time from one stage to next, but two studies (n = 18) quantified whole generation development time. There were deficient data available to record parasite survival under different temperature perturbations, as many papers did not record standard deviation/errors, and thus could not be included in the analysis. To investigate the effect of a temperature perturbation on parasites, a baseline or 'ambient' temperature was defined as the temperature at which the parasite was cultured or sampled, i.e. that is was adapted to (see Appendix A.1, Table A.1.1 for ambient temperatures for all parasites). If the parasite was a laboratory strain, the temperature of the laboratory stated in the publication was used as the ambient reference. For field strains, the ambient climate was quantified according to the regional data reported in the publication. For studies where a range of ambient temperatures was given, the mean was calculated.

In addition to the overall effects of temperature on parasites, we determined whether certain host or parasite taxa could explain the heterogeneity in the observed effect of temperature on parasites by broadly classifying host taxa into: amphibians, fish, invertebrates, reptiles or mammals and parasite taxa into arthropods, nematodes and platyhelminths. When considering host and parasite taxa, only samples sizes greater than 5 were considered for robust data interpretation.

2.3.2 Meta-analysis

The effect size; the relative change in parasite life-history traits between the experimental group relative to the control group was measured using the response ratio, R, reported here as the natural logarithm of R ($\ln R$). The logarithm linearizes the metric such that deviations in the numerator and denominator of the response ratio are treated in the same way, normalising the data, thus providing a standardised effect size

allowing cross-comparison (Hedges *et al.*, 1999; Rosenburg *et al.*, 2000; Harrison, 2011).

The mean effect sizes (ln*R*, ± bootstrapped 95% confidence intervals, CIs) of parasite abundance, fecundity and development time were calculated and compared to identify the effect of a change in temperature on parasitic life-history traits using MetaWin 2.1 (Rosenburg *et al.*, 2000), with sample size used as a weighting function. If CIs did not overlap zero, the temperature perturbation was determined to have a significant effect on parasite performance (Adams *et al.*, 1997). A random effects model was chosen over a fixed-effects model when calculating effect sizes, because the latter assumes that all observed variation is due to sampling error (Maestre *et al.*, 2005). In the current meta-analysis, however, a broad range of studies were used, which included multiple parasite and host species and so a random effects model was deemed the most appropriate to deal with the variation resulting from using data derived from multiple studies (Rosenburg *et al.*, 2000). A negative effect size for parasite abundance and fecundity indicated a reduction in these variables, whilst a positive effect size indicated an increase. Alternatively, when investigating parasite development time, a negative effect size indicated faster parasite development time, whereas a positive effect size indicated slower development time. To assess whether there was a bias in the publication of articles showing one type of result, publication bias was tested for, using Kendall's Tau and Spearman's rank correlation tests which are included within the MetaWin programme (Begg and Mazumdar, 1994; Sokal and Rohlf, 1995; Rosenburg *et al.*, 2000).

2.4 Results

To investigate the effect of temperature perturbations on parasite abundance, fecundity and development time, 443 data-points were collated from 36 publications, some of which contained results on more than one trait (for details see Appendix A.1, Table A.1.2). As such, 11 studies investigated parasite abundance; nine investigated parasite fecundity; and 18 examined parasite development time.

Table 2.1 Meta-analysis of published studies reporting temperature perturbation effects on parasite life-history traits. A positive ln*R* indicated abundance and fecundity increased and a negative ln*R* indicated both traits reduced, whereas a positive ln*R* indicated slower development and a negative ln*R* suggested faster development. Life-history traits were significantly affected by a temperature perturbation where bootstrapped 95% CIs did not overlap zero (indicated with an asterisk). The degrees of freedom (df) are also given.

Parasite life-history trait	Temperature change	df	ln*R*	Bootstrapped CI
Abundance	> 10.1°C	2	0.615	1.544 to -0.660
	5.1 to 10°C	8	-1.202	-2.809 to 0.315
	0.1 to 5°C	26	0.762	1.103 to 0.399 *
	Average increase	37	0.207	0.766 to -0.414
	Overall average	66	0.994	1.686 to 0.313 *
	Average decrease	28	2.146	3.437 to 0.814 *
	-0.1 to -5°C	6	1.107	2.018 to 0.303 *
	-5.1 to -10°C	9	3.564	5.166 to 1.851 *
	< -10.1°C	10	1.409	4.543 to -1.965
Fecundity	> 10.1°C	10	-1.695	-0.259 to -3.266 *
	5.1 to 10°C	16	-0.049	0.101 to -0.218
	0.1 to 5°C	22	-0.091	0.451 to -0.672
	Average increase	48	-0.402	-0.607 to -0.765 *
	Overall average	71	-0.343	-0.676 to 0.065
	Average decrease	22	-0.160	0.207 to -0.783
	-0.1 to -5°C	8	0.516	0.615 to 0.416 *
	-5.1 to -10°C	9	0.542	0.615 to -0.619
	< -10.1°C	5	0.096	0.202 to -0.740
Development time	> 16.1°C	24	-1.670	-1.368 to -2.030 *
	14.1 to 16.0°C	20	-1.532	-1.263 to -1.812 *
	12.1 to 14.0 °C	4	-0.811	-0.583 to -1.086 *
	10.1 to 12.0°C	18	-1.782	-1.350 to -2.389 *
	8.1 to 10.0°C	30	-1.747	-1.454 to -2.381 *
	6.1 to 8.0°C	12	-0.966	-0.449 to -1.833 *
	4.1 to 6.0°C	33	-0.623	-0.396 to -0.860 *
	2.1 to 4.0°C	12	-0.281	-0.175 to -0.386 *
	0.1 to 2.0°C	5	-0.128	-0.046 to -0.210 *
	Average increase	162	-1.231	-1.075 to -1.404 *

Table 2.1 (continued)

Parasite life-history trait	Temperature change	df	ln*R*	Bootstrapped CI
Development time	Overall average	235	-0.915	-0.727 to -1.118 *
	Average decrease	76	-0.125	0.230 to -0.525
	-2.1 to -4.0°C	18	-0.222	0.132 to -0.583
	-4.1 to -6.0°C	14	0.261	0.673 to -0.163
	-6.1 to -8.0°C	11	0.094	0.556 to -0.337
	-8.1 to -10.0°C	12	0.384	1.091 to -0.558
	-10.1 to -12.0°C	4	-1.684	1.325 to -5.184
	-12.1 to -14.0°C	4	-1.803	1.118 to -4.816
	-14.1 to -16.0°C	7	-1.702	0.724 to -3.927
	< -16.1°C	4	-0.993	2.902 to -5.385

2.4.1 Parasite abundance

Across the studies analysed, temperature was perturbed, on average, by (mean ± S.E.) 5.2°C ± 1.05°C above ambient (range = 0.1°C to 20°C) and by 8.81°C ± 1.17°C below ambient (range = -2°C to -20°C). Temperature increases were examined in five degree increments; parasite abundance significantly increased with temperatures up to and including 5°C above ambient temperature, but no significant change in effect size was found beyond this, although sample sizes were small (Fig. 2.1, Table 2.1). On average, decreased temperatures significantly increased parasite abundance by 79.63 ± 53.66 parasites/host (range: 13.8 to 814.6 parasites/host). At five degrees increments there was heterogeneity across the observed temperature range (-0°C to -20°C), whereby increased abundance in parasites was observed when temperatures were reduced to -10°C, but further temperature reductions had no significant effect (Fig. 2.1, Table 2.1). No publication bias was detected.

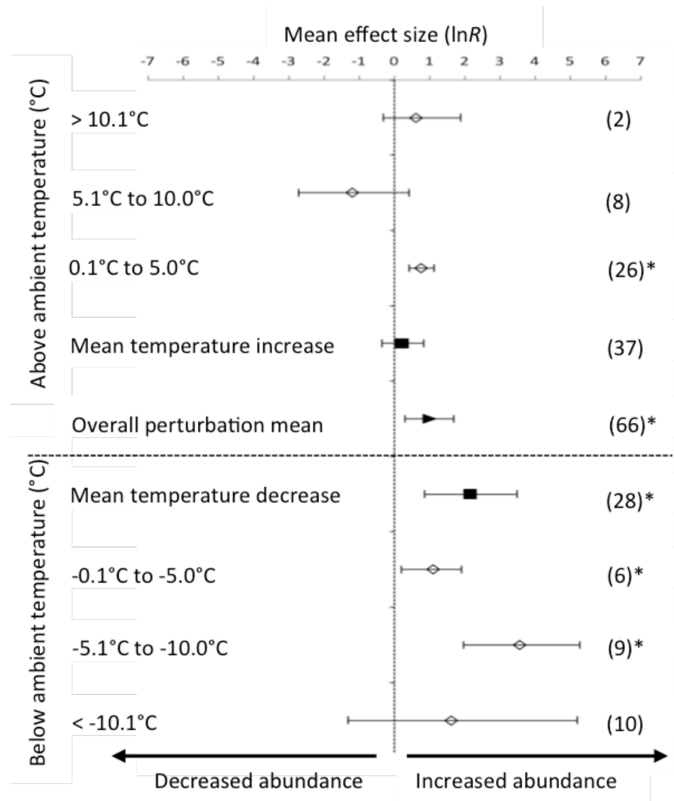

Figure 2.1 Meta-analysis of published studies reporting temperature perturbation impacts on parasite abundance. Effect sizes (± bootstrapped 95% CI) calculated from mean abundance of parasites after an increase or decrease in the ambient temperature that the parasite was adapted to. A negative effect size indicates a reduction in parasite abundance and a positive effect size an increase in abundance. Effect sizes were significantly different from an ambient climate where CIs do not overlap zero (indicated with an asterisk). The average effect size of an increase and decrease in a temperature perturbation (square symbol), and the overall effect of a temperature perturbation (triangle symbol), are given. The number of observations is indicated in parentheses for each temperature increment.

2.4.2 Parasite fecundity

Temperature was perturbed on average by 5.0°C ± 4.47 (range = 2° to 20°C) above and 9.0°C ± 4.46°C (range = -3°C to -16°C) below ambient. Elevated temperatures significantly reduced parasite fecundity by 14.42 ± 36.73 eggs/host (Fig. 2.2; Table 2.1), although this pattern was driven only by temperatures greater than 10.1°C above ambient (Fig. 2.2). Parasite fecundity was also reduced by decreases in temperature, but only to -5°C below ambient; further reductions in temperature had no significant effect (Fig. 2.2; Table 2.2). Publication bias was found, suggesting that publications finding a negative effect of temperature perturbations on parasite fecundity had a greater influence than those finding a positive effect (Kendall's Tau: Tau = -0.317; Z = -3.846; $p < 0.001$; Spearman rank-order correlation: Rs = -0.217; $p = 0.033$).

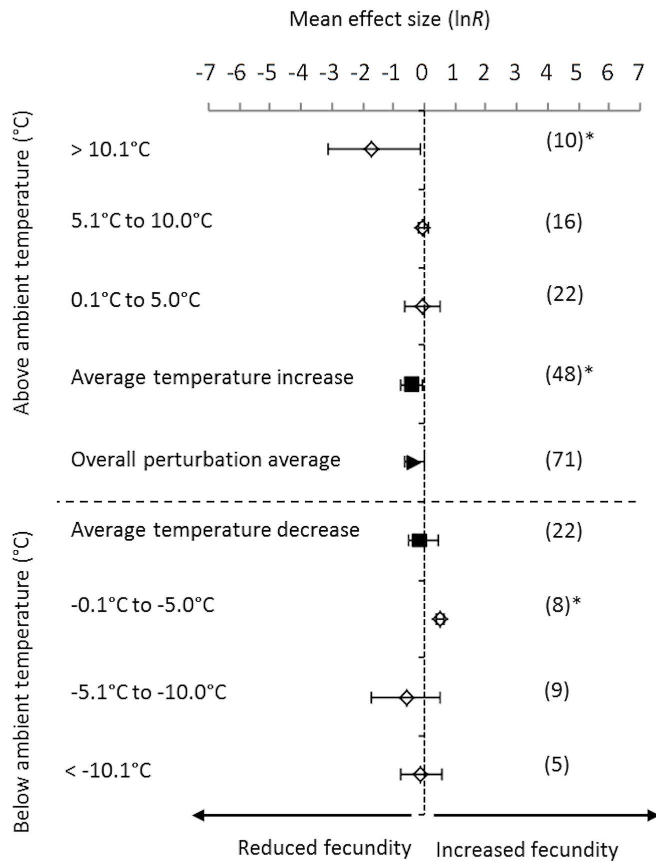

Figure 2.2 Meta-analysis of published studies reporting temperature perturbation impacts on parasite fecundity. Effect sizes (± bootstrapped 95% CI) calculated as the mean number of eggs after increases or decreases in the ambient temperature that the parasite was adapted to. A negative effect size indicates a reduction in parasite fecundity and a positive effect size indicates fecundity increased. Effect sizes were significantly different from an ambient climate where CIs do not overlap zero (indicated with an asterisk). The average effect size of an increase and decrease in a temperature perturbation (square symbol), and the overall effect of a temperature perturbation (triangle symbol), are given. The number of observations is indicated in parentheses for each temperature increment.

2.4.3 Parasite development time

Temperature was perturbed on average by 10.79°C ± 0.50 above (range: 2°C to 27.5°C) and by 6.87°C ± 0.75 below the parasite's ambient temperature (range: -2°C to -30°C). Temperature increases resulted in significantly faster parasite development times by an average of 12.36 ± 1.51 days (range = -102.9 to 42.8 days; Fig. 2.3; Table 2.1) whilst temperature decreases had, on average, no effect (Fig. 3; Table 1). Due to the comparatively large amount of data available for parasite development, 2°C increments were examined. Initial temperature increases up to 2.0°C above ambient, did not significantly affect parasite development time, but above this threshold temperature, development time increased, but only up to 10°C where the effect size reached an asymptote, and additional temperature increases did not lead to any further changes in development time (Fig. 2.3). Reduced temperatures had no significant effect on parasite

development times, but a large degree of variation was present above 10°C, and sample sizes were small (Fig. 2.3). No publication bias was detected.

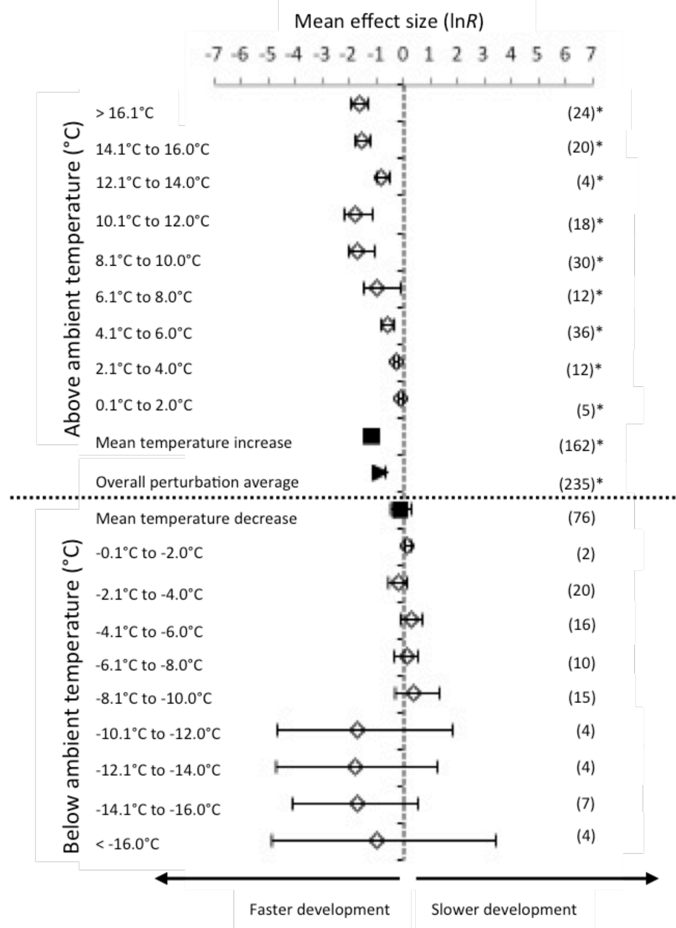

Figure 2.3 Meta-analysis of published studies reporting temperature perturbation impacts on parasite development time. Effect sizes (ln*R*, ± bootstrapped CI) were calculated from a change in parasite development time when temperatures were perturbed from an ambient temperature that the parasite was adapted to. A negative effect size indicates a faster parasite development time and a positive effect size a slower development time. Overlapping CI's denote statistical significance (indicated with an asterisk). The average effect size of an increase and decrease in a temperature perturbation (square symbol), and the overall effect of a temperature perturbation (triangle symbol) are shown. The number of observations is indicated in parentheses for each temperature increment.

2.3.5 Host-parasite taxa and parasite abundance, fecundity and development time

Hosts were divided into broad taxonomic groups of amphibians, fish, invertebrates, reptiles and mammals, and parasite taxa into arthropods, nematodes and platyhelminths. The effect of increased and decreased temperatures on each of the three life-history traits in relation to host and parasite taxa are presented in full in Table 2.2. Not all taxa

could be investigated at increased and reduced temperatures, due to a lack of empirical data or small samples sizes (see Table 2.2 for details).

The abundance of parasites infecting mammals (endotherms) reduced whether temperatures were increased or decreased, though it should be noted that only temperature increases were significant (Table 2.2). The abundance of parasites infecting fish and invertebrates (ectotherms) increased under either type of temperature perturbation, although again results were not always significant (Table 2.2). The abundance of nematodes and platyhelminths also increased, whether they were exposed to warmer or cooler temperatures (Table 2.2).

Increases in temperature always reduced fecundity and accelerated development time for each host and parasite taxa (Table 2.2). Decreases in temperature increased fecundity of parasites of all host and parasite taxa, and, development was slowed down for the majority of taxa. The one exception was the parasites of arthropods that accelerated their development when exposed to cooler temperatures (Table 2.2).

Table 2.2 Meta-analysis of published studies reporting the responses of parasites to temperature increases or decreases according to host and parasite taxa. A positive ln*R* (effect size) indicates the parasite trait increased (black shading) and a negative ln*R* indicates the trait reduced (grey shading). Significance occurs where CIs do not overlap zero (denoted by an asterisk). The degrees of freedom (d.f.) are also given in parentheses.

Parasite and host type/taxa	Temp.	Abundance ln*R* (df) and CI	Fecundity ln*R* (df) and CI	Development time ln*R* (df) and CI
Amphibians	Increase	NA	NA	-1.406 (12) 2.146 to -0.885
	Decrease	NA	NA	NA
Fish	Increase	1.066 (18) 0.719 to 1.404*	-0.449 (7) -0.823 to -0.106	-0.230 (6) -0.383 to -0.085*
	Decrease	NA	NA	NA
Invertebrates	Increase	0.229 (10) -0.537 to 1.182	NA	NA
	Decrease	2.950 (22) 1.481 to 4.300*	NA	NA
Reptiles	Increase	NA	-0.251 (13) -1.016 to 0.275	NA
	Decrease	NA	NA	0.061 (5) -1.644 to 1.088
Mammals	Increase	-1.979 (5) -3.769 to -0.276*	-0.534 (20) -1.193 to -0.003*	-1.313 (137) -1.515 to -1.138*
	Decrease	-0.900 (5) -3.354 to 0.409	0.133 (11) -0.240 to 0.341	-0.431 (67) -0.972 to 0.081
Arthropods	Increase	NA	-0.476 (30) -1.058 to -0.026*	-1.452 (99) -1.712 to -1.217*
	Decrease	NA	NA	-0.661 (51) -1.294 to -0.052*
Nematodes	Increase	NA	NA	-1.046 (47) -1.316 to -0.809*
	Decrease	3.828 (19) 2.687 to 4.988*	0.133 (11) -0.261 to 0.331	0.330 (24) -0.153 to 0.817
Platyhelminths	Increase	0.635 (29) 0.181 to 1.090*	-0.569 (6) -0.912 to -0.290*	-0.532 (13) -0.747 to -0.328*
	Decrease	NA	NA	0.535 (7) 0.299 to 0.771*

2.5 Discussion

This meta-analysis provided broad insight into the effect of temperature on parasite abundance, fecundity and development time, but these traits, of course, are not independent. Increases in parasite abundance likely reduced the fecundity of parasites in response to intraspecific competition limiting the production of eggs (Stear *et al.*, 1995; Tompkins and Hudson, 1999; Richards and Lewis, 2001; Chylinski *et al.*, 2009; Luong *et al.*, 2010). Warmer temperatures were shown to increase development times, but this

occurred only up to a threshold, as excessive temperatures have been shown to affect parasite survival detrimentally (Heath, 1979; Gibson, 1981; Salih and Grainger, 1982; Boag and Thomas, 1985; Rueda *et al.*, 1990; Bayoh and Lindsay, 2004; Ogden *et al.*, 2004; Hudson *et al.*, 2006; van Dijk and Morgan, 2008; Azam *et al.*, 2012). Increases in parasite abundance were associated with ectothermic hosts, which may be due to increased susceptibility compared to endotherms as a function of invertebrate immunity (Seppälä and Jokela, 2011). Indeed, invertebrates become infected with parasites more quickly when exposed to higher temperatures (Studer *et al.*, 2010), as well as display higher levels of disease when temperatures exceed or are lower than the optimum temperature, due to inefficacy of the innate immune system at these temperature extremes (Avtalion, 1969; Fryer *et al.*, 1976; Magnadóttir *et al.*, 1999; Xu *et al.*, 2011).

Climate-induced increases in parasite loads and accelerated parasite development times, may result in a greater number of parasites being produced per season (Aho *et al.*, 1982), which may have serious consequences for host health. An increased abundance of the parasitic fungus *Batrachochytrium dendrobatidis* has been correlated to warmer temperatures resulting in mass amphibian mortality (Pounds *et al.*, 2006). Parasite-induced extinction, due to climate-mediated increases in parasite loads is, however, an unlikely scenario because theoretically, significant numbers of host mortalities should reduce the population size to below the critical community size required for parasite persistence (de Castro and Bolker, 2005). The effects of parasites instead tend to be sub-lethal, for example, reductions in host fecundity (Hudson *et al.*, 1992a). As such, long term monitoring of the life history traits of both hosts and parasites are required to provide insight into the changing dynamics of parasites on their host populations.

The range of temperature increases and decreases that were employed by some of the studies included in this meta-analysis extend well beyond the average temperature increases that have been predicted (1.1-4.8°C, IPCC, 2013). Extreme temperature increases (more than 10°C above ambient) had no effect on abundance, yet increased fecundity and accelerated parasite development time. Conversely, extreme temperature reductions (less than 10°C below ambient) had no significant effect on any of the parasite life-history traits. An increase in extreme climatic events has been predicted, which include very low or high variations in local temperature (Easterling *et al.*, 2000; Karl and Trenberth, 2003), as well as more frequent heat wave events (Kunkel *et al.*, 1999; Easterling *et al.*, 2000). There is a hemispheric asymmetry in temperature warming that undoubtedly will contribute to heterogeneity in ecological dynamics

across systems; warming is greater at northern latitudes, and temperatures in the arctic are increasing at twice the global average rate compared with other regions (Kutz *et al.*, 2009). As such, host-parasite interactions in these key biomes are expected to show greater response to climate than temperate regions.

Out of 36 studies included in this meta-analysis, only one investigated temperature effects on parasites in the field, and five used parasites collected from wild hosts, as oppose to laboratory strains. As such, there was insufficient data available for any of the life-history traits to examine whether there was a significant difference between wild and laboratory strains. Wild parasite strains are adapted to stochastic environmental conditions and host availability (Crofton *et al.*, 1965; van Dijk and Morgan, 2012), whereas laboratory parasites are not exposed to the same selection pressures. The response of wild parasite strains to temperature perturbations, therefore, may be different compared with laboratory strains (Herrmann and Gern, 2010). Indeed it may only be from field observations that key responses of parasites to climate changes may be observed, for example increased biting and infection rates of mosquitoes at elevated temperatures (Leeson, 1939; Dohm *et al.*, 2002; Soverow *et al.*, 2009; Paaijmans *et al.*, 2010). The majority of the studies used in this meta-analysis investigated a constant change in temperature (n = 31); rarely are diurnal or seasonal weather fluctuations typical of free-living systems considered. Stochastic temperatures may result in faster parasite development (Saunders *et al.*, 2002), and it has been suggested that experiments and models based on constant temperatures over-estimate the risk of parasitic disease in warm environments, and under-estimate it in cooler temperatures (Paaijmans *et al.*, 2009). The studies included in the meta-analysis focused on just one climatic variable – temperature. Yet in the natural environment, parasites are affected by a range of climate-related variables including, humidity, precipitation, frost cover and degree-days (Allen and Ingram, 2002; Kutz *et al.*, 2005; Dore, 2005; Jylhä *et al.*, 2008).

Likely related to low sample sizes, we found a publication bias in some of our analyses; the effect of a temperature perturbation on parasite fecundity. Bias can arise in meta-analyses for a number of reasons: for example, researchers/journal editors unwilling to publish negative results or sample sizes being too small, which result in low statistical power (Cohen, 1988; Thornton and Lee, 2000; Jennions and Møller, 2002), or they are simply understudied. Publication bias is a widespread problem throughout the scientific literature (Thornton and Lee, 2000), and the results of this meta-analysis where bias was detected should be cautiously interpreted.

To summarise, temperature increases in line with those predicted by the IPCC resulted in greater parasite abundance and accelerated development time, yet had no effect on fecundity, whilst decreases in temperature increased abundance but did not affect fecundity or development time. The broad patterns observed suggests predictable patterns of parasites in response to climate warming, which could be used to inform disease management strategies. There was, however, a paucity of field experiments investigating the response of parasites to a range of stochastic climatic conditions, such as temperature and humidity (but see Gordon, 1948), and to understand how these factors alter parasite life history traits is an important area for future research.

2.6 Author acknowledgements

The manuscript resulting from this chapter is authored by:

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Chapter 3

The effect of climate, host and parasite factors on parasite fecundity

3.1 Abstract

A changing global climate affects both parasite and host life-history traits. Some parasites have shifted their distributions northwards or to higher altitudes in response to warmer temperatures, while others display altered development. One key trait, parasite fecundity may be influenced by climate, but also by host factors, such as sex, body mass and breeding condition, and parasite inter- and intraspecific competition. Fecundity may also be affected by the sex ratio of male to female parasites, and sex ratios are also known to be affected by host biology and parasite infection. Using a common nematode (*Heligmosomoides polygyrus*) infecting wild yellow-necked mice (*Apodemus flavicollis*) as a model system, this study assessed how fecundity may be affected by all three drivers (climate, host and parasite) under natural conditions. Across three mountains in the Italian Alps, altitude was used as an *in situ* proxy measure for climate, with low altitude sites (500 m) significantly warmer than high altitude sites (1400 m). A multi-model averaging approach revealed that parasite fecundity (measured as the number of eggs per mm length) was lowest in female hosts at low altitude compared to males at low altitude, and either sex at high altitude. The fecundity-altitude interaction in females was not explained by parasite inter- or intraspecific competition. Instead, it is posited that this pattern may be driven by the sex ratio of *H. polygyrus*, as female hosts at low altitude were infected with a male-biased parasite infrapopulation. With future climate change, parasite fecundity may be indirectly affected by host biology and directly by parasite sex ratios.

3.2 Introduction

Climate-induced changes in parasite life-history traits are evident in both aquatic and terrestrial ecosystems (Marcogliese, 2001; Harvell *et al.*, 2002; Brooks and Hoberg, 2007). Warmer temperatures have increased the prevalence of some parasites (van Riper III *et al.*, 1986; Jouda *et al.*, 2004; Freed *et al.*, 2005; Laaksonen *et al.*, 2010; Zamora-Vilchis *et al.*, 2012), or lengthened the time period when the climate is optimal for development to occur (Halvorsen and Skorping, 1982), whilst developmental rates of some Arctic nematodes have accelerated (Kutz *et al.*, 2005). Perturbation experiments, which increased temperatures, have tended to reduce fecundity in a range of parasite taxa (Chapter 2); however, all of the studies from this meta-analysis were conducted on laboratory parasite strains exposed to constant temperatures. As parasites

may respond differently to constant compared with fluctuating temperatures (Saunders *et al.*, 2002; Hernandez *et al.*, 2013), it is important to examine the effect of climate on parasite fecundity under natural conditions. Parasite fecundity is an important life-history trait, because it can be a proxy for host infectiousness, and hosts that harbour highly fecund parasites have the potential to shed the greatest number of infective stages. A high number of infective stages can lead to an increase in the number of secondary infectious cases, the basic reproductive number (R_0), resulting in increased infection rates in the population (Woolhouse *et al.*, 1991, 1997; Smith *et al.*, 2007; Luong *et al.*, 2010). Any variation in parasite fecundity, due to biotic or abiotic factors, therefore has the potential to change R_0.

Biotic effects, such as host factors, also play a role in affecting parasite fecundity (Ferrari *et al.*, 2004; Luong *et al.*, 2009; Corlatti *et al.*, 2012). Smaller hosts tend to support more fecund parasites (Luong *et al.*, 2010), and host sex and breeding condition can significantly affect parasite fecundity. For instance, mammalian male hosts in breeding condition typically shed more eggs than other individuals in the population (Ferrari *et al.*, 2004; Luong *et al.*, 2009; Corlatti *et al.*, 2012). When sex-specific parasite burdens were experimentally manipulated anthelminthics administered to adult male mice significantly reduced infection levels in untreated females, but not vice-versa, suggesting that male mice support high helminth fecundity (Ferrari *et al.*, 2004).

Simultaneous infection of hosts with multiple parasite species, termed coinfection, has also been associated with changes in parasite fecundity (Matthews *et al.*, 2006b; Cattadori *et al.*, 2007; Chylinski *et al.*, 2009; Lass *et al.*, 2013). Coinfecting parasites may directly compete or interact indirectly via host immunity and resources, which can yield antagonistic or synergistic effects on the abundance and prevalence of parasites (Cox, 2001; Lello *et al.*, 2004; Graham, 2008). Coinfection has also been shown to alter parasite fecundity such that it may produce ‘super-shedders’ (individuals that produce more infective stages than average; Lass *et al.*, 2013). Alternatively, intraspecific parasite competition may be the driving mechanism affecting parasite fecundity, rather than and also in addition to coinfection (Stear *et al.*, 1995; Tompkins and Hudson, 1999; Richards and Lewis, 2001; Chylinski *et al.*, 2009; Luong *et al.*, 2010). Observational data support the hypothesis that intraspecific competition may affect fecundity, as hosts with high intraspecific parasite burdens harbour shorter and less fecund worms than hosts with low infection loads (Stear *et al.*, 1995; Tompkins and Hudson, 1999; Richards and Lewis, 2001).

For dioecious parasites, fecundity may be affected by the sex ratio of male to female parasites. The sex ratio of nematodes infecting vertebrates can often be biased towards females, as many species demonstrate an excess of females (Seidenberg *et al.*, 1973; Haukisalmi *et al.*, 1996; Craig *et al.*, 2010; D'Ávila *et al.*, 2012), which may be attributed to male nematodes dying earlier than females (Waller and Thomas, 1978; Poulin, 1997; Stien *et al.*, 2005). Host factors, such as age and body mass, have been suggested as a mechanism affecting sex ratios, and ratios become more female-biased in older and heavier individuals, which again may be related to female nematodes surviving longer than males (Roche and Patrzec, 1966; Seidenberg *et al.*, 1973; Shimura, 1983; Craig *et al.*, 2010). Intensity of infection is also considered to be a major driver affecting sex ratios, with female-biased ratios typical when infection intensity is low, and male-biased ratios common at high infection intensity (Poulin, 1997), although a negative correlation between male-biased ratios and parasite intensity has been reported (Craig *et al.*, 2010). Additionally, the ambient temperature at which parasites develop can also affect these ratios, with a decrease in typical ambient temperatures leading to female-bias and increase in temperatures causing the ratios to become more equal (Tingley and Anderson, 1986).

The effects of climate, host factors and parasite coinfection on parasite fecundity have been previously investigated as individual variables within laboratory host-parasite systems. To the best of our knowledge, the potential interaction between climate, host and parasite factors on parasite fecundity in natural systems has not been investigated, despite the potential for all three to interact. This study assessed whether the fecundity and sex ratio of a common dioecious nematode (*Heligmosomoides polygyrus)* infecting wild yellow-necked mice (*Apodemus flavicollis*) was affected by climate, host and/or parasitic factors. Three specific hypotheses were tested: (1) there would be no effect of climate changes on parasite fecundity; (2) host factors (host sex, body mass and breeding condition) would significantly increase parasite fecundity; (3) parasitic factors (intra- and inter-specific competition and sex ratio) would significantly increase parasite fecundity.

3.3 Materials and Methods

3.3.1 Field Sampling

Live-trapping of yellow-necked mice (*Apodemus flavicollis*) was conducted between May and August 2012 on three geographically distinct mountains in the Italian Dolomitic Alps, Trentino; at Monte Bondone (46°2'13.38N, 11°0'12.64E), Panarotta (46°0'58.72N, 11°18'46.70E) and Monte Baldo (45°51'52.93N, 10°53'33.21E). On each mountain, trapping grids were selected at low (500 m) and high (1400 m) altitude, with three replicated transects per altitude. Each transect was separated by at least 300 m, representing non-overlapping ranges for the mice, and thereby ensuring sampling of independent populations. Trapping transects were all located in similar habitat; broadleaf woodlands with a west-facing aspect. At each of the 18 transects, 20 Ugglan multi-capture live traps (Grahnab, Sweden) were set 10 m apart for a total of 16 days per mountain, amounting to a total of 5760 trap nights (defined as the total number of traps set multiplied by the number of nights that traps were set). Each trap was baited with a standardized amount of seed and a slice of potato as a source of moisture. Trapping was conducted on Monte Bondone between May and June, on Panarotta in July, and on Monte Baldo in August. For each mouse captured, the sex, body mass and breeding condition was recorded. Males with descended testes and females with perforated vaginas were classed as adults; those with non-descended testes and imperforate vaginas were classed as sub-adults. Juveniles were individuals that were in grey pelage and under 15g body mass; they were not included in this study due to their low age-related exposure to parasites. Host density did not differ between transects. Individuals (n = 257) were euthanized *in situ* using isofluorane followed by cervical dislocation. Gastrointestinal tracts were extracted by dissection and stored in 90% ethanol. All animal-handling procedures and ethical issues were approved by the Provincial Wildlife Management Committee (authorization n. 595 issued on 04.05.2011). Approval to trap animals was granted by the Comitato Faunistico of the Autonomous Province of Trento.

Temperature-humidity data recorders (iButton®, Maxim Integrated Products, USA) were placed at the trapping sites; one at each of the 18 transects across the three mountains. iButtons were secured to a tree facing northwards, approximately 20 cm from the ground in the middle of each transect, and recorded hourly for the duration of the experiment.

3.3.2 Quantifying nematode abundance and fecundity

Nematodes recovered during dissection of the gastrointestinal tract were removed, examined under a stereo-microscope with fibre optic illumination and identified. *Heligmosomoides polygyrus* was selected as the focal nematode to quantify fecundity as its eggs are clearly visible within females, and preliminary work established it to be one of the most common nematodes infecting rodents. From the 147 rodents caught that were infected with *H. polygyrus,* each individual female (n = 1291) and male (n = 820) *H. polygyrus* was mounted with a coverslip onto a microscope slide. Nematodes were photographed using a colour video camera (JVC^{TM} TK-C1380), and the images were used to measure the length of each nematode in millimetres to two decimal places using ImageJ (Schneider *et al.*, 2012). Females were measured from the anterior to posterior tip and males were measured from the anterior tip to the tip of the longest finger of the copulatory bursa. Eggs *in utero* of female nematodes were visually counted using a stereo-microscope under x10 magnification and fibre-optic illumination. Two counts of eggs performed on a subset of nematodes (n = 30) revealed the method was reproducible to 1.35 ± 0.55. The number of eggs *in utero* was utilized as an approximation of parasite fecundity (Tompkins and Hudson, 1999; Irvine *et al.*, 2001; Luong *et al.*, 2010; Grear *et al.*, 2012; Sherrard-Smith *et al.*, 2015). This approach has previously been used as a proxy for fecundity when data on the number of eggs shed in host faeces are not available (Richards and Lewis, 2001; Sherrard-Smith *et al.*, 2015).

3.3.3 Climate data

The temperature and relative humidity data were averaged for 18 sites at low (500 m) and high (1400 m) altitude, and generalised linear models (GLM) were used to assess the climatic differences between these two altitudes. Temperature was significantly warmer at low (mean = 19.67 ± 0.44°C) compared to high altitude (mean = 15.99 ± 0.14°C; $F_{1, 84} = 28.30$, $p < 0.001$; Fig. 1). Furthermore, low altitude was significantly less humid (mean = 77.04 ± 0.60%) than high altitude (mean = 80.90 ± 0.50%; $F_{1, 84} = 30.81$, $p < 0.001$; Fig. 1). To summarize, low altitude was 3.68°C ± 0.30 warmer and 3.86% ± 0.11 less humid than high altitude. By 2100, global surface temperatures are predicted to increase by 1.1-4.8°C (IPCC, 2013), making the difference in climate

recorded between our high and low field sites within the range of predicted temperature increase, and the temperatures at low altitude representative of a future climate, when compared to high altitude. As such, the term altitude was used in subsequent analyses as a proxy for climate change.

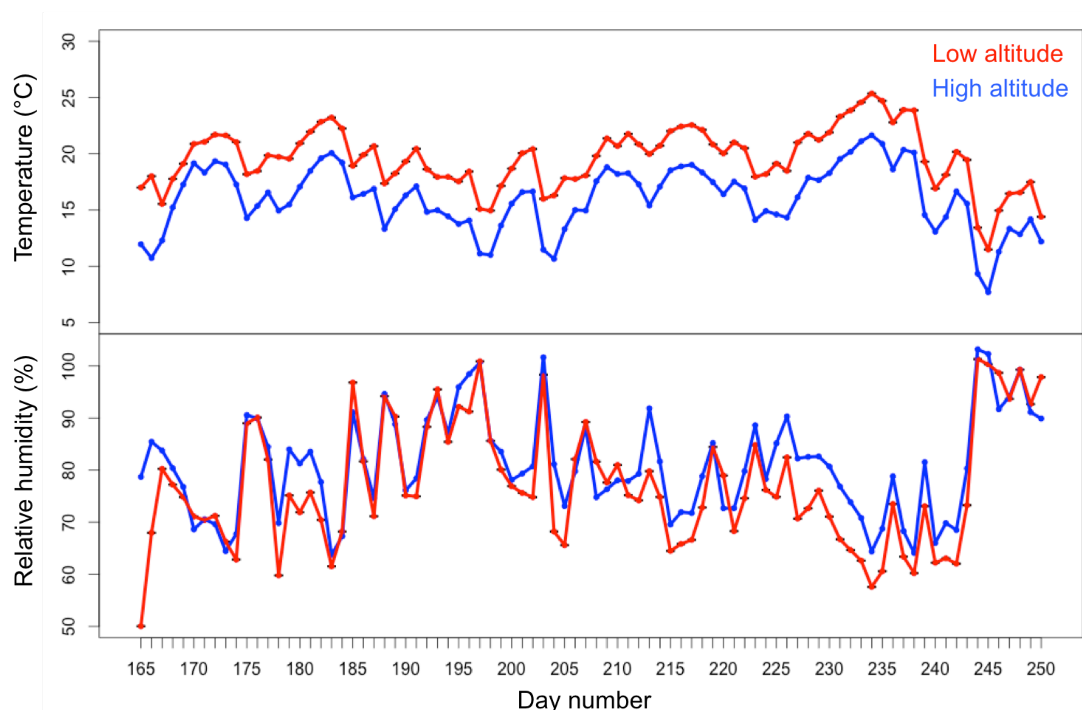

Figure 3.1 Temperature (top graph) and relative humidity (bottom graph) between low (500 m; red) and high (1400 m; blue) altitude in the Italian Dolomitic Alps measured between 165 and 250 Julian days

3.3.4 Statistical analysis

All statistical analyses were carried out in R, version 3.1.1 (R Core Development Team, 2013). To determine how climate, host and parasite factors may affect *in utero* parasite fecundity, a global model was constructed based on the fecundity per unit length (mm) of female *H. polygyrus* as the response variable, fitted with a Gamma distribution and a log function using the “lme4” function in R. Fecundity per mm length was used to prevent bias due to the positive relationship observed previously between nematode size and fecundity (Stear *et al.*, 1995), and will from hereon be referred to as fecundity. The global model included: altitude (used to represent the change in climate), host sex, host body mass, host breeding condition (sub-adult or adult), the sum total of coinfecting macroparasites as a measure of interspecific pressure, the intensity (excluding zero, see Bush *et al.*, 1997) of female and male *H. polygyrus* as a measure of intraspecific

competition and the sex ratio of *H. polygyrus.* The sex ratio was calculated by dividing the number of males by the number of females. Two-way interactions were also included in the model. As fecundity counts from multiple worms within the same host were used, the unique identification code of each host was also included as a random effect within the model to avoid pseudoreplication. The location of the sample site and mountain where sampling took place were included as random factors within the model. The model variables were standardized using the arm library in R (Gelman and Su, 2013) and the dredge function in the MuMIn package was used to create a set of models. The most important explanatory variables were determined using model averaging of the top models with delta AIC < 2.5 (Grueber *et al.*, 2011; Bartón, 2013). The relative importance of each explanatory variable was calculated by summing the Akaike weights for each variable across each of the models in which the variable occurs (Burnham and Anderson, 2002). Explanatory variables were considered important predictors of fecundity if the 95% confidence intervals did not overlap zero, and the greater the difference in non-overlapping confidence intervals, the more important the predictor.

3.4 Results

In total, 147 mice were infected with 2125 *Heligmosomoides polygyrus* individuals, with a mean intensity (± S.E.) of 10.54 ± 1.42 female (n=1303) and 6.46 ± 0.98 male (n=807) worms. Hosts were coinfected with up to five endoparasite species: *Rictularia proni*, *Mastophorus muris*, *Trichuris muris*, *Syphacia* spp. and the cestode *Rodentolepis straminea* (Table 3.1), although not every host was coinfected.

Table 3.1. Summary of the prevalence, mean intensity (excluding zeros) and mean abundance (including zeros, ± S.E.) of infection of five species of endoparasites infecting wild yellow-necked mice (*Apodemus flavicollis*) that were also infected with the nematode *Heligmosomoides polygyrus* at low (500 m) and high (1400 m) altitude.

	Prevalence (%)	Intensity ± S.E.	Abundance ± S.E.
Low altitude (n=66)			
Heligmosomoides polygyrus	100	18.41 ± 2.68	18.41 ± 2.68
Rodentolepis straminea	14.67	6.27 ± 1.28	0.92 ± 0.32
Syphacia spp.	61.33	76.63 ± 29.26	47.00 ± 18.39
Mastophorus muris	3.11	1.00 ± 0.00	0.03 ± 0.02
Rictularia proni	1.33	1.00 ± 0.00	0.01 ± 0.01
Trichuris muris	12.00	2.33 ± 0.99	0.28 ± 0.14
High altitude (n=48)			
Heligmosomoides polygyrus	100	12.73 ± 3.24	12.73 ± 3.24
Rodentolepis straminea	22.39	4.60 ± 1.01	1.03 ± 0.32
Syphacia spp.	71.64	45.38 ± 10.91	32.52 ± 8.19
Mastophorus muris	0.00	0.00	0.00
Rictularia proni	0.00	0.00	0.00
Trichuris muris	2.99	1.00 ± 0.00	0.03 ± 0.02

Altitude as a single factor was not an important predictor of *H. polygyrus* fecundity (Table 3.2). Host sex was important, with male hosts (n = 67) harbouring nematodes that were significantly more fecund (mean = 7.99 ± 0.13 eggs/mm) than female hosts (n = 47; mean = 6.60 ± 0.15 eggs/mm; Table 3.2). The interaction between altitude and host sex was the most important variable in the model, highlighted by the greatest difference between the 95% confidence intervals (Table 3.2). Female hosts at low altitude (n = 21, mean = 5.04 ± 0.22) harboured significantly less fecund nematodes than those at high altitude (n = 26, mean = 6.94 ± 0.17) or male hosts at either altitude (low altitude: n = 45, mean = 7.35 ± 0.13; high altitude: n = 22, mean = 6.98 ± 0.25; Table 3.2; Fig. 3.2). The low fecundity of nematodes infecting female hosts at low altitude was not due to a significant difference in body mass or breeding condition of these individuals. In addition, neither the sum of coinfecting macroparasites (interspecific measure of competition) nor *H. polygyrus* intensity (intraspecific competition measure) were important predictors of *H. polygyrus* fecundity (Table 3.2).

Table 3.2. Summary of the averaged-model standardised coefficients, unconditional standard errors (S.E.) and 95% confidence intervals of predictors for fecundity of female *Heligmosomoides polygyrus* infecting yellow-necked mice (*Apodemus flavicollis*) along an altitudinal gradient in the Italian Alps. Coefficients were standardised using the arm library in R. Averaged model is based on the top models with ΔAIC <2.5 (35 top models). A colon (:) indicates an interaction. Predictors for which the confidence intervals do not overlap zero are considered statistically significant and are shaded.

Predictor	**Standardised coefficient**	**Unconditional S.E.**	**95% Confidence Intervals**		**Relative importance of predictor**
Altitude	0.02	0.12	-0.22	0.26	0.54
Host sex	0.32	0.13	0.06	0.58	1.00
Body mass	0.32	0.18	-0.03	0.67	0.73
Breeding condition	-0.31	0.19	-0.67	0.06	0.69
Macroparasite coinfection	0.01	0.09	-0.18	0.18	0.08
H. polygyrus intensity	0.25	0.23	-0.20	0.70	0.28
Sex ratio	0.39	0.09	0.21	0.58	1.00
Altitude: Sex	-0.49	0.24	-0.95	-0.02	0.48
Altitude: Body mass	0.16	0.28	-0.39	0.70	0.06
Altitude: Breeding condition	0.06	0.25	-0.42	0.55	0.02
Sex: Body mass	-0.24	0.28	-0.79	0.31	0.08
Sex: Breeding condition	-0.24	0.25	-0.72	0.25	0.13
Mass: Breeding condition	-0.37	0.36	-1.07	0.33	0.18

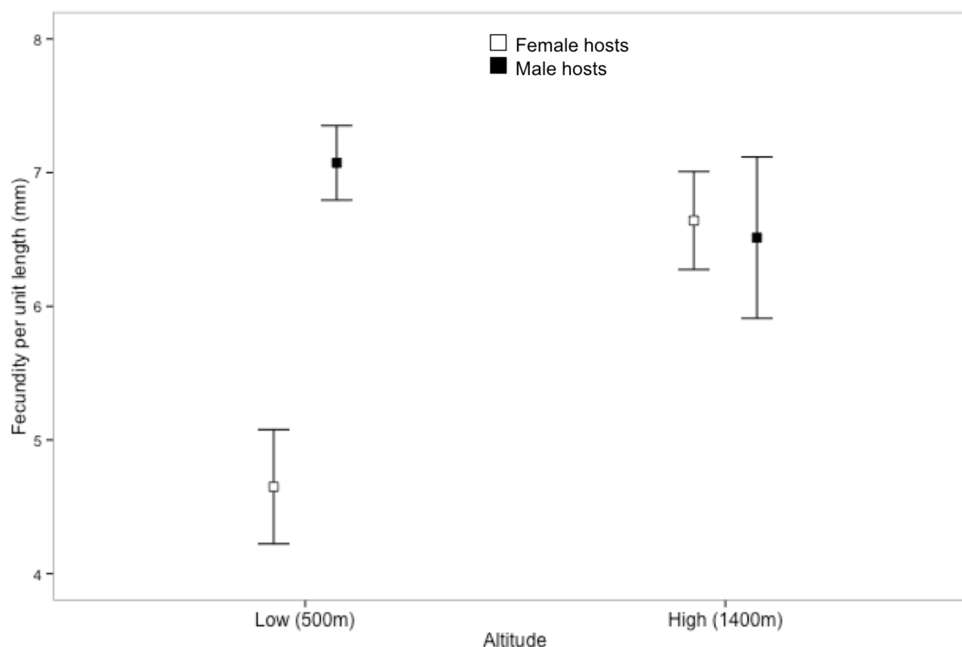

Figure 3.2 Fecundity per unit length (mm) of female *Heligmosomoides polygyrus* infecting female (white) and male (black) yellow-necked mice (*Apodemus flavicollis*) at low (500 m) and high (1400 m) altitude in the Italian Dolomitic Alps. The data represent the overall mean and the error bars are the 95% confidence intervals surrounding the mean.

The sex ratio increased (i.e. became male-biased) as nematode fecundity increased (Table 3.2). This relationship, however, was host sex-specific; for female hosts, when the sex ratio increased and became more male-biased, fecundity increased; but for male hosts, fecundity stayed relatively constant when the sex ratio increased (Fig. 3.3). This pattern held for both host sexes at either altitude. A *post-hoc* test revealed that female hosts at low altitude had a significantly male-biased sex ratio compared with male hosts at low altitude ($F_{3,1039} = 2.30$, $p = 0.031$; Fig. 3.4). No other two-way interactions included in the final averaged model were considered important predictors of *H. polygyrus* fecundity (Table 3.2).

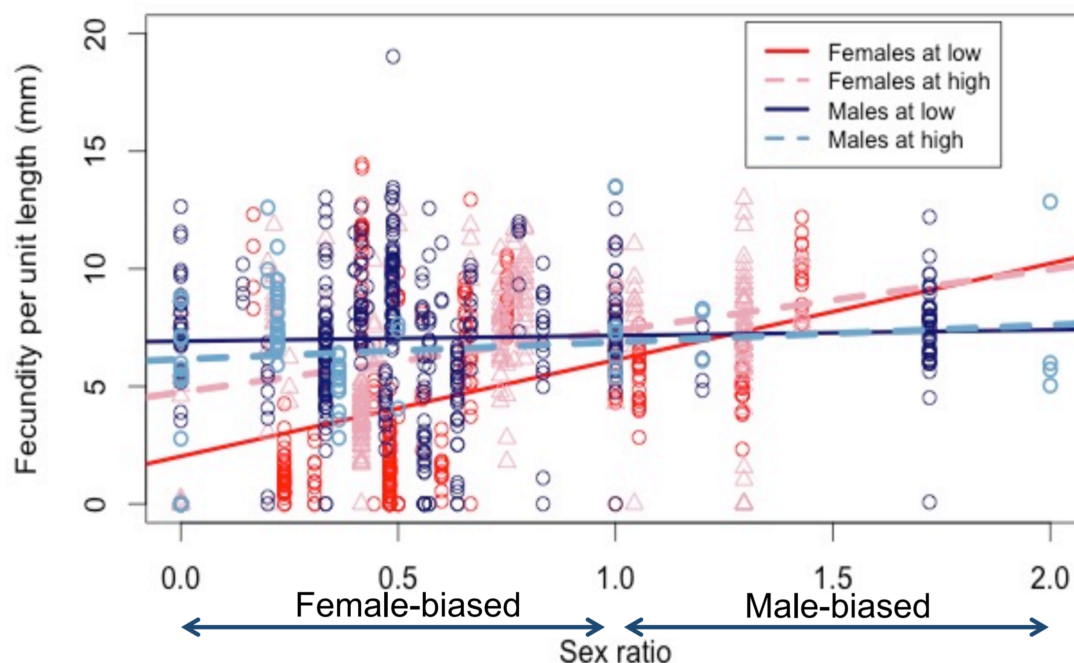

Figure 3.3 The relationship between the fecundity per unit length (mm) and the sex ratio (number of males divided by the number of females) of *Heligmosomoides polygyrus* infecting male and female yellow-necked mice (*Apodemus flavicollis*) at low (500 m) and high (1400 m) altitude in the Italian Alps. Sex ratios <1 indicate female-bias and >1 indicate male-bias.

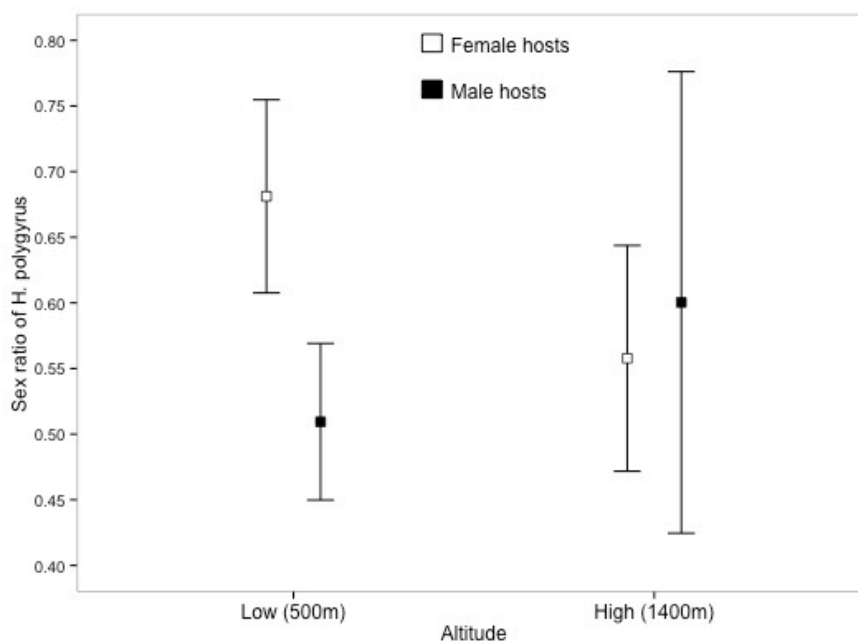

Figure 3.4 The sex ratio (number of males divided by the number of females) of *Heligmosomoides polygyrus* infecting female and male yellow-necked mice (*Apodemus flavicollis*) at low (500 m; white box) and high (1400 m; black box) altitude in the Italian Dolomitic Alps. The data represent the overall mean and the error bars are the 95% confidence intervals surrounding the mean.

3.5 Discussion

This study examined the effect of climate, host and parasite factors on *Heligmosomoides polygyrus* fecundity, a measure of host infectiousness. The mean difference in climate recorded along three altitudinal gradients in the Italian Alps during this study fell within the predicted range of global surface temperature increases (IPCC, 2013), making it a good proxy for forecasted climate change. There was a significant interaction between altitude and host sex, such that at low altitude, female hosts were infected with less fecund parasites than males. This sex-specific reduction in parasite fecundity at low altitude was not due to variation in host body mass or breeding condition, nor parasite inter- or intraspecific competition, as predicted. For female hosts, parasite fecundity increased when the *H. polygyrus* sex ratio became more male-biased, whereas for male hosts, fecundity stayed relatively constant when the sex ratio increased; a pattern that held true for female and male hosts at both altitudes.

In general, *H. polygyrus* exhibits female-biased sex ratios (Müller-Graf *et al.*, 1999), yet other nematodes particularly those of the Heligmosomidae family (Haukisalmi *et al.*, 1996) have previously demonstrated more male-biased infections at high mean intensities (Poulin, 1997; D'Ávila *et al.*, 2012). Given that there was a male-bias in parasite sex ratios within female hosts at low altitude, we might have expected to see high mean intensities in these individuals. Yet, *H. polygyrus* intensity did not differ between host sex-altitude cohorts, suggesting that the interaction between climate and host sex may play a role in driving fecundity at low altitude. Whilst host age and body size are known drivers of parasite sex ratios (Roche and Patrzec, 1966; Seidenberg *et al.*, 1973; Shimura, 1983; Craig *et al.*, 2010), the role of host sex is relatively unknown. It has been suggested that perturbations in temperature can directly affect parasite sex ratios, although there is a dearth of research in this area. In the case of the nematode, *Romanomermis culicivorax,* when temperatures increased from ambient, parasite sex ratios became more equal than at lower temperatures, when they were more female-biased (Tingley and Anderson, 1986). Female nematodes require more nutrients than males, and, as has been shown for mosquitoes and their nematodes, if parasite development relative to their hosts is slower at lower temperatures, there will be more nutrients available for the parasites, resulting in female-biased ratios (Tingley and Anderson, 1986). Here, a male-biased ratio of *H. polygyrus* was found in female hosts at warmer temperatures. This may be because there were fewer nutrients available for *H. polygyrus* infecting female hosts at low altitude, although there is currently no

empirical data to support this hypothesis. The results from the current study suggests that climate may be an indirect driver of parasite sex ratios, but suggests host sex plays a significant role too.

Whilst an interaction between host sex and nematode fecundity was apparent in this experiment, no direct effect of the altitudinal gradient on nematode fecundity was observed. Only a portion of the *H. polygyrus* lifecycle is spent outside of the host: eggs are shed into the environment in the faeces of an infected host, and they hatch and develop into an infective stage that is ingested by the host. It is only during this stage of the lifecycle that *H. polygyrus* is vulnerable to direct climatic effects. A meta-analysis revealed that a temperature increase above an 'ambient' temperature to which parasites were adapted, reduced parasite fecundity (Chapter 2). A significant effect on fecundity, however, was only apparent once temperatures exceeded 10°C above ambient (Chapter 2). The difference in temperature that was recorded along the altitudinal gradient for the duration of this study was, on average, 4°C. This may not have been sufficient to affect fecundity directly, but reflects predicted temperature rises (IPCC, 2013).

Evaluating the relative role of host characteristics in driving fecundity patterns, male mice were found to harbour more fecund nematodes than female mice, as previously reported by Luong *et al.* (2010). Increased fecundity of parasites infecting males may be related to sex-specific differences in the host immune response (Tompkins and Hudson, 1999; Viney, 2002; Wilkes *et al.*, 2004). In particular, testosterone in males is generally considered immunosuppressive, making them more susceptible to parasitic infection (Folstad and Karter, 1992; Poulin, 1996; Zuk and McKean, 1996) and able to produce more fecund worms (Seivwright *et al.*, 2005). In the current study, host body mass had no significant effect on parasite fecundity. Body mass can be a measure of host age, and previous research found hosts with larger body mass (i.e. older individuals) harboured the least fecund nematodes (Luong *et al.*, 2010). This may be related to developing acquired immunity or a reduction in the number of infections that were acquired by the host in early life (Gregory *et al.*, 1990; Luong *et al.*, 2010). Host breeding condition also had no effect on *H. polygyrus* fecundity. Previously, males in breeding condition were found to be infected with the most fecund parasites (Luong *et al.*, 2010), which may be linked to elevated testosterone during breeding, resulting in increased immune-suppression and more fecund worms (Poulin, 1996; Zuk and McKean, 1996). Males in breeding condition harbouring the most fecund parasites could be driven more by host

sex than breeding condition, which could explain why the effect of host breeding condition was not detected in the current study.

Previous studies have found that increasing *H. polygyrus* intensity resulted in reduced fecundity, possibly due to intraspecific competition between individuals limiting egg production as intensity increases (Chylinski *et al.*, 2009; Luong *et al.*, 2010), but this was not the case in our system. This study, however, does agree with previous work with respect to interspecific competition between coinfecting parasites (Luong *et al.* 2010), which was not seen to affect fecundity. Whilst the abundance of multiple macroparasitic infections was investigated in the current study, it was not used as a method for examining interactions between parasites (see Lello *et al.*, 2004 and Pedersen and Fenton, 2007), but it is more a measure of interspecific competition within the gastrointestinal tract.

In conclusion, male hosts were infected with the most fecund parasites, making them the most infectious individuals, whereas female hosts at low altitude were least infectious. Because parasite fecundity can be used as a measure of host infectiousness (Tompkins and Hudson, 1999; Irvine *et al.*, 2001; Luong *et al.*, 2010; Grear *et al.*, 2012; Sherrard-Smith *et al.*, 2015), the influence that climatic, host and parasite factors have on fecundity can affect how an infection spreads throughout a population (Woolhouse *et al.*, 1991, 1997; Smith *et al.*, 2007; Luong *et al.*, 2010). As such, these results suggest that with future climate change, parasite fecundity may be indirectly affected by the host and the sex ratios of the parasite. Future work investigating how climate change affects parasite life-history traits needs to consider the effects of the host, as much as those of a changing global climate.

3.6 Author Acknowledgements

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Chapter 4

Are the most infected hosts also the most infectious? The effect of coinfection on helminth egg shedding

4.1 Abstract

For many macroparasitic helminths, eggs are the infective stages, and the number of eggs shed by a host can be used as a proxy measure of host infectiousness. In addition to measuring infectious output of an individual, faecal egg counts is a well used non-invasive method employed to estimate the intensity and diversity of helminths infecting a host. This proxy of parasite intensity, of course, assumes a positive linear relationship between parasite load and infectious propagule output. Yet, empirical studies have shown that intraspecific competition and host immunity can reverse the relationship between parasite intensity and infectiousness. Coinfection (i.e. simultaneous infection with multiple parasite species) has been shown to alter both parasite intensity and egg shedding (infectiousness) as individual factors. As such, we propose that coinfection may also alter the intensity-infectious relationship of helminths. The aim of this study was to investigate whether the intensity-infectiousness relationship of helminths infecting wild yellow-necked mice (*Apodemus flavicollis*) was altered by coinfection. As an extension of this, we examined whether the diversity of helminth eggs detected in the faeces was a reflection of those helminth species found in the gastrointestinal tract. A positive relationship between *Heligmosomoides polygyrus* intensity and infectiousness was observed for both single and coinfected hosts. A negative relationship was detected in hosts that were single-infected with *Rodentolepis straminea*, but this was reversed by coinfection. Only two species out of a possible six were found in the faeces, suggesting that faecal egg counts do not accurately represent the full helminth infracommunity in the gastrointestinal tract. This study highlights that faecal egg counts do not accurately represent parasite diversity in the gastrointestinal tract, and they can be mis-representative of parasite intensity in coinfected hosts.

4.2 Introduction

Parasites are a crucial component of biodiversity as they are able to control host population cycles (Hudson *et al.*, 1998), increase their vulnerability to predation (Hudson *et al.*, 1992b) and control the host's reproductive output (Hudson *et al.*, 1992a). For parasite transmission to occur, a susceptible host has to come into contact with either an infected individual or an infective propagule. Many mathematical models assume that the rate of transmission (β) of infectious propagules is constant (Anderson and May, 1992; Grenfell and Dobson, 1995; Rodríguez and Torres-Sorando, 2001). Yet,

a number of empirical studies have found heterogeneity in the number of infectious stages shed by a host (termed 'infectiousness' from hereon), including the parasite burden (Quinn *et al.*, 2000; Matthews *et al.*, 2006a; Chase-Topping *et al.*, 2008; Lass *et al.*, 2013). Assuming the rate of transmission to be constant, therefore, may fail to describe the dynamics of disease epidemics (Cuadros *et al.*, 2011).

Hosts that are most infectious are also thought to harbour the greatest parasite burden (Quinn *et al.*, 2000; Matthews *et al.*, 2006a; Chase-Topping *et al.*, 2008; Lass *et al.*, 2013), thereby assuming a positive relationship between intensity and infectiousness. Positive density dependent patterns have been reported across multiple taxa, including red grouse (Seivwright *et al.*, 2005), domestic sheep (Lejambre *et al.*, 1971), cattle (Matthews *et al.*, 2006a) and humans (Elkins *et al.*, 1991; Sithithaworn *et al.*, 1991). This relationship suggests that the most infected individuals are also the most infectious and may occur due to a lack of intraspecific competition. In other host-parasite systems, however, negative relationships between intensity and infectiousness occur (Michael and Bundy, 1989; Irvine *et al.*, 2001; Dezfuli *et al.*, 2002; Chylinski *et al.*, 2009; Romeo *et al.*, 2014), and are proposed to be driven by intraspecific competition between parasites (Stear *et al.*, 1995; Tompkins and Hudson, 1999; Richards and Lewis, 2001; Chylinski *et al.*, 2009; Luong *et al.*, 2010).

Whilst intraspecific competition, or the lack thereof, appears to be driving host infectiousness, it is important to recall that in natural systems, infection with a single parasite species is rare; instead hosts tend to be coinfected with at least two parasite species (Lello *et al.*, 2004; Graham, 2008; Behnke *et al.*, 2009; Telfer *et al.*, 2010; Chapter 5), as such, interspecific competition is also expected to be a mechanism underlying infectiousness. Coinfecting parasites can interact with one another in the same manner that intraspecific interactions can occur, including competition for resources, crowding or immune-mediated effects (Behnke *et al.*, 2001a; Cox, 2001; Lello *et al.*, 2004; Pedersen and Fenton, 2007; Telfer *et al.*, 2010). Whilst coinfection leads to both positive and negative relationships between parasites, altering their intensity, it can also create 'super-shedders'; individuals that produce more infective stages than the average host (Lass *et al.*, 2013). Coinfection has been previously observed to increase infectious output of one coinfecting parasite species, but not the other (Cattadori *et al.*, 2014). Whilst both of these studies have provided insight into the effect that coinfection has on the relationship between intensity and infectiousness, they have only investigated the interactions between two coinfecting parasite species.

Previous work has found that wild rodents may be coinfected with as many as five helminth species and these species interact with one another (Telfer *et al.*, 2010; Chapter 5); as such, to understand the relationship between intensity and infectiousness for helminths, it is essential to take into account the whole helminth community.

Eggs of helminths represent the infective stages, and the number of eggs released in the host's faeces can be used as a proxy for host infectiousness. Typically, these faecal egg counts are also used as an estimate for helminth intensity when it is not possible to directly measure them through experimental constraints (Ferrari *et al.*, 2004; Pedersen and Fenton, 2007). Whilst helminth abundance has been observed to relate to *in vivo* parasite loads, it is unclear whether the diversity of parasite species that is found in the gastrointestinal tract is accurately represented by the species of eggs shed in the faeces. Furthermore, quantifying the relationship between parasite intensity and egg-shedding in single-infected versus coinfected hosts may provide fundamental information on the difference in parasite transmission potential in coinfected hosts. Using a wild host-parasite community, we examined whether the most infected hosts were also the most infectious, and if an individual was coinfected then this may increase host infectiousness. Second, we determined if the diversity of parasite species in the gastrointestinal tract may be the same as those found in the faeces.

4.3 Materials and Methods

4.3.1 Field sampling

Live-trapping of yellow-necked mice (*Apodemus flavicollis*) was carried out between May and August 2012 on three separate mountains (replicates) in the Italian Dolomitic Alps, Trentino; at Monte Bondone (46°2'13.38N, 11°0'12.64E), Panarotta (46°0'58.72N, 11°18'46.70E) and Monte Baldo (45°51'52.93N, 10°53'33.21E). Field work was carried out on Monte Bondone between May and June, on Panarotta in July, and on Monte Baldo in August. Trapping was located in broadleaf woodlands at six distinct sites on each mountain. A distance of at least 300 m between each site ensured that independent mouse populations were sampled.

At each site, multi-capture live Ugglan traps (Grahnab, Sweden) were set in transects of 20 for a total of 16 days per mountain, totalling 5760 trap nights (defined as the total number of traps set multiplied by the number of nights that traps were set). Each trap

was baited with a standardized amount of seed and a slice of potato as a source of moisture. Faeces were removed from traps containing one individual and used to gain a quantitative count of egg shedding once back in the laboratory. Faecal samples were weighed and 1 ml of saturated salt (NaCl) solution per 1 g of faeces was added, and homogenised using pestle and mortar. The solution was then passed over a mesh of 0.5 mm pore to remove debris. The solution was added to a two-chambered McMaster slide and after 2 minutes, eggs observed on the surface were counted, following standard methods of (Holmes, 1961). Individuals were only included in analysis if a faecal sample was available (n = 89). Individuals were euthanized using isofluorane and cervical dislocation, and transported to the laboratory in a portable refrigerator, where the gastrointestinal tracts were extracted by dissection. Helminths found during dissection were removed, examined under a stereo-microscope with fibre optic illumination and identified to species level. All animal-handling procedures and ethical issues were approved by the Provincial Wildlife Management Committee (authorization n. 595 issued on 04.05.2011).

4.3.2 Statistical analysis

A combination of generalised linear modelling (GLM) and generalised linear mixed modelling (GLMM) was carried out using the "lme4" function in R version 3.1.2 (R Core Development Team, 2013). The abundance of eggs per gram of faeces (including zeros; as defined by Bush *et al.*, 1997) of each parasite species was treated, in turn, as the dependent variable and was log transformed (log(x+1)), which resulted in normalised residuals in all cases. The explanatory variables included in each model were the month of sampling, the intensity (log(x), excluding zeros; as defined by Bush *et al.*, 1997) of helminths of the same species of egg (intensity was used here as hosts did not shed eggs unless helminths were present), the abundance (log(x+1)) of each coinfecting parasite species that had a prevalence of above 10%, to ensure sufficient statistical power (see Table 6.1), and Simpson's and Shannon's diversity indices. The parasite species infecting any given host was also included as a factor within the model to determine whether the presence of any given coinfecting species altered the intensity-egg shedding relationship, where sample sizes were adequate (i.e. n > 5). Random factors included in the GLMMs were the field site and the mountain where trapping took place, and these were assessed using Akaike's Information Criterion (AIC). If

inclusion of random terms produced AIC values that were greater than models where random terms were excluded, the model was rerun as a GLM. Fixed model terms were assessed by the conditional F-statistic and associated p-values; the most insignificant term ($p > 0.05$) was removed in a step-wise manner, until a minimal model was produced. All models were fitted with Gaussian distributions and identity link functions.

To investigate whether there was a significant difference in the diversity of parasite species found in the gut compared with the diversity of eggs shed, Simpson's and Shannon-Weiner diversity indices were calculated. Both indices measure the changes in the numbers of species in a population, yet Simpson's index is a more sensitive measure for abundant species, whilst Shannon-Weiner takes account of rarer species (Krebs, 1999; Honarvar *et al.*, 2011; Larsen and Mouritsen, 2014). Two GLMM's were fitted using Gamma distributions and log link functions. Each diversity index was treated as the dependent variable in a GLMM, with the location of the parasites (either gastrointestinal tract or faeces) included as the only explanatory variable. As each individual host was included in the model twice, with a measure of diversity of parasites from the gastrointestinal tract and another from the faeces, the unique identification code of each host was included as a random term within the model.

4.4 Results

In total, six species comprised the parasite community. Five species of nematodes were found: *Heligmosomoides polygyrus*, *Rictularia proni*, *Mastophorus muris*, *Syphacia* spp., and *Trichuris muris,* and one species of cestode, *Rodentolepis straminea.* The most prevalent parasite species was *H. polygyrus*, and the most abundant was *Syphacia* spp. (Table 4.1). Three species had a prevalence of less than 10% (*M. muris*, *R. proni* and *T. muris;* Table 4.1); they were included in the diversity indices calculations, but their abundances were excluded from models due to their low prevalence. Despite, the parasite community consisting of six species, the eggs of only two parasite species, *H. polygyrus* and *R. straminea*, were detected in the faeces; the relationship between intensity and infectiousness, and the role coinfection played in altering this relationship was considered for these two parasites in turn.

Table 4.1 Prevalence (%) and abundance (including zeros) of helminths found in the gastrointestinal tract, and the number of eggs (per gram of faeces) found in the faeces of yellow-necked mice (*Apodemus flavicollis*).

Parasite species	**Prevalence (%)**	**Abundance (± S.E.)**	**Number of eggs per gram of faeces (± S.E.)**
Heligmosomoides polygyrus	82.76	12.47 ± 2.28	497.15 ± 99.35
Rodentolepis straminea	34.48	2.20 ± 0.59	3474.17 ± 709.16
Syphacia spp.	48.26	25.43 ± 9.25	0
Mastophorus muris	2.30	0.02 ± 0.02	0
Rictularia proni	4.60	0.31 ± 0.19	0
Trichuris muris	8.64	0.28 ± 0.13	0

4.4.1 Heligmosomoides polygyrus *intensity-infectiousness relationship*

A positive relationship was found between *H. polygyrus* intensity and the number of eggs shed in faeces (infectiousness; $F_{1, 62} = 31.45$; $p < 0.001$; Fig. 4.1), suggesting that individuals infected with the greatest *H. polygyrus* burden were also the most infectious. In total, 60% of hosts infected with *H. polygyrus* were coinfected with at least one other parasite species. Of these hosts, 20.83% were coinfected with *R. straminea*, and 47.22% with *Syphacia* spp. The maximum number of coinfecting parasite species was three. Individuals coinfected with all three helminth species shed the same number of *H. polygyrus* eggs but at a lower intensity than hosts single- or coinfected with two species (Fig 4.1). There was, however, no significant difference in the intensity-infectiousness relationship of *H. polygyrus* in hosts that were coinfected with *R. straminea* ($F_{1, 41} = 4.20$, $p = 0.949$) or *Syphacia* spp. ($F_{1, 42} = 2.79$, $p = 0.600$), or all three species ($F_{3, 58} = 1.76$, $p = 0.167$; Fig. 4.1). Neither the Simpson's ($F_{1, 47} = 3.45$, $p = 0.560$) nor Shannon-Weiner diversity indices ($F_{1, 43} = 1.96$, $p = 0.661$) were significantly associated with *H. polygyrus* infectiousness. Sampling month also had no significant effect on infectiousness ($F_{3, 44} = 7.59$, $p = 0.523$), and nor did host sex ($F_{1,42} = 5.43$, $p = 0.561$), body mass ($F_{1, 39} = 1.53$, $p = 0.698$) or breeding condition ($F_{1, 57} = 8.43$, $p = 0.362$).

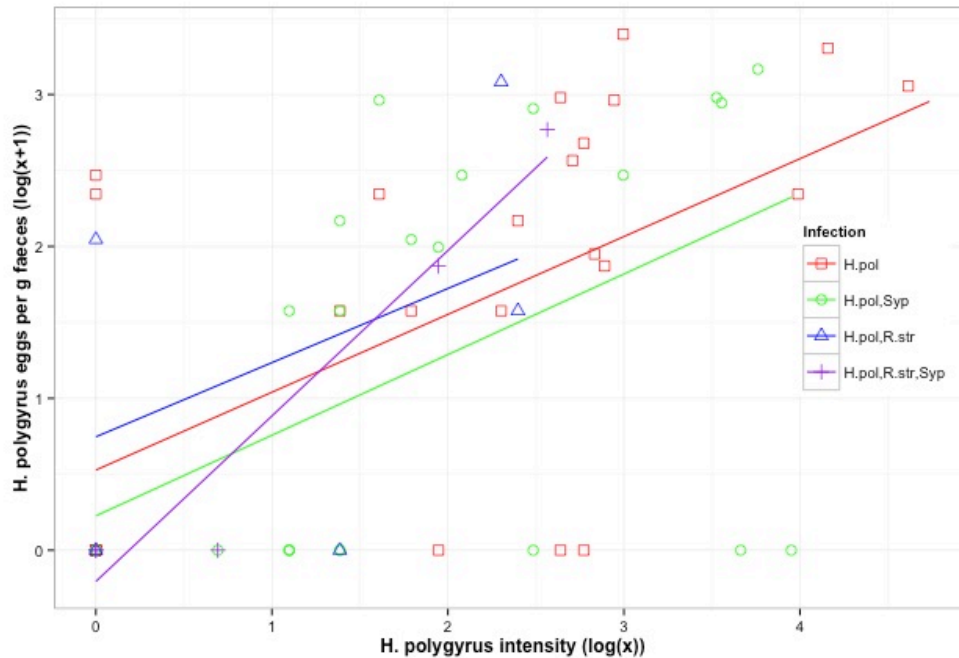

Figure 4.1 The relationship between the number of *Heligmosomoides polygyrus* eggs shed (log(x+1)) and *H. polygyrus* intensity (log(x)) in individuals single-infected with *H. polygyrus* (red); or *H. polygyrus* and *Syphacia* spp. (green), or *H. polygyrus and R. straminea* (blue) or individuals infected with *H. polygyrus, R. straminea and Syphacia* spp. (purple)

4.4.2 Rodentolepis straminea *intensity-infectiousness relationship*

Individuals that were single-infected with *R. straminea* displayed a negative relationship between intensity and infectiousness (Fig. 6.2), suggesting that those with the lowest parasite intensity were the most infectious. In total, 80% of hosts infected with *R. straminea* were coinfected with at least one other parasite species. Of these hosts, 48.38% were coinfected with *H. polygyrus*, and 27.59% with *Syphacia* spp. The negative intensity-infectiousness relationship was reversed when any other coinfecting parasite species was present, as such, there was a significant difference in the intensity-infectiousness relationship between single-infected and coinfected hosts ($F_{1, 24} = 1.65$, $p = 0.0195$; Fig. 4.2). There was no significant difference in the intensity-infectiousness relationship of *R. straminea* in hosts coinfected with *H. polygyrus* ($F_{1, 14} = 15.53$, $p = 0.698$) or *Syphacia* spp. ($F_{1, 15} = 1.59$ $p = 0.226$), or with all three species ($F_{3, 24} = 1.98$, $p = 0.467$; Fig. 4.2). Neither Simpson's ($F_{1, 28} = 4.12$, $p = 0.530$) nor Shannon-Weiner ($F_{1, 28} = 33.81$, $p = 0.571$) diversity indices were associated with *R. straminea* infectiousness. Sampling month had no significant effect on *R. straminea* egg shedding ($F_{3, 24} = 1.69$, $p = 0.220$), neither did host sex ($F_{1, 24} = 2.90$, $p = 0.926$), body mass ($F_{1, 24} = 1.73$, $p = 0.686$) nor breeding condition ($F_{1, 24} = 11.26$, $p = 0.743$).

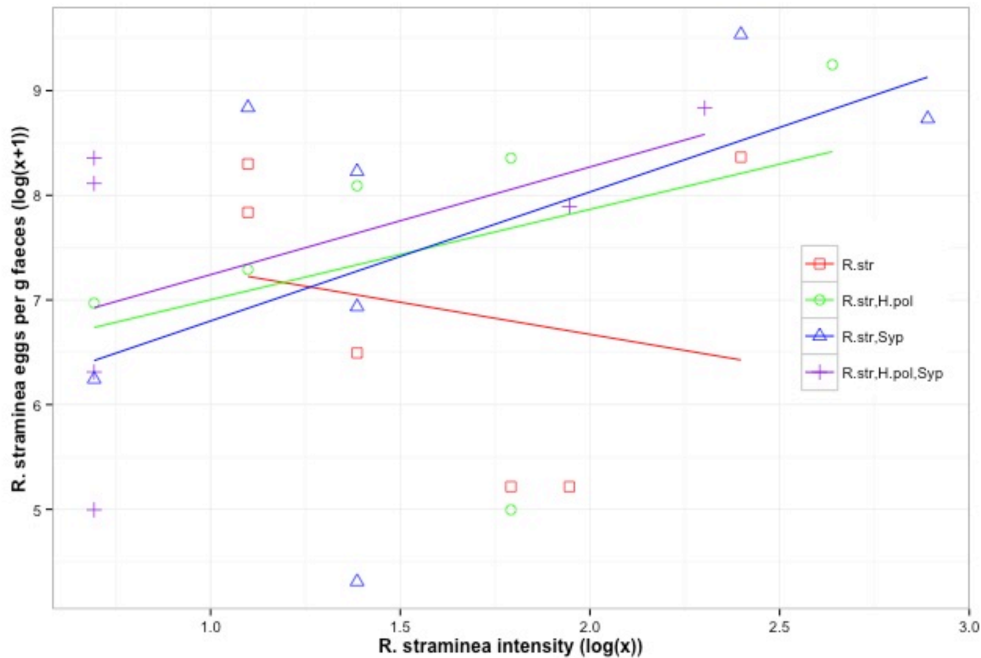

Figure 4.2 The relationship between the number of *Rodentolepis straminea* eggs shed (log(x+1)) and *R. straminea* abundance (log(x+1)) in hosts single-infected with *R. straminea* (red), individuals infected with *R. straminea* and *H. polygyrus* (green), hosts infected with *R. straminea* and *Syphacia* spp. (blue), and individuals infected with *R. straminea, H. polygyrus* and *Syphacia* spp. (purple)

4.4.3 Diversity of helminths in the gastrointestinal tract versus the faeces

Eggs from only two of the six species of gastrointestinal helminths were found in the faeces: one nematode, *H. polygyrus,* and the cestode, *R. straminea* (Table 4.1). Both the Simpson's (gastrointestinal mean ± S.E. = 0.51 ± 0.03; faeces mean ± S.E. = 0.02 ± 0.01; LRT = 40.32; $p < 0.001$) and Shannon-Weiner (gastrointestinal mean ± S.E. = 0.13 ± 0.02; faeces mean ± S.E. = 0.06 ± 0.01; LRT = 191.65; $p < 0.001$) diversity indices indicated that the diversity of parasites found in the gastrointestinal tract was significantly greater than that found in the faeces (Fig. 4.3, Table 4.1).

4.5 Discussion

Coinfection altered the relationship between *Rodentolepis straminea* intensity and infectiousness, from a negative to positive one, such that coinfected individuals that harboured the greatest parasite load shed the most eggs. Hosts coinfected with the three helminths species *Heligmosomoides polygyrus*, *R. straminea* and *Syphacia* spp. shed

more *H. polygyrus* eggs, but at a lower intensity than hosts coinfected with two of these species or single-infected hosts, although there was no difference in egg shedding whether hosts were single- or coinfected. These results suggest that coinfection can contribute to the heterogeneity seen in host infectiousness, and this has important implications for understanding disease epidemics. A positive relationship between coinfection and infectiousness can lead to an increase in the prevalence of infection (Woolhouse *et al.*, 1997; Perkins *et al.*, 2003; Chase-Topping *et al.*, 2008; Luong *et al.*, 2010; Lass *et al.*, 2013). Comparison of the Simpson and Shannon-Weiner diversity indices suggest that the gastrointestinal tract is considerably more diverse than the faeces indicate. Six parasite species were present in the gastrointestinal tract, whilst eggs from only two species were shed in the faeces, namely *H. polygyrus* and *R. straminea*.

Coinfection is known to affect the number of infectious stages that a host produces (Bassetti *et al.*, 2005; Matthews *et al.*, 2006a). The switch from a negative to positive relationship between *R. straminea* intensity and infectiousness that was seen with coinfection could be driven by the immune system, whereby the presence of coinfecting parasite species suppress cytokine response, resulting in greater parasite intensity, establishment, susceptibility and infectious output (Cox, 2001; Lello *et al.*, 2004; Graham, 2008; Montes *et al.*, 2009; Luong *et al.*, 2010). For example, rabbits infected with the poxvirus myxoma were more susceptible to nematode infection and had greater nematode burdens than individuals not infected with the virus (Boag, 1988; Cattadori *et al.*, 2007). Coinfection may disrupt the ability of the immune system to clear infections (Cattadori *et al.*, 2007), and as a result, parasites in coinfected hosts may shed more eggs compared with those in single-infected hosts. Coinfected individuals could be responsible for the majority of egg shedding of *R. straminea*, thus driving the infection (Woolhouse *et al.*, 1991, 1997; Smith *et al.*, 2007; Luong *et al.*, 2010).

Whilst a difference between the intensity-egg shedding relationship between single-infected and coinfected hosts was detected for *R. straminea*, no such association was found in hosts predominately affected by *H. polygyrus*. The lack of contrasting intensity-infectiousness relationships in single-infected and coinfected hosts for *H. polygyrus* is somewhat surprising. To avoid detection, *H. polygyrus* is known to manipulate the immune system of the host (Jenkins and Behnke, 1977; Behnke *et al.*, 1978, 1983). Coinfection with *Syphacia* spp. and *Hymenolepis nana*, a well-studied relative of *R. straminea*, are known to induce a Th2 immune response in hosts, which

produces interleukin 4 (IL-4) and IL-5 cytokines (Conchedda *et al.*, 1996; Michels *et al.*, 2006). The immuno-modulatory effects of *H. polygyrus* may, therefore, be lost in coinfected hosts, and so we might have expected to see a reduction in egg shedding when a second or third parasite species was present. The negative density dependent relationship apparent in hosts infected with *R. straminea* only might be due to the immune system concentrating on just that species, and causes a reduction in the intensity-infectiousness relationship. In coinfected hosts, however, the immune response may be spread between multiple parasites, and the effect on *R. straminea* is much reduced, resulting in a positive infected-infectiousness relationship.

Parasite egg shedding can be affected by a range of factors including seasonal and daily variation, which can be a major cause of heterogeneity in helminth infections, and can affect the survival, transmission and, therefore, availability of infective eggs (Gregory *et al.*, 1992; Abu-Madi *et al.*, 1998; Morgan *et al.*, 2004). The prevalence and intensity of *H. polygyrus* is at its highest during the winter and spring, and then declines in the autumn (Gregory *et al.*, 1990; Abu-Madi *et al.*, 2000), whereas the presence of *Hymenolepis diminuta*, which is related to *R. straminea*, is lowest during the spring (Easterbrook *et al.*, 2008). Fieldwork in the current study was carried out between May and August; repeating this experiment during the spring when *H. polygyrus* is at its highest intensity and *R. straminea* at its lowest intensity may generate different results. For *H. polygyrus*, this could mean a change in the intensity-egg shedding relationship to a negative one, as intraspecific competition for resources and/or space may reduce worm development and cause fewer eggs to be produced (Tompkins and Hudson, 1999). As parasite intensity is known to be affected by seasonality and has been shown in the current study to affect egg shedding of both *H. polygyrus* and *R. straminea*, we suggest that egg shedding may also be affected by seasonality, although this has not yet been looked at in these species.

There are conflicting reports from empirical studies about whether faecal egg outputs accurately represent parasite infection load, yet in the current study, for host that were predominantly infected with *H. polygyrus* at least, the most infected hosts were also the most infectious, which is in agreement with previous studies reporting similar correlations (Lejambre *et al.*, 1971; Coadwell and Ward, 1982; Elkins *et al.*, 1991; Sithithaworn *et al.*, 1991; Seivwright *et al.*, 2005). One other study has examined the role of coinfection on host infectiousness (Cattadori *et al.*, 2014), and the authors reported a positive relationship between the intensity and infectiousness of

Trichostrongylus retortaeformis, but not *Graphidium strigosum* in coinfected rabbits. The heterogeneity caused by coinfection on the intensity-infectiousness relationships reported in Cattadori *et al.* (2014) and the current study suggests that unless it is known whether a host is coinfected, faecal egg counting might not accurately represent *R. straminea* intensity.

A secondary aim of this study was to determine whether the diversity of parasite species in the gastrointestinal tract was accurately represented by species of eggs detected in the faeces. In studies of parasites in wild host populations, faecal egg counts are the main method used when monitoring how the parasite population changes over time (e.g. Hudson *et al.*, 1992a; MacIntosh *et al.*, 2010; Raharivololona and Ganzhorn, 2010; Winternitz *et al.*, 2012), or when the parasite community is manipulated (e.g. Ferrari *et al.*, 2004, 2009; Knowles *et al.*, 2013; Pedersen and Antonovics, 2013). In the current study, six parasite species were present in the gastrointestinal tract, yet only two of these species were shed as eggs in the faeces. The three least common species (*M. muris, R. proni and T. muris*) and the most abundant helminth, *Syphacia* spp. (see Table 4.1) were not detected in the faeces. These species may not have been shedding eggs during the experiment, or it might be that there were no females present. This was not the case for *Syphacia* spp., however, as females were more common in hosts than males (Gillingham, pers. obs). Failure to detect *Syphacia* spp. eggs in the faeces may relate to the method of egg laying carried out by female pinworms; they migrate to the anus and deposit embryonated eggs on the skin, and hosts become infected by ingesting eggs from the perianal region of an infected animal (Taffs, 1976). The results from the current study, however, suggest that at the time of sampling in this population, faecal egg counting did not accurately represent the diversity of the gastrointestinal tract and this should be considered in studies using faecal egg counts as a measure of the parasite community.

In conclusion, these results provide empirical evidence that coinfection can alter the infectiousness of hosts. Faecal egg counts do not accurately represent the presence of parasites in the gastrointestinal tract, as only two out of six species were detected in the faeces. Infection load, therefore, cannot be predicted from egg shedding in hosts that are coinfected.

4.6 Authorship Acknowledgement

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Chapter 5

Interactions between the parasite communities of wild mice at high and low altitude

5.1 Abstract

Interactions occurring between multiple parasite species simultaneously infecting a host, termed coinfection, have the potential to shape and influence the parasite community. These interactions may be positive, negative or neutral. Biotic factors, such as host sex, breeding condition and body mass are known to alter host-parasite interactions in single infection scenarios. In addition, abiotic factors (e.g. climate) have been found to alter parasite abundance, but again, only single infections have been addressed. Given the pervasiveness of coinfection within hosts, changes in parasite abundance caused by these factors in any given parasite species could have a knock-on effect on others. As such, using high and low altitude as a proxy for climate change, the interplay between coinfecting parasites, climate and host biology of wild yellow-necked mice (*Apodemus flavicollis*) was quantified using a series of general linear models. All species were involved in at least one predicted interaction with one other. Four broad patterns were identified. First, the intensity of most ectoparasites was reduced in the presence of other coinfecting ectoparasite species, which we hypothesise is due to a negative feedback loop induced by increased grooming in response to local inflammation. Second, the intensity of the helminth, *Heligmosomoides polygyrus* increased with body mass in single-infected hosts, yet reduced with increasing body mass when hosts were also coinfected with *Syphacia* spp., which is suggestive of an immuno-modulatory effect of the coinfecting species. Third, cross-boundary interactions were evident (i.e., those that occurred between internal helminths and external ectoparasites). Finally, climatic differences were associated with changes in parasite intensity, with five of eight parasite species having a greater intensity at low compared to high altitude, which ultimately changed interactions between coinfecting parasite species. Overall, this study demonstrates that parasite communities are highly interactive, host factors play a role in community structure and community interactions change under differing climatic conditions.

5.2 Introduction

The interactions that occur between multiple species sharing the same resources and/or habitat have the potential to shape and influence community structure (Berg and Smalla, 2009).. Parasites are no exception to this rule. In free-living populations, the majority of individuals are simultaneously infected with more than one parasite, known as

coinfection (Lello *et al.*, 2004; Graham, 2008; Telfer *et al.*, 2010). These coinfecting parasites may have no effect upon one another (Montgomery and Montgomery, 1988; Graham, 2008) or they may interact, for example, through competition for resources, crowding or immune-mediated effects (Behnke *et al.*, 2001a; Cox, 2001; Lello *et al.*, 2004; Pedersen and Fenton, 2007; Telfer *et al.*, 2010). Broad patterns of interspecific parasite interactions have been demonstrated; for example, a meta-analysis investigating interactions between macroparasites and microparasites across a range of parasite taxa infecting mice found that the majority of interactions occurring between parasite species were positive (50%), with 27% of interactions having no effect, and 23% having a negative effect on microparasite density (Graham, 2008).

Wild rodents are infected with a diverse community of ectoparasites and helminths, making them a useful model species for studying the interspecific interactions of coinfecting parasites (e.g. Montgomery and Montgomery, 1990; Haukisalmi and Henttonen, 1993a; b; Behnke *et al.*, 2001, 2005, 2009; Pedersen and Antonovics, 2013). Previous field surveys and manipulation experiments in wild rodents have reported a range of interactions between coinfecting parasites. For example, wood mice (*Apodemus sylvaticus*) infected with the helminth *Heligmosomoides polygyrus* had a higher prevalence of other helminth species (Behnke et al. 2009), suggesting synergistic effects yet in both yellow-necked (*Apodemus flavicollis*) and wood mice, antagonism is evident as experimental removal of nematodes with anthelminthics increased the prevalence of cestodes, coccidial protozoans and ticks (Ferrari *et al.*, 2009; Pedersen and Antonovics, 2013), whilst null interactions were found between helminths using observational data (Montgomery and Montgomery, 1990). One empirical study that investigated the interactions between coinfecting microparasites in wild rodents found that each microparasite was connected with at least one other species, with a mixture of positive and negative interactions occurring between the community (Telfer *et al.*, 2010). While it is possible that the lack of consensus in coinfection studies is due to heterogeneities inherent in host-parasite systems, such as variation in host sex, body mass and abiotic factors, it is also important to note that it is harder to assess interactions from observational studies, especially cross-sectional ones, compared to data from experimental studies (Fenton *et al.*, 2010, 2014). Regardless, there is clear evidence that the presence or absence of one parasite species has the potential to affect another, and this may have knock-on effects for the community as a whole.

The interactions between coinfecting parasites may ultimately be driven by biotic factors, including host sex, breeding condition, body mass and immune response. Comparative studies have found that male hosts tend to be the most infected sex (Poulin, 1996; Zuk and McKean, 1996; Moore and Wilson, 2002), which may be related to the immuno-suppressant effect of testosterone resulting in males being more susceptible to infection (Folstad and Karter, 1992; Poulin, 1996; Zuk and McKean, 1996). In general, larger hosts may be more parasitized due to greater exposure through consumption of more infected food than smaller hosts, larger home ranges or individual size presenting a larger target for ectoparasites (Zuk and McKean, 1996; Perkins *et al.*, 2003; Harrison *et al.*, 2010). Similarly, age is also an important influence in many host-parasite systems, with different age intensity curves demonstrated in similar species (Woolhouse, 1998; Cattadori *et al.*, 2005).

Whilst the mechanisms driving the interactions between coinfecting parasites can be biotic, environmental fluctuations may also play a role in interspecific interactions involving macroparasites because the majority of macroparasite species have a free-living phase when they are directly exposed to varying climatic conditions. As such, we may expect climate to influence life-history traits of these parasites. Generally, increases in temperature accelerate parasite development times (Kutz *et al.*, 2005; Chapter 2) and lengthen the period of the year when hosts may be exposed to the infective stages of parasites (Laaksonen *et al.*, 2010; Hernandez *et al.*, 2013). Extreme temperature increases, however, can result in desiccation and may reduce parasite survival (Gibson, 1981; Boag and Thomas, 1985; van Dijk and Morgan, 2008). Further, the response of different parasite species to climate is not uniform and parasite species infecting the same host may respond differently to the same perturbation. Exposure of eggs of the rabbit parasites *Trichostrongylus retortaeformis* and *Graphidium strigosum* to increased temperatures resulted in both species exhibiting accelerated egg development, increased hatching rate and increased larval survival, *T. retortaeformis* was more responsive than *G. strigosum* (see Hernandez *et al.*, 2013). These two helminth species are known to interact with one another and other coinfecting parasites of the rabbit (see Lello *et al.*, 2004, 2008), and any changes to either parasite induced by climatic perturbations may affect the parasite community as a whole.

Host biology, environment and coinfection may all interact to shape the parasite community interactions of wild hosts. The seasonal variation in parasite intensity in a host, driven by climate, may differ between the sexes because the climatic effects are

moderated by host susceptibility. For example, in rabbits, the cestode *Mosgovoyia pectinata* shows substantial seasonal variation and is associated with an increased intensity of the pinworm *Passalurus ambiguous* in female but not in male wild rabbits. This is particularly noteworthy because the pinworm is largely transmitted through grooming and is therefore little influenced by seasonal events (Lello *et al.*, 2004). In this study, we expand on previous literature by carrying out an empirical study to explore the interplay between coinfection, climate and host biology using free-living wild yellow-necked mice (*Apodemus flavicollis*). As climatic perturbations significantly affect parasite abundance (Chapter 2), we will address the hypothesis that an increase or decrease in temperature and/or humidity will significantly increase the number of parasites (abundance) and so alter interactions between ectoparasitic and endoparasitic (helminth) species infecting *A. flavicollis*.

5.3 Materials and methods

5.3.1 Field sampling

Live-trapping of yellow-necked mice (*A. flavicollis*) was conducted between May and August 2012 on three geographically distinct mountains in the Italian Dolomitic Alps, Trentino; at Monte Bondone (46°2’13.38N, 11°0’12.64E), Panarotta (46°0’58.72N, 11°18’46.70E) and Monte Baldo (45°51’52.93N, 10°53’33.21E). Trapping was carried out on Monte Bondone between May and June, on Panarotta in July, and on Monte Baldo in August. On each mountain, three replicated live-trapping transects were located at low (500 m) and high altitude (1400 m). Altitudinal gradients are used as a system for studying the effects of climate change on species where long-term data are not available (Pickett, 1989; Jouda *et al.*, 2004; Hodkinson, 2005; Gilbert, 2010). There were at least 300 m between each transect, representing non-overlapping ranges for the mice, to ensure that independent mouse populations were sampled. Transects were located in similar habitat; broadleaf woodlands with a west-facing aspect. At each of the transects, 20 multi-capture live Ugglan traps (Grahnab, Sweden) were set 10 m apart for approximately 16 days per mountain, amounting to a total of 5760 trap nights (defined as the total number of traps set multiplied by the number of nights that the traps were set).

Each trap was baited with a standardised amount of seed and a slice of potato as a source of moisture. For each mouse captured, the sex, body mass and breeding condition was recorded: males with descended testes and females with perforated vaginas were classed as adults. Those with non-descended testes and imperforate vaginas were sub-adults. Juveniles were classified as those that were under 15 g body mass, and due to their low exposure to parasites, juveniles were excluded from this study. Host density did not differ between transects. Individuals (n = 257) were euthanised *in situ* using isofluorane, followed by cervical dislocation. Gastrointestinal tracts were removed from the animals and stored in 90% ethanol, and carcasses were stored at -20°C. All animal-handling procedures and ethical issues were approved by the Provincial Wildlife Management Committee (authorization n. 595 issued on 04.05.2011). Approval to trap animals was granted by the Comitato Faunistico of the Autonomous Province of Trento.

5.3.2 Quantifying helminth and ectoparasite intensity and prevalence

All helminths found during dissection of the gastrointestinal tract were removed, examined under a stereo-microscope with fibre optic illumination and identified. Five species of nematode were identified: *Heligmosomoides polygyrus* (Heligmosomoidae); *Mastophorus muris* (Spiruridea); *Rictularia proni* (Rictulariidae); *Syphacia* spp. (Oxyuridae); and *Trichuris muris* (Trichuridae). Two species of tapeworm were also identified: *Rodentolepis straminea and Hymenolepis murissylvatici* (Hymenolepididae). Parasite species were only included in analyses if they were prevalent in more than 10% of animals; as such, *M. muris*, *R. proni*, *T. muris* and *H. murissylvatici* were excluded from analyses.

Ectoparasites were removed from the rodent's body by placing individuals over a sieve with an aperture of 710 μm and washing them under running water for 5 min. The sieve contents were transferred with a Pasteur pipette into a petri dish and examined under a stereo-microscope with fibre optic illumination. Six species of ectoparasites were identified: larval and nymphal *Ixodes ricinus* (Ixodidae); *Ctenophthalmus solutus*; *Doratopsylla dasycnema* (Hystrichopsyllidae); *Leptotrombidium europaeum* (Trombiculidae); *Laelaps agilis* (Laelapidae); and lice (not identified to species). The prevalence of nymphal ticks was less than 10%, and no adult ticks were found; as such nymphs and the more highly prevalent tick larvae were combined numerically for the

purposes of analysis and from hereon are referred to as 'total ticks'. Similarly, one of the flea species *D. dasycnema* had a prevalence of less than 10%, and so the two flea species found were combined for analysis and are referred to as 'total fleas'. Details of the prevalence and mean intensity of all parasites are shown in Table 5.1.

5.3.3 Climate data

Temperature-humidity data recorders (iButton®, Maxim Integrated Products, USA) were placed at each of the 18 transects across the three mountains. The recorders were secured onto a tree facing northwards, approximately 20 cm from the ground in the middle of each transect and were set to record hourly for the duration of the experiment. The temperature and relative humidity data were averaged for 18 sites at low and high altitude, and generalised linear models (GLM) were used to assess the climatic differences between these two altitudes. Temperature was significantly warmer at low (mean = 19.67 ± 0.44°C) compared to high (mean = 15.99 ± 0.14°C; $F_{1, 84} = 28.30$, $p < 0.001$) altitude. Furthermore, low was significantly less humid (mean = 77.04 ± 0.60%) than high (mean = 80.90 ± 0.50%; $F_{1, 84} = 30.81$, $p < 0.001$) altitude. To summarize, low altitude was 3.68°C ± 0.30 warmer and 3.86% ± 0.11 less humid than high altitude. By 2100, global surface temperatures are predicted to increase by 1.1-4.8°C (IPCC, 2013), making the difference in climate recorded between high and low field sites within the range of predicted temperature increase, and the temperatures at low altitude representative of a future climate, when compared to high altitude.

5.3.4 Statistical analysis

A combination of generalised linear modelling (GLM) and generalised linear mixed modelling (GLMM) was performed in R version 3.1.2 (R Core Team 2013) and using the ASReml-R v3 package for mixed modelling (VSN International Ltd., Hemel Hempstead, UK). The intensity of each parasite species (excluding zeros, as defined by Bush *et al.*, 1997) was treated as the dependent variable for each species in turn and was log transformed (log(x)) which resulted in normalised residuals in all cases. In brief, the explanatory variables included in each model were: the Julian Day since the start of the sampling period (as sampling began in May, 164 equated to the first day of sampling), the altitude where the individual was captured, host sex, host body mass, breeding

condition (sub-adult or adult) and parasite abundance (including zeros, as defined by Bush *et al.*, 1997) of coinfecting species, which were normalised by log transformation (log(x+1)) were included as explanatory variables in the model (Fenton *et al.*, 2010). If data transformation was not possible, presence/absence data were used. Second order interactions were also included in the model where sufficient data were available (with ten or more data points per factor level; see Appendix A.2, Table A.2.1 for a list of terms excluded from the models). Random factors included in the ASReml models were the field site and the mountain where trapping took place. In addition, a continuous variable describing the Julian Day was included in the random terms using a cubic smoothing spline, as well as a linear term in the fixed effects. Insignificant terms in both the random and fixed model components were removed in a step-wise manner to produce a minimal model. Random terms were assessed using likelihood ratio tests. Where random terms were insignificant (i.e. at the $p = 0.05$ level), the model was rerun as a GLM. Fixed model terms were initially assessed by the conditional F-statistic and associated *p*-values (using the Wald test for GLMMs). Fixed model terms were removed from the model if their *p*-value was >0.1; thereafter models were refined using bootstrapping which generated 95% confidence intervals for each coefficient. A term was considered significant if the confidence intervals for that coefficient did not overlap zero. In order to assess the change in the dependent variables as predicted from the model, the absolute predicted percentage change was calculated as the percentage difference between the predicted level of the back-transformed dependent variable when the coinfecting species count was zero, compared with the predicted level of the back-transformed dependent variable when the coinfecting species was either at its geometric mean (in the case of abundance data) or was present (in the case of presence/absence data; see Fenton *et al.*, 2010).

5.4 Results

Yellow-necked mice were infected with 13 macroparasite species, of which five were excluded as they had a prevalence of less than 10% (Table 5.1). Every parasite species was involved in at least one predicted interaction with another parasite (Fig. 5.1). Notably, a range of largely negative interactions occurred among the ectoparasites; cross-boundary (internal-external) interspecific relationships are evident and several

interactions are subject to alteration by the drivers of climate and host biology (Fig. 5.1).

Table 5.1. The species, location of infection, prevalence (% infected) and mean intensity (excluding zeros, ± S.E.) of parasites infecting a population of yellow-necked mice (*Apodemus flavicollis*) in the Italian Alps. Species that were included in the models are shaded in grey (although *Ixodes ricinus* larvae and nymphs, and *C. solutus* and *D. dasycnema* were included in analyses, they were combined and included within the model as 'Total ticks' and 'Total fleas', respectively, and hence are not shaded).

Parasite spp.	**Site of infection**	**Prevalence (%)**	**Mean intensity (± S.E.)**
Heligmosomoides polygyrus (nematode)	Intestine	57.03	14.5 ± 1.96
Rodentolepis straminea (cestode)	Intestine	21.40	6.56 ± 0.94
Hymenolepis murissylvatici (cestode)	Intestine	0.38	1.00 ± 0.00
Mastophorus muris (nematode)	Stomach	3.80	3.20 ± 1.67
Rictularia proni (nematode)	Intestine	6.84	5.89 ± 1.21
Syphacia spp. (nematode)	Intestine, caecum, colon	63.88	53.33 ± 9.21
Trichuris muris (nematode)	Caecum	8.75	2.22 ± 0.49
Ixodes ricinus larvae	Pelage; ears, tail and feet	69.96	14.28 ± 1.30
Ixodes ricinus nymphs	Pelage; ears, tail and feet	9.89	1.37 ± 0.17
Total ticks	Pelage; ears, tail and feet	70.34	14.34 ± 1.31
Leptotrombidium europaeum (mite)	Pelage; ears	50.19	18.79 ± 1.57
Laelaps agilis (mite)	Pelage	98.86	21.86 ± 1.22
Ctenophthalmus solutus (flea)	Pelage	38.78	1.78 ± 0.13
Doratopsylla dasycnema (flea)	Pelage	3.80	1.10 ± 0.10
Total fleas	Pelage	41.44	1.75 ± 0.12
Lice	Pelage	42.21	7.42 ± 1.00

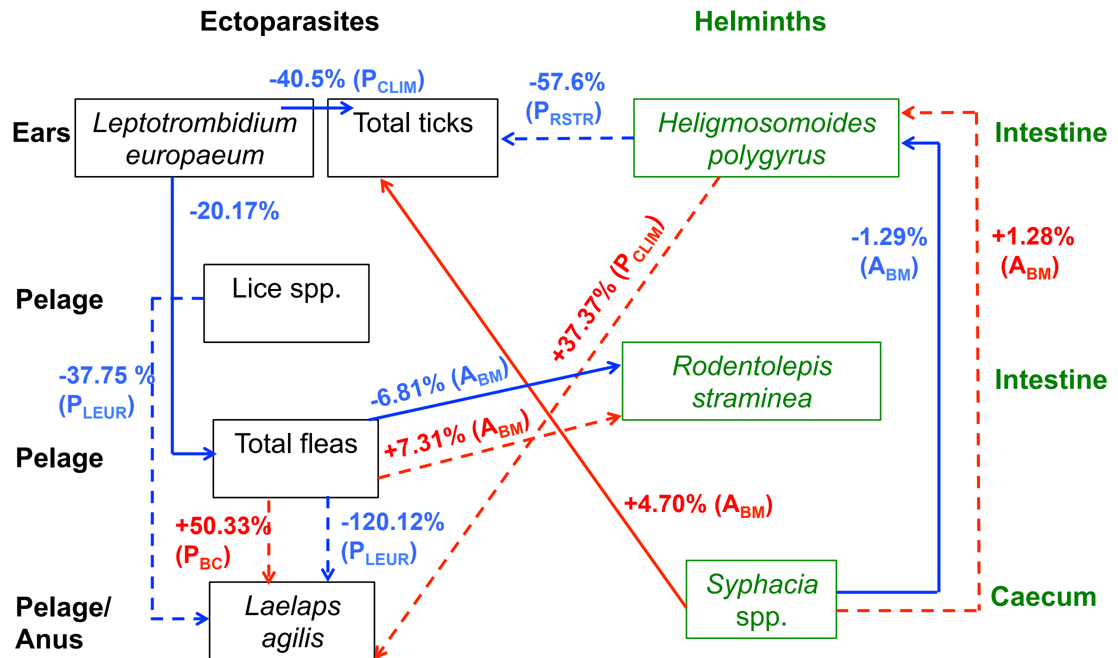

Figure 5.1 Relationships between ectoparasites (black) and helminths (green) infecting yellow-necked mice (*Apodemus flavicollis*) at low (500m) and high (1400m) altitude in the Italian Dolomitic Alps. Negative relationships are represented in blue and positive interactions in red. Whether the relationship is due to the presence of a parasite species (solid lines) or the absence of a species (dashed lines) are depicted. The predicted absolute percentage change (see Materials and Methods for definition) of the dependent variable is given for each interaction. Parentheses identify whether the relationship is based on presence/absence (P) or abundance (A) data; sub-scripts represent interactions dependent upon climate (CLIM), body mass (BM), breeding condition (BC), *Leptotrombidium europaeum* presence/absence (LEUR); or *Rodentolepis straminea* presence/absence (RSTR).

A range of largely negative interactions occurred between ectoparasite species (Fig. 5.1). Flea intensity was reduced by 20.17% when hosts were coinfected with the mite *L. europaeum* ($F_{1, 104} = 4.87$, $p = 0.030$). The mite *L. europaeum* was involved in another significant interaction, whereby the intensity of a second mite species, *L. agilis,* was reduced by 37.75% when hosts were coinfected with both lice and *L. europaeum* ($F_{1, 241} = 8.35$, $p = 0.004$), but reduced by 120.12% when fleas were present and the mite *L. europaeum* was absent ($F_{1, 241} = 6.06$, $p < 0.001$). Focusing solely on the helminth-helminth relationships (Fig. 5.1): only one association was observed; *H. polygyrus* intensity was positively associated with host body mass when *Syphacia* spp. was absent, but the relationship was negative when *Syphacia* spp. was present ($F_{1, 141} = 4.94$, $p = 0.028$; Fig. 5.2).

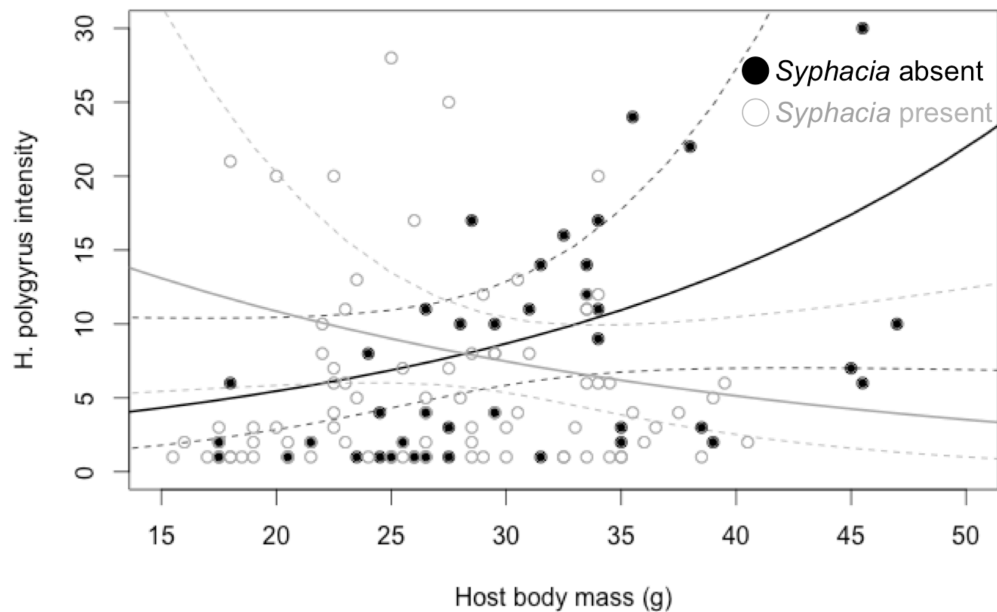

Figure 5.2 The relationship between *Heligmosomoides polygyrus* intensity (excluding zeros) and host body mass when *Syphacia* spp. was absent (black dots) and present (grey dots). The solid lines represent mean predictions from the linear model, and the dashed lines are the 95% confidence intervals surrounding the means.

Focusing on the cross-boundary (internal-external) interplay between ectoparasites and helminths, we found four significant interactions (Fig. 5.1). The cestode *R. straminea*, showed a positive correlation with host body mass when fleas were absent, but this relationship was reversed when fleas were present ($F_{1, 162} = 5.96$, $p = 0.018$). Conversely, there was a positive correlation between tick intensity and body mass when hosts were coinfected with *Syphacia* spp. ($F_{1, 167} = 10.58$, $p = 0.001$), but there was no significant association when *Syphacia* spp. was absent. Tick intensity was also involved in a significant interaction with *H. polygyrus* and *R. straminea*, whereby the intensity of ticks was reduced by 57.6% when hosts were coinfected with *H. polygyrus* but *R. straminea* was absent ($F_{1, 162} = 6.01$, $p = 0.015$). Finally, hosts were infected with 37% more *L. agilis* when *H. polygyrus* was absent, compared to when they were coinfected, but this relationship was only evident at low altitude ($F_{1, 241} = 6.17$, $p = 0.014$).

The relationship between *L. agilis* mite intensity and *H. polygyrus* was one of several interactions where the difference in climate between low and high altitude populations had a significant direct effect on the predicted interactions within the parasite community (Fig. 5.1). There was a reduction in the intensity of both *L. europaeum* mites (57.58%; $F_{1, 126} = 13.98$, $p < 0.001$) and ticks (85.62%; $F_{1, 167} = 7.34$, $p = 0.007$) at high altitude. These two species were also involved in an interaction with one another,

whereby the intensity of ticks at low altitude was reduced by 40.5% in hosts that were coinfected with *L. europaeum* mites ($F_{1, 167} = 7.34$, $p = 0.007$), but at high altitude there was no significant difference between singly-infected and coinfected hosts. In total, five out of eight parasite species were significantly affected by the difference in climate between low and high altitude (Table 5.2). Four of these species were ectoparasites, and *H. polygyrus* was the only helminth species to be affected by the climate. Controlling all other variables (coinfecting species set to zero, body mass set to the mean, host sex set to males and breeding condition set to adults) showed that parasite intensity was lower at high altitude, and intensity increases ranged between 31.46% and 79.97% (Table 5.2).

Table 5.2 The back transformed predicted parasite intensity from the GLM models (and 95% confidence intervals that were directly affected by the change in climate between sites at low (500 m) and high (1400 m) altitude. Model predictions were made with any coinfecting species set to zero abundance or absence, body mass set to the mean, host sex set to males and breeding condition set to adults.

Parasite species	Altitude	Geometric mean	95% confidence intervals	Predicted percentage change from high to low altitude (%)
Heligmosomoides polygyrus	Low	28.57	424.04 to 1.92	56.59
	High	12.40	180.02 to 0.85	
Total ticks	Low	21.48	71.18 to 6.48	79.75
	High	4.35	14.31 to 1.32	
Laelaps agilis (mite)	Low	20.85	32.21 to 13.49	31.46
	High	14.29	20.26 to 10.08	
Leptotrombidium europaeum (mite)	Low	8.20	17.35 to 3.87	55.42
	High	3.65	7.20 to 1.86	
Lice spp.	Low	7.83	16.66 to 3.68	41.39
	High	4.59	8.48 to 2.48	

5.5 Discussion

Our analyses suggest a web of interactions occur between and within the ectoparasite and helminth community of wild mice. When considering only parasite species with a prevalence greater than 10%, hosts were coinfected with up to eight species, with each involved in at least one interaction with another species. These interactions caused substantial changes in the parasite community, with a maximum reduction in the intensity of one parasite species by another of 120.12%, to a maximum increase of

50.33%. Specifically, negative interactions characterised the majority of ectoparasite-ectoparasite relationships; of the twelve interactions observed, there were five predicted cross-boundary (internal-external) interactions between ectoparasites and helminths, and eight interactions were driven by either the change in climate along the altitudinal gradient or the biology of the host.

Four of the five interactions that occurred between ectoparasitic species were negative, such that, where interactions were evident, the intensity of each ectoparasite was generally lower in the presence of interspecific interactions. We posit that this overall pattern of negative correlations may be due to negative feedback induced by grooming in response to local inflammation. The main function of grooming in rodents is to remove ectoparasites (Hawlena *et al.*, 2007, 2008) and can be stimulus-driven. When a host's skin is bitten by an ectoparasite, histamine is released, and the animal grooms in response to the irritation (Willadsen, 1980; Mooring and Samuel, 1998), as such, an increasing ectoparasite burden is expected to induce grooming (Mooring and Samuel, 1998). One exception is observed, however, where the presence of the mite *L. europaeum* appears to cancel out the negative association observed between two of the ectoparasites (lice and fleas) and *L. agilis* intensity. *Laelaps agilis* is a free-moving mite found all over the body, but is concentrated around the base of the tail, whereas *L. europaeum* is located on the ears of the host. A possible explanation for the relationship detected could be that grooming is concentrated on the ears when *L. europaeum* is present, because the irritation caused by *L. europaeum* is greater than the stimulus produced by other ectoparasites located elsewhere on the body. The host may therefore concentrate grooming on the ears, and reduce its efforts on the rest of the body.

Some helminth species manipulate the immune system in order to avoid being expelled by the host (Maizels *et al.*, 2004). One such example in small rodents is immune suppression by *H. polygyrus,* which allows the helminth to avoid detection (Jenkins and Behnke, 1977; Behnke *et al.*, 1978, 1983). In this study, *H. polygyrus* intensity significantly increased with body mass in single-infected individuals, suggesting that there is a peak in the body mass-intensity relationship in older hosts (Woolhouse, 1998; Cattadori *et al.*, 2005). When coinfected with *Syphacia* spp., however, there was a negative correlation between *H. polygyrus* intensity and host body mass. *Heligmosomoides polygyrus* is known to suppress the immune system to avoid detection by the host (Jenkins and Behnke, 1977; Behnke *et al.*, 1978, 1983). Infection with *Syphacia* spp. has been shown to induce a T helper cell 2 (Th2) immune response

in hosts, with elevated interleukin 4 (IL-4), IL-5 and IL-13 cytokine production, and mice deficient in ILs demonstrated chronic parasite infection compared to hosts mounting an IL immune response (Michels *et al.*, 2006). The immune modulatory effects of *H. polygyrus* may therefore be lost due to the Th2 immune response stimulated by the coinfecting *Syphacia* spp., thereby exposing *H. polygyrus* to immune cells that can reduce helminth numbers.

In addition to the within-group (i.e. ectoparasite-ectoparasite and helminth-helminth) relationships observed, there were also five significant cross-boundary interactions between ectoparasite and helminth species, which have only been investigated previously in one other rodent study (Ferrari *et al.*, 2009). Cross-boundary interactions are unlikely to arise from competition for space, as the two parasite types occupy different parts of the host's body. Similarly, they are not likely to be mediated by resource competition, although this is still possible (e.g. if both species feed on blood). Due to the different locations occupied by the parasites (gastrointestinal system and skin), these interactions are most likely to be mediated by a systemic response of the immune system (Lello *et al.*, 2004; Graham, 2008; Lass *et al.*, 2013), but this hypothesis requires further investigation as the design of the study did not provide the opportunity to identify causal mechanisms of community interactions.

In this study, the difference in climate between high and low altitude fell within the range of changes that have been predicted by the year 2100 (IPCC, 2013), and the conditions at low altitude, therefore, are representative of future climatic predictions in comparison to high altitude. For all ectoparasites (with the exception of fleas) and one helminth species (*H. polygyrus*), climate had a significant affect on parasite intensity. In total, five out of eight parasite species were significantly affected by the difference in climate between the two altitudes. Overall, there was a greater intensity of parasite species at low altitude compared with high altitude, with increases ranging from approximately 30% to 80%. Similar increases in ectoparasite load from high to low altitudinal sites has previously been reported in single parasite species studies (Jouda *et al.*, 2004; Morán Cadenas *et al.*, 2007; Gern *et al.*, 2008; Gilbert, 2010; Zamora-Vilchis *et al.*, 2012), although these studies have only examined ectoparasites and the pathogens they transmit. The reason for the reductions in parasite intensity reported at high altitude may be because ectoparasites spend their entire life-cycle in the external environment, and the climatic conditions at low altitude may be more favourable for survival (Daniel, 1993; Jouda *et al.*, 2004; Gilbert, 2010). The only helminth species affected by the

climate was *H. polygyrus*, and hosts at low altitude were infected with the highest intensity. Eggs of *H. polygyrus* hatch and develop through two larval stages in the environment. In comparison, female *Syphacia* spp., which were unaffected by the difference in climate, migrate to the anus and deposit embryonated eggs on the skin, which become infective within 5-20 hours; hosts become infected by either ingesting eggs from the perianal region of an infected animal or from contaminated faeces (Taffs, 1976), therefore, they are little exposed to environmental pressures. Eggs of *R. straminea* are ingested by an insect intermediate host, which is later ingested by the rodent (Casanova *et al.*, 2001); the length of time that eggs are able to remain in the environment is unknown but it may be that they are buffered from the climatic conditions by being inside their intermediate host. As *H. polygyrus* eggs develop into larvae directly in the soil, the larvae may be more affected by the different climatic conditions than the other two species, so leading to the within-host patterns observed.

The intensity of over half of the parasite species investigated in this study were affected by the climate, and with increases in global surface temperatures that have been predicted for the future (IPCC, 2013), there would be an increase in the intensity of many of the parasites involved in this community. In addition to the direct affects of climate change on parasite intensity, two interactions between parasite species were also affected. The highly interactive nature of the microparasite communities of wild rodents has previously been reported (Telfer *et al.*, 2008, 2010), and the current study suggests that this relationship extends across multiple parasite taxa. Due to the interactions between the ectoparasite and helminth community, changes that directly affect the intensity of one species, therefore, could have knock-on effects for the intensity of another species. For example, *L. europaeum* intensity significantly increased at low altitude. In turn, *L. europaeum* decreased the intensity of fleas, which would ultimately lead to a decrease in *R. straminea*. So, the climatic influence on *L. europaeum* leads to a change in *R. straminea*, which is not directly affected by the climate, although the exact degree of effect needs to be determined through modelling. Climate is, therefore, having both direct and indirect effects on the parasite community.

In summary, the ectoparasite and helminth community of wild mice is characterised by a complex web of interactions. This is the first study to investigate the interactions that occur between multiple ectoparasite and helminth communities (rather than focusing on a single ectoparasite or helminth interaction), and the number of cross-boundary interactions suggests that these two sectors of the community are interactive. Changes to

one type of parasite will not only affect other species of the same parasite type (i.e. ectoparasite-ectoparasite or helminth-helminth), but due to the complex web of cross-boundary interactions that exist, there will be knock-on effects for the entire parasite community. Indeed, one aspect not included here was the microparasite community, which within rodents is also known to be interactive (Telfer *et al.*, 2010), and so future work should consider incorporating microparasites into the analysis to determine the effect it might have on the parasite community. This is especially pertinent if we consider that the location of this field study, Trentino, has been identified as a hotspot for tick-borne diseases (Hudson *et al.*, 2001; Carpi *et al.*, 2009), which are pathogenic to humans and includes *Borrelia burgdorferi sensu lato*, Apicomplexa, *Ehrlichia* spp., and tick-borne encephalitis (Beninati *et al.*, 2002; Perkins *et al.*, 2003; Kallio-Kokko *et al.*, 2006; Rizzoli *et al.*, 2009). In addition, in this area recently, an unprecedented increase in the prevalence of the rodent-borne zoonotic infection, hantavirus has been documented, and although the mechanism behind this increase has not been identified, climate has been suggested as playing a role (Rizzoli *et al.*, 2014).

In our study, climate significantly affected the majority of parasite species, and due to the interactive nature of the community, the intensity of other species were indirectly affected. This study points to the necessity of taking a whole community approach when considering the consequences of climate change on parasite communities. It is essential to note, for example, that had the intensity of *R. straminea* alone been investigated, we would have concluded this species was unaffected by climate. Through its interaction with other parasite species, however, this conclusion is invalid. Future investigations should consider taking account of community relationships when examining the affect of climate on host-parasite interactions.

5.6 Author acknowledgements

The manuscript resulting from this chapter is authored by:

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Chapter 6

The role of tick endosymbionts in altering the emigration rates of ticks

6.1 Abstract

Ticks are host to a diverse microbial community comprising both mutualistic (endosymbiont) and pathogenic species, of which the former are mostly vertically transmitted, and the latter are horizontally transmitted via the vertebrate hosts from which they take a blood meal. Both endosymbionts and tick-borne pathogens may be expected to facilitate their own persistence and maximise fitness by increasing the likelihood that a tick will find and attach to a vertebrate host. It has been previously assumed that ticks do not rapidly emigrate from a vertebrate host post-death. Upon host death, however, we posit that ticks infected with endosymbionts and/or tick-borne pathogens could initiate rapid transfer of the ticks to new, viable vertebrate hosts to ensure their continued persistence. To test this hypothesis, this study quantified how quickly and how many *Ixodes ricinus* ticks emigrated from yellow-necked mice (*Apodemus flavicollis*) up to 24 hours post-host death, and examined whether infection with endosymbionts, which includes tick-borne pathogens affected tick emigration behaviour. Rodent host blood samples (n = 20) and the ticks emigrating from them (n = 39) were screened for *Ehrlichia* spp. and Apicomplexa using 18S or 16S rDNA PCR. Ticks began to emigrate from rodents within 2 hours post-death, and within 24 hours, 54.7% of the tick population had left the carcasses. Thus, tick intensity reported from animals that have been dead for 24 hours or more is likely an underestimate, but prevalence data may still be informative. Emigration rates were significantly faster for *Ehrlichia*-positive ticks compared to those without infection, whilst there was no such link with Apicomplexa. An endosymbiont species, therefore, appears to drive emigration of ticks away from dead hosts, potentially increasing the likelihood of the tick finding a new host, which is ultimately of benefit to the endosymbiont.

6.2 Introduction

Ticks play host to a diverse microbial community, including pathogenic agents, commensals and mutualistic endosymbionts (Carpi *et al.*, 2011). Some endosymbionts appear to have no pathogenic activity and form long-term associations with the tick (Duron *et al.*, 2008; Clay *et al.*, 2008; Beninati *et al.*, 2009), although their presence has been implicated in pathogen transmission (Childs and Paddock, 2002; Ahantarig *et al.*, 2013). Ticks typically acquire endosymbionts via vertical or transovarial transmission, especially in the case of obligate symbionts (Beninati *et al.*, 2004; Lo *et al.*, 2006;

Klyachko *et al.*, 2007; Rynkiewicz *et al.*, 2015), but also during blood feeding on a vertebrate host (Bremer *et al.*, 2005; Munderloh and Kurtti, 2011; Carpi *et al.*, 2011). Pathogenic endosymbionts, for example, *Borrelia* spp., the causative agent of Lyme disease, are transmitted between ticks horizontally, via their vertebrate blood host (Ostfeld and Keesing, 2000). In addition, endosymbionts appear to play an important role in the biology of ticks; engorged female ticks, for example, treated with an antibiotic to remove endosymbionts laid a smaller proportion of eggs and successfully hatched fewer eggs than untreated females, and of those hatching the proportion of viable larvae that developed was also reduced (Zhong *et al.*, 2007). Bacterial symbionts, particularly those that are vertically transmitted (e.g. *Midichloria mitochondrii*; see Beninati *et al.*, 2004; Lo *et al.*, 2006), stand to maximise their own fitness if they can influence their tick host in a manner that enhances their own transmission.

Whilst many endosymbionts are mutualistic and benefit the invertebrate host (e.g. ticks), others can be pathogenic to the vertebrate host from which the invertebrate takes a blood meal, as such, these invertebrates are vectors (Bourtzis and Miller, 2003; Hosokawa *et al.*, 2008). These pathogenic endosymbionts, also known as vector-borne pathogens, may alter the behaviour of vectors to facilitate their own transmission (Azad and Beard, 1998; Koella *et al.*, 1998). Infection with tick-borne pathogens, for example, has been shown to increase the activity of questing (host-seeking) ticks, making them more likely to find a host and successfully transmit the pathogen (Alekseev *et al.*, 2000; Herrmann and Gern, 2012; Belova *et al.*, 2012). Given the benefits to both endosymbionts and their tick hosts of the mutualistic relationship, and the evidence of behavioural manipulation of ticks by pathogens, we posit that infected ticks may exhibit behavioural adaptations to optimise their probability of maintaining this association, for example active host-seeking, a corollary of which would be active host emigration following host death. Pervasive in the literature, however, is an assumption that upon death of a vertebrate host on which a tick is feeding, ticks do not emigrate from their host (McCay and Durden, 1996; Sherrard-Smith *et al.*, 2012); a poor strategy for maximum fitness of both tick and endosymbiont survival.

The assumption that tick vectors do not emigrate from their host rapidly after host death has been used previously to justify the hypothesis that tick counts from dead hosts are a good estimate of the burden of the live host (e.g. McCay and Durden, 1996; Sherrard-Smith *et al.*, 2012). Yet, in some cases, the prevalence and abundance of some flea and tick species infesting dead hosts has been observed to be lower than those on live

animals (Gross and Bonnet, 1949; Colbo and MacLeod, 1976; Millán *et al.*, 2004). This observation suggests that ticks and other vectors are likely to emigrate from dead hosts, but to the best of our knowledge, it is not known when this process begins. Yet, knowing when and at what rate emigration occurs is vital to prevent underestimating the prevalence, abundance and/or intensity of vectors infesting dead hosts that have been left in the environment, either by experimental design (e.g. snap-trapping; Gilbert *et al.*, 2000) or whilst using opportunistic survey methods (e.g. roadkill; Lorusso *et al.*, 2011; Sherrard-Smith *et al.*, 2012).

Vectors that do not leave a host carcass risk being ingested by a scavenger, resulting in a dead-end for the transmission of endosymbionts, unless transmission can occur via the consumption of infected vectors. One such example of this is louping ill virus in ticks (Gilbert *et al.*, 2004), but in general, this mechanism of pathogen transmission is considered rare. It would, therefore, be advantageous for an endosymbiont or vector-borne pathogen to facilitate rapid vector emigration post-host death, allowing a new host to be located quickly. There is evidence to suggest that infection with tick-borne pathogens alters tick behaviour, for example, by increasing questing (Belova *et al.*, 2012), and motor activity (Alekseev *et al.*, 2000; Herrmann and Gern, 2012). Yet, whether infected ticks emigrate faster from dead hosts than uninfected ticks, or whether certain endosymbionts may be fundamental in initiating these behaviours, is not known.

The aims of this study were, firstly, to quantify how quickly and how many ticks (*Ixodes ricinus*) left a rodent host (yellow-necked mouse, *Apodemus flavicollis*) 24 hours post-host death. Secondly, we examined whether ticks that emigrated from hosts were more likely to be infected with endosymbionts, which can be either mutualistic or pathogenic (*Ehrlichia* spp., Apicomplexa), or tick-borne pathogens (*Anaplasma phagocytophilum and Borrelia burgdorferi* sensu lato). These pathogens can cause debilitating infections in humans, including Lyme disease and human granulocytic anaplasmosis (Burgdorfer *et al.*, 1982; Chen *et al.*, 1994). The prevalence of these infections in the ticks of wild rodents has been used previously to assess the risk to humans (Courtney *et al.*, 2004), but the risk may be underestimated if the presence of these pathogens results in infected ticks leaving the host faster than uninfected ticks, which would exclude them from pathogen prevalence studies.

6.3 Materials and methods

6.3.1 Vector-host system

The yellow-necked mouse (*Apodemus flavicollis*) is a common small mammal found throughout Europe. In Trentino, Italy, it is host to the sheep tick, *Ixodes ricinus.* Trentino is a hotspot for tick-borne diseases (Hudson *et al.*, 2001; Carpi *et al.*, 2009), with ticks in this region known to transmit a range of pathogens, including *Borrelia burgdorferi* s.l., Apicomplexa, *Anaplasma* spp. and *Ehrlichia* spp. (see Gray *et al.*, 1992; Hillyard, 1996; Porretta *et al.*, 2013).

Live-trapping of yellow-necked mice was carried out for 7 days in June 2012 on Monte Bondone, Trentino, Italy (46°2'13.38N, 11°0'12.64E), using 60 multi-capture live Ugglan traps (Grahnab, Sweden). Trapping occurred at three distinct trapping sites, located in broadleaf woodlands, with at least 300 m between each site to ensure independent mouse populations were sampled. Traps were baited with a standardised amount of seed with a slice of potato as a water source, and were set daily. For each mouse caught, sex and breeding condition (sub-adult or adult) were recorded. Adult status (defined as males with descended testes and females with perforated vaginas) and the presence of ticks were criteria for inclusion in the current study (n = 20). Mice were euthanized *in situ* using isofluorane followed by cervical dislocation, and an ocular blood sample was taken immediately post-death. All the mice included in this study were euthanised for the purposes of another study (Chapter 5). All animal-handling procedures and ethical issues were approved by the Provincial Wildlife Management Committee (Authorization Number 595 issued on 04.05.2011). Approval to trap animals was granted by the Comitato Faunistico of the Autonomous Province of Trento.

Each carcass was placed immediately into an individually-sealed plastic bag for transportation to the laboratory (< 1 hour), where mice were individually placed onto a suspended net over a smooth-sided dish (15cm x 25cm x 5cm), filled with 20 ml of water for 24 hours. Ectoparasites that moved off the carcass became suspended in the water, allowing them to be collected. All ticks in the initial transportation bag were counted as the first time period (1 hour post-death). At 3, 4, 6, 8, 10, 13, 22 and 24 hours post-death, ticks leaving the carcass were collected from the water, counted, identified to species level (using Hillyard, 1996) and stored in 90% molecular grade ethanol. After 24 hours, each mouse was washed with running water for 5 min over a

sieve with a 710 µm aperture, to remove any remaining ticks from the carcass. The carcass was also finely combed and microscopically examined to ensure full removal of all ticks, yielding a maximum tick count for each host. Hosts were simultaneously infected with fleas and mites, but for the purpose of this study, only ticks and their tick-borne endosymbionts and pathogens were considered.

6.3.2 Detecting vector-borne endosymbionts and pathogens

Both the infection status (defined here as presence of either endosymbionts or pathogens) of the mouse and the ticks themselves were investigated as drivers for a change in emigration of the ticks. Mice were infested with both tick larvae (prevalence = 100%; mean intensity (excluding zeros, based on the definition of Bush *et al.*, 1997) ± S.E. = 18.9 ± 1.9) and, to a lesser extent, nymphs (prevalence = 7.0%; mean intensity ± S.E. = 1.1 ± 0.1). Due to the low prevalence of nymphs infesting the mice, which is typical for rodents (Randolph, 1998; Perkins *et al.*, 2003; Ferrari *et al.*, 2009) only larvae were examined for the presence of endosymbionts. The presence or absence of endosymbionts was established using molecular techniques; ticks were selected in terms of the first individual to leave each carcass (n = 20) plus one engorged tick removed from the host during carcass washing (>24 hours post-death, n = 19), and a blood sample was taken from each mouse (n = 20). DNA was extracted from single ticks and 100 µl blood using the ammonia method according to Bown *et al.* (2003). Ticks placed in the base of an Eppendorf tube were crushed with a sterile pin tip prior to extraction. Ammonia hydroxide (1.25% v/v) solution (100 µl for larval ticks or 500 µl for blood) was added to each sample and incubated at 100°C for 20 minutes. Following brief centrifugation (60 rpm for 20 seconds), the samples were left at 24°C for a further 20 minutes or until approximately half of the solution had evaporated.

Each DNA sample was tested for two taxa of endosymbionts, which can be either mutualistic or pathogenic strains (*Ehrlichia* spp. and Apicomplexa; see Simpson *et al.*, 2005) and two species of tick-borne pathogens: (*Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l.; see Courtney *et al.*, 2004). The *Ehrlichia* primers were not specific and have been shown to amplify both mutualistic and pathogenic endosymbionts and we were unable to determine species. The Apicomplexa primers were also non-specific and have been shown to amplify *Babesia* spp. but also *Hepatozoon* spp. and various other Apicomplexa (Simpson *et al.*, 2005). Different

amplification methods were necessary for each endosymbiont/pathogen. A single-step PCR was used to amplify a 75 bp 16S rRNA gene fragment of *Ehrlichia* bacterial DNA (Courtney *et al.*, 2004). A total of 25 µl volume – including 2 µl DNA extract, 12.5 µl Taq DNA polymerase Master Mix (Boehringer Mannheim, Germany) and primers, EHR521F and EHR747r (20 ρmol/µl, Belongia *et al.*, 1997) – was subjected to PCR conditions involving an initial denaturation step at 94°C for 5 minutes followed by 40 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 15 seconds, with a final extension time of 5 minutes.

A two-step nested PCR was used to detect Apicomplexa DNA using BmF1 and F2, and BmR1 and R2 primers to target a 700 bp 18S rRNA gene fragment (following Simpson *et al.*, 2005). False-negatives can occur in Apicomplexa infections from blood samples because of DNA from non-pathogenic strains in the host. Presence or absence of *Ehrlichia* and Apicomplexa infections were confirmed by electrophoresis of the PCR products on 1.5% agarose gels.

To identify positive cases of *A. phagocytophilum* and *B. burgdorferi* s.l., real-time PCR was applied to extracted DNA samples. A total volume of 25 µl, containing Taq polymerase Master Mix (Boehringer Mannheim, Germany), the primers (20 ρmol/µl) Bb235F and Bb235R for *Borrelia* and 2 µl DNA template, was prepared separately for each pathogen type. The real-time PCR had an initial denaturation at 94°C for 5 minutes, followed by 40 cycles of 94°C for 30 seconds, an annealing step at 55°C for 30 seconds and extension step at 72°C for 15 seconds, with final extension for 5 minutes (Courtney *et al.*, 2004).

6.3.3 Statistical analysis

The mean emigration rate of ticks from dead hosts was estimated by fitting a function describing tick loss over time, selected using log likelihood tests. Whether the relative tick loss over time was dependent on the infection status of the ticks was explored graphically by comparing the relative functions for emigration rates of ticks with and without infection. To test whether the infection status of the host was associated with tick emigration from the dead host, an exact binomial test on the mean count of ticks that were infected with each endosymbiont was compared to ticks without infection. If infection status was shown to affect tick emigration significantly, the time of the first

tick to leave was recorded and compared for ticks leaving infection-positive mice to infection-negative mice (Mann-Whitney Test). All statistical analyses were performed using R version 14.2 (R Core Development Team, 2013).

6.4 Results

On average, yellow-necked mice (n=20) were infected with 26.4 ± 3.5 *Ixodes ricinus* ticks. Ticks were first recorded leaving the hosts within 2 hours post-death, with most ticks emigrating between 8 and 10 hours post-death (mean intensity ± S.E. = 3.6 ± 0.9 ticks). Within 24 hours post-death, over half (54.7%) of the total tick burden infecting any given host had emigrated. In only one case had all ticks emigrated from the host within 24 hours.

The logistic function fitted best to the emigration rate data (Fig. 6.1); the predicted number of ticks to have left the carcass after 24 hours was 63%, and 18.5% of ticks were predicted to have left the carcass after 10 hours, but the wide 95% confidence intervals indicated that the emigration rate was very variable.

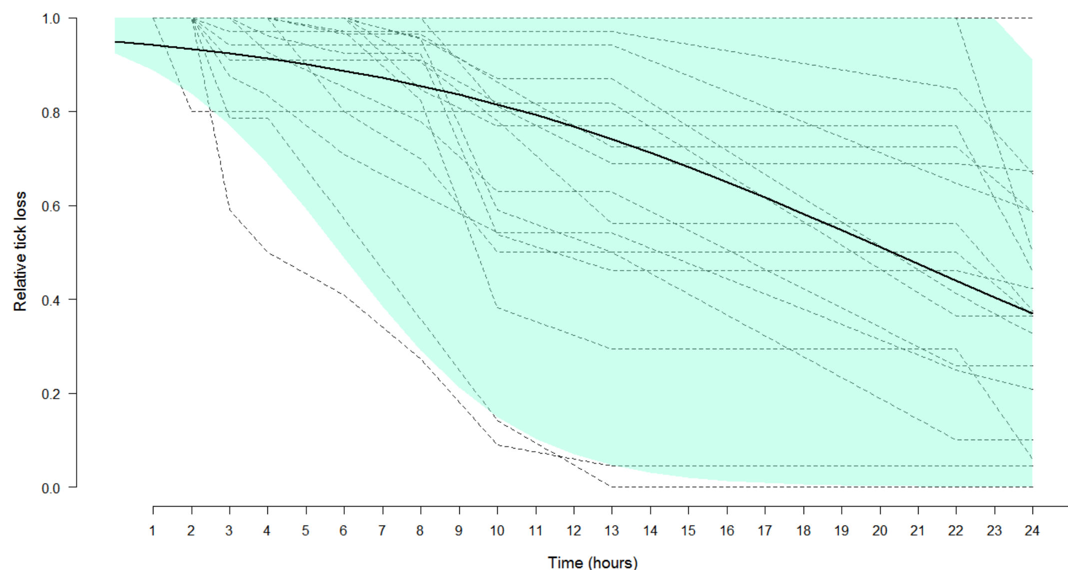

Figure 6.1: The relative tick (*Ixodes ricinus*) loss from yellow-necked mice (*Apodemus flavicollis*) over 24 hours. The dotted grey lines represent tick loss from each individual mouse, the black line represents the logistic fit for emigration rate, and the shaded area is the upper and lower 95% confidence intervals.

6.4.1 Tick infections

In total, 64.1% (25 out of 39) of ticks were infected with *Ehrlichia* spp. and 51.3% (20 out of 39) with Apicomplexa (Table 6.1). Of the 20 ticks that were first to leave their host, 65% (13 out of 20) were infected with *Ehrlichia* spp. and 35% (7 out of 20) with Apicomplexa, whilst of the 19 ticks removed from hosts during washing (at 24 hours), 63.2% (12 out of 19) were infected with *Ehrlichia* spp. and 68.4% (13 out of 19) with Apicomplexa. The functional fits describing the relative tick loss with and without infection indicated that ticks that were positive for *Ehrlichia* spp. and to a lesser extent Apicomplexa, did indeed leave the host at a more rapid rate than those ticks that were negative (Fig. 6.2). A greater proportion of ticks infected with *Ehrlichia* spp. had left the host at 24 hours than uninfected ticks (probability of infected ticks having left within 24 hours = 0.6 [95% CI: 0.39-0.78], vs uninfected probability = 0.34 [Exact Binomial test: p = 0.008]). Conversely, there was no significant difference between ticks infected with Apicomplexa and uninfected ticks leaving hosts within 24 hours (probability of infected ticks having left within 24 hours = 0.6 [95% CI: 0.39-0.79], vs uninfected probability = 0.409, [Exact Binomial test: p = 0.066]). *Anaplasma phagocytophilum* was not detected in any of the ticks sampled. Just one tick was positive for *B. burgdorferi* s.l. and this tick was the first to leave its host 6 hours post-death.

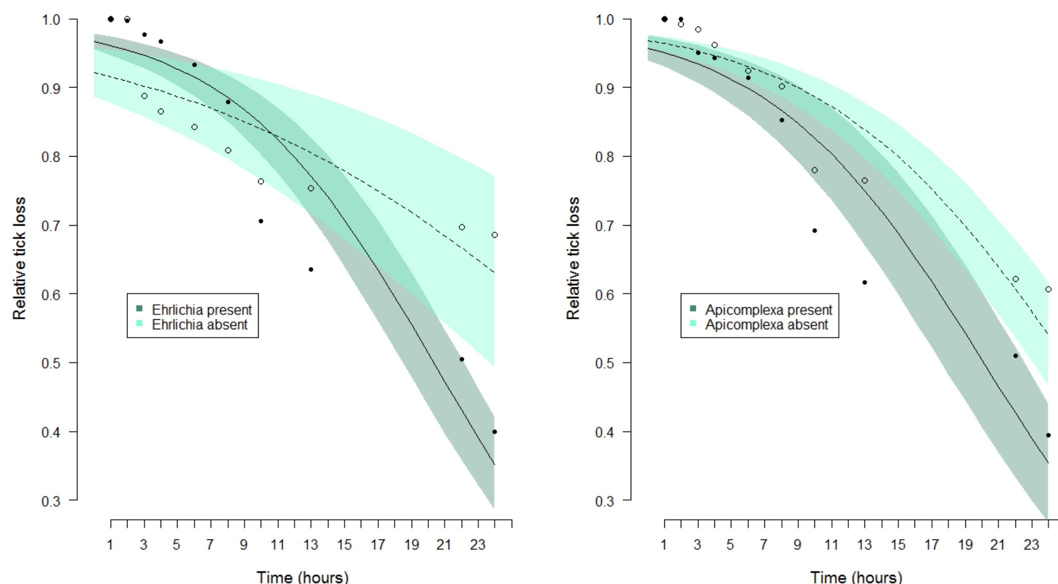

Figure 6.2 The mean (and standard error shown as shaded areas) relative loss of ticks (*Ixodes ricinus*) leaving a dead host (yellow-necked mouse, *Apodemus flavicollis*) with (solid lines; fitted using the logistic function as described in methods) or without (dashed lines) *Ehrlichia* (left figure) or

Apicomplexa (right figure) infection over a 24-hour period. If any tick on a given mouse was infected then that mouse was also considered to be infected.

6.4.2 Host infections

From blood serum analyses, 20% of hosts were infected with *Ehrlichia* spp. (Table 6.1), suggesting that there was no transovarial transmission. The average time for the first tick to leave was 4.2 hours for *Ehrlichia*-positive mice (n = 5) compared to 6.4 hours for *Ehrlichia*-negative hosts (n = 15; Mann-Whitney test $W_{20} = 17.5$, $p = 0.08$). Of the 10 ticks analysed from *Ehrlichia*-positive mice, 70% were infected with *Ehrlichia* spp., but 62% (18 out of 29) of ticks infesting *Ehrlichia*-negative hosts were also infected. There was no significant difference in the emigration rate of infected ticks from *Ehrlichia*-positive or *Ehrlichia*-negative hosts (Exact binomial test: $p = 0.42$). Despite over half of the ticks testing positive for Apicomplexa, all hosts tested negative (Table 6.1), and neither *B. burgdorferi* s.l. nor *A. phagocytophilum* were detected in any host specimens sampled.

Table 6.1: Number of ticks (*Ixodes ricinus*) infected with pathogens that were the first to emigrate from a dead host (yellow-necked mice, *Apodemus flavicollis*), were removed from the host 24 hours post-death and the number of infected blood samples.

	Number of hosts	Pathogen infection			
		Apicomplexa	*Ehrlichia* spp.	*Borrelia burgdorferi* s.l.	*Anaplasma phagocytophilum*
First tick to emigrate	20	7	13	5	0
Engorged tick removed from host	19	13	12	0	0
Host blood sample	20	0	5	0	0

6.5 Discussion

The behaviour of *Ixodes ricinus* ticks appeared to be influenced by infection with *Erhlichia* spp., but not Apicomplexa. Ticks positive for *Erhlichia* spp. emigrated away from dead hosts faster than those that were uninfected, which has potential fitness advantages for both the tick and endosymbiont. This was not, however, the case for ticks or hosts infected with Apicomplexa. Over half of the ticks had emigrated from a

dead host within 24 hours of host-death regardless of tick or host infection status. This relatively rapid emigration of ticks has consequences for studies that estimate tick intensity from carcasses.

Ehrlichia-infected ticks emigrated from dead mice at a significantly faster rate than ticks infected with Apicomplexa, or uninfected ticks, supporting previous studies that have indicated microbes can alter tick behaviour (Hosokawa *et al.*, 2008; Belova *et al.*, 2012; Alekseev *et al.*, 2000; Herrmann and Gern, 2012). Ticks sampled from dead hosts, therefore, may not be suitable for estimating the prevalence of endosymbionts in a population as infected ticks may have emigrated from the dead host faster than uninfected ticks. Although we cannot yet comment on the endosymbiont or pathogenic status of the *Ehrlichia* species in our study, infection with endosymbionts has been found to increase the activity of infected ticks (Hosokawa *et al.*, 2008), and tick-borne pathogen infection was observed to alter vector behaviour: tick-borne encephalitis (TBE), for example, causes infected ticks to become more active during questing (Alekseev *et al.*, 1988; Belova *et al.*, 2012). Such behavioural changes can increase the reproductive potential of pathogens through increased likelihood of finding a subsequent host successfully.

When a tick infected with a tick-borne pathogen emigrates from a dead host, it would be logical to conclude that the host is infected with the same pathogen because *I. ricinus* individuals feed only once per life stage (e.g. Cotté *et al.*, 2008). Yet, not all ticks that emigrated from *Ehrlichia*-positive mice were positive (3 of the 10 were negative). An explanation for this result could be preferential PCR amplification of infections from the ticks rather than the blood, because the entire tick was sampled compared to just a 100 µl sample of the host's blood, introducing the possibility of false negatives (e.g. Schulz *et al.*, 2003). This may also explain the low prevalence of *Ehrlichia* spp. detected in mouse blood samples, and similar mis-matches between infection detected in ticks compared to their hosts have been reported elsewhere (e.g. Chu *et al.*, 2008). Alternatively, *Ehrlichia* spp. infection may simply increase activity rates of the ticks (e.g. Herrmann and Gern, 2012) such that they switch hosts more regularly, perhaps without feeding to repletion, although there is no evidence yet to support this hypothesis. No mice were infected with Apicomplexa and there was no evidence that ticks infected with Apicomplexa (51.3%) left the carcass earlier than uninfected conspecifics. Mice were not infected with *A. phagocytophilum*, and there is recent evidence suggesting that yellow-necked mice may not be competent hosts of the

pathogen (Baráková *et al.*, 2014). None of the mice were infected with *B. burgdorferi* s.l.

Ticks began to emigrate from the carcass within 2 hours post-death. This suggests that tick intensity studies using any method where lethal traps remain unchecked for more than two hours (e.g. Gilbert *et al.*, 2000; Mihalca *et al.*, 2012), or those reliant on opportunistic sampling such as road-kills (e.g. Lorusso *et al.*, 2011; Sherrard-Smith *et al.*, 2012), are unlikely to represent accurate tick burdens of living hosts. If the observed rapid emigration rate in our study is a generality then this has clear implications for data interpretation. Our data do suggest, however, that tick prevalence studies are representative of natural *in situ* tick prevalence; on average, 54.7% of the total tick population emigrated from a host within 24 hours. The wide confidence intervals also suggest that emigration rates are highly variable. Whilst increasing the sample size to understand better such a pattern could tighten these conclusions, it is ethically necessary to restrict sampling during destructive studies and these results still provide a useful insight into tick behaviour.

In conclusion, *Ehrlichia* spp. infection facilitates earlier movement of ticks away from a dead host implying that this endosymbiont manipulates host behaviour to maximise its own fitness. In addition, this work questions whether ticks sampled from dead hosts are suitable for estimating the prevalence and the intensity of endosymbionts and tick-borne pathogens in a population given that over half of the ticks infesting hosts had emigrated within 24 hours post-host death. The model we present could be used to calibrate counts of ticks on hosts that have been dead for less than 24 hours if a given study's purpose is to estimate intensity, but confidence intervals were large for our data. Overall, this study highlights the complexity of host-parasite relationships and suggests that the presence of endosymbionts alters the behaviour of their tick host.

6.6 Author acknowledgements

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Chapter 7

General discussion

Changes in the global climate alter ecological traits, including the phenology, distribution and survival of species (Pounds *et al.*, 1999; Root *et al.*, 2003; Jenni and Kéry, 2003; Hickling *et al.*, 2006; Carvalho *et al.*, 2010). As the majority of parasite species spend at least a portion of their lifecycle exposed to the ambient climate, they will not be exempt from similar climate-induced variation. As global surface temperatures continue to increase, the response of parasites to future climatic changes will have implications for the health and survival of wildlife, agricultural and human populations (Harvell *et al.*, 2002; Epstein, 2005; Patz *et al.*, 2005). By the year 2100, global surface temperatures are predicted to increase by 1.1-4.8°C (IPCC, 2013). This range of predicted temperature increases typically occurs along altitudinal gradients (Körner, 2007), as such altitude can be used as an empirical test of climate change on ecological systems (e.g. Hodkinson, 2005; Körner, 2007; Garibaldi *et al.*, 2011; Michalet *et al.*, 2014).

This study has investigated the effect that climate has on host-parasite interactions using a comparative analysis, followed by field experiments on wild yellow-necked mice (*Apodemus flavicollis*) and their parasite community along an altitudinal gradient. Increases in temperature within the range predicted by the Intergovernmental Panel on Climate Change (IPCC) to occur by the year 2100 were found to increase parasite abundance and accelerate development time (Chapter 2). Although no broad effect of temperature was found across host-parasite taxa on parasite fecundity within the meta-analysis, empirical work led to the finding that altitude (climate) had a regulatory effect on fecundity, whereby female hosts at low altitude were infected with the least fecund nematodes due to a male-biased sex ratio (Chapter 3). Importantly, coinfection with multiple parasite species was found to alter the relationship between infection load and infectiousness, such that hosts infected with just one helminth species had a negative relationship between parasite load and infectious output, but when coinfected the relationship was reversed, although this relationship was species-specific (Chapter 4). Following on from determining how coinfection changes infectious output, a whole macroparasite community analysis was carried out to determine the extent to which the parasite community interacts, in the same manner that free-living communities do. This analysis represents the first full macroparasite community analysis of any host-parasite system. The parasite community was found to be fully interacting, with strong negative feedback loops between ectoparasites and 'cross-boundary' interactions between helminths and ectoparasites. In addition, the community interactions were different at

high and low altitude, suggesting a potential impact of climate. Extending these findings to other host-parasite systems the implications for management of such a fully interacting system is that control, via removal, of any given parasite has the potential to alter the whole parasite community, which could have cascade effects elsewhere in the community and negative effects on human health if zoonotic pathogens increase (Chapter 5). Finally, whilst the host has a community of interacting parasites it was recognised that parasites themselves can harbour a community of hyperparasites, akin to a microbiota, and that these endosymbionts may alter their parasite host's behaviour. It was found that ticks infected with key endosymbionts altered tick behaviour, possibly as an evolutionary adaptation to facilitate their own transmission to a new host (Chapter 6).

The broad effects of temperature on parasite life-history traits were dependent upon the degree of warming or cooling. Temperature increases between 0.1°C and 5°C, which fall within the range of warming for predicted global surface temperatures (IPCC, 2013), resulted in greater parasite abundance and increased development times. The increased infection load on hosts is expected to reduce host fecundity, but for species which are also being negatively impacted by other factors, for example, pollution, the combination of factors can result in greater host mortality, as has been seen for amphibians (Pounds *et al.*, 2006). Climate change alone does have negative impacts of species, and in response some change their distribution, but others are geographically bounded, for example, montane species (Grabherr *et al.*, 1994). It is these climate 'refugee' species that may be most vulnerable to the effects of elevated parasitism and any reductions in their numbers or even extinction may lead to reductions in biodiversity (Brooks and Hoberg, 2000; Daszak *et al.*, 2000; Fayer, 2000; Marcogliese, 2001; Harvell *et al.*, 2002; Lafferty, 2009a). Whilst broad patterns were observed with the meta-analysis, expanding these findings to other free-living host-parasite systems is problematic. The majority of studies investigated parasite response to a constant change in temperature. In the natural environment, however, parasites are continually exposed to stochastic climatic conditions, and laboratory studies involving only constant temperatures may over- or underestimate climate-induced effects on parasite life-history traits (Saunders *et al.*, 2002; Paaijmans *et al.*, 2009; Hernandez *et al.*, 2013). Parasitic nematodes are also known to evolve rapidly and adapt to warmer temperatures, and so the effect of temperature perturbations on parasite life-history traits may not be reduced (Crofton *et al.*, 1965; Thomas, 1991; van Dijk and Morgan, 2010). In addition, one host

and one parasite was investigated in Chapter 2, but Chapters 4 and 5 showed that variation in life-history traits – namely fecundity and abundance can be driven by coinfecting parasites within an individual host.

Other studies have determined that coinfecting parasites interact (Behnke *et al.*, 2001a; Cox, 2001; Lello *et al.*, 2004; Pedersen and Fenton, 2007; Graham, 2008; Telfer *et al.*, 2010), but here the effect of climate on parasite communities was investigated for the first time. In total, four ectoparasites and one helminth (*H. polygyrus*) were significantly affected by the change in climate between low and high altitude, and parasite intensity was always greater at low altitude, supporting the finding from Chapter 2 that parasite abundance increases under warmer temperatures. Whilst previous work has examined how the prevalence of ticks and the pathogen *Borrelia burgdorferi* changes under different climatic scenarios along altitudinal gradients (Jouda *et al.*, 2004; Morán Cadenas *et al.*, 2007; Gern *et al.*, 2008; Gilbert, 2010; Zamora-Vilchis *et al.*, 2012), the affects of altitude/climate on the parasite community as a whole was unknown (Chapter 5). Here, the parasite community was found to be highly interactive (Chapter 5), such that changes in one species could produce cascading effects though the community; a factor well-studied in free-living communities and shown to give rise to trophic cascades with impacts throughout the food web (e.g. Polis, 1994; Bascompte *et al.*, 2005; Dunne and Williams, 2009). The interactive nature of this infracommunity suggests that climate has both direct and indirect effects on the parasite community, and this finding emphasises the importance of considering the response of multiple parasite species to climatic perturbations; a factor that has not been considered to date.

In Chapter 5, the role that host biology plays in the structure of the parasite community was also identified. Three interactions between parasites were identified that were driven by host body mass. In two of these interactions (*R. straminea* and flea presence/absence; *H. polygyrus* intensity and *Syphacia* spp. presence/absence), parasite intensity was reduced in heavier hosts when they were coinfected. This suggests that intensity is greatest in younger individuals, and is followed by an acquired immunity-induced reduction in older hosts (Cattadori *et al.*, 2005). The third interaction (tick intensity and *Syphacia* spp. presence/absence) resulted in parasite intensity increasing in older, coinfected hosts. This may be as a result of a build up in parasite intensity over time, and/ or due to immunosenescence, whereby the immune response is less successful in older individuals, and culminates in these individuals harbouring the greatest parasite burden (Wilson *et al.*, 2002; Roberts and Hughes, 2014).

Host characteristics such as sex, have also been shown to be important drivers in parasite life-history traits, where male hosts have been found to be infected with the most fecund parasites (Ferrari *et al.*, 2004; Luong *et al.*, 2009; Corlatti *et al.*, 2012). Similarly, interactions between coinfecting parasites can result in an increase in parasite fecundity (Matthews *et al.*, 2006b; Cattadori *et al.*, 2007; Chylinski *et al.*, 2009; Lass *et al.*, 2013). Parasite fecundity is a particularly important life-history variable to understand because it represents a proxy for host infectiousness, as such, it is a key focus in disease ecology (Ferrari *et al.*, 2004; Matthews *et al.*, 2006b; Luong *et al.*, 2010). The interplay between climate, host biology and parasite infection on parasite fecundity was investigated with a field experiment. Fecundity, was greatest in male hosts, which is supported by previous work putatively driven by the immunosuppressive effect of testosterone (Ferrari *et al.*, 2004; Luong *et al.*, 2009; Corlatti *et al.*, 2012). Female hosts at low altitude were infected with significantly less fecund parasites than male hosts at the same altitude, and either sex at high altitude, which was found to be due to a male-biased sex ratio of the *H. polygyrus* infrapopulation. The role of a male-biased infection driving fecundity is a unique finding; typically the sex ratio is female-biased (Seidenberg *et al.*, 1973; Waller and Thomas, 1978; Haukisalmi *et al.*, 1996; Poulin, 1997; Stien *et al.*, 2005; Craig *et al.*, 2010; D'Ávila *et al.*, 2012). This male-bias may be due to a strategy to increase mating probability, which can be observed at low parasite intensities (Seidenberg *et al.*, 1973; Haukisalmi *et al.*, 1996; Craig *et al.*, 2010). Whilst work reported in Chapter 2 found no direct effect of temperature perturbations on fecundity, that in Chapter 3 shows an indirect effect caused by the interaction between host sex and *H. polygyrus* sex ratio. The study reported in Chapter 3 presents the first empirical study that has considered the interactions between host biology, parasite factors (e.g. sex ratio) and climatic effects on parasite fecundity. These findings emphasise the importance of continuing to consider the interplay occurring between these three drivers, rather than treating them independently.

Much insight into the population dynamics of parasites in their host population has come from mathematical modelling where a major assumption is that every individual in a population has an equal probability of transmitting a parasite to a naïve, susceptible host (Anderson and May, 1992; Grenfell and Dobson, 1995; Rodríguez and Torres-Sorando, 2001). There is, however, much heterogeneity that can affect the transmission process (Woolhouse *et al.*, 1997; Perkins *et al.*, 2003), and assuming all hosts in a population contribute equally to transmission may fail to describe the dynamics of

disease epidemics (Cuadros *et al.*, 2011). One such mechanism that may affect host infectiousness, measured by the number of eggs shed by a host, is coinfection. Whilst coinfecting parasites have been shown to alter the parasite community significantly (Chapter 5), they have also been shown to increase the intensity-infectiousness relationship for the cestode *R. straminea* (Chapter 4). Specifically, for single-infected hosts, the least infected hosts were the most infectious, yet in coinfected hosts this relationship was reversed (Chapter 4). For *H. polygyrus*, however, the most infected hosts were the most infectious, and this relationship did not change in coinfected or single-infected hosts. Coinfection may effectively overwhelm the immune system, preventing it from clearing parasite infections, allowing coinfected hosts to shed eggs over a longer period of time (Cattadori *et al.*, 2007; Lass *et al.*, 2013). The mechanism behind coinfection driving higher egg shedding (infectiousness) of *R. straminea* but not *H. polygyrus* is unlikely to be linked to an immunomodulatory effect. For example, *Hymenolepis nana*, a relative of *R. straminea* induces a T helper cell 2 (Th2) immune response and so would reduce egg shedding, whilst *H. polygyrus* is able to prevent the host from producing an immune response and so single-infected hosts would be likely to shed the most infectious eggs (Jenkins and Behnke, 1977; Behnke *et al.*, 1978, 1983; Conchedda *et al.*, 1996). There is only one other study that has investigated the effect of coinfection on the intensity-infectiousness relationship (Cattadori *et al.*, 2014), and this study investigated coinfection with only two parasite species, whereas here five have been used. Chapter 4, therefore, reports the first study to consider the entire helminth parasite community (up to 5 species) on the intensity-infectiousness relationship and adds to the growing body of literature suggesting that coinfection contributes to the heterogeneity that is seen in parasite fecundity (Cattadori *et al.*, 2007, 2014; Lass *et al.*, 2013).

The parasite community is a complex web of associations, with negative correlations between ectoparasites (Chapter 5), which is posited to be due to a negative feedback loop induced by grooming in response to local inflammation (Willadsen, 1980; Mooring and Samuel, 1998; Hawlena *et al.*, 2007, 2008). There was also an interaction pattern between endoparasites that is likely to be a function of immune-driven interactions; in single-infected hosts, *H. polygyrus* avoided detection by the immune system (Jenkins and Behnke, 1977; Behnke *et al.*, 1978, 1983), but coinfection with *Syphacia* spp. exposed *H. polygyrus* to the immune system, resulting in *H. polygyrus* intensity reducing in coinfected hosts. Cross-boundary interactions that occurred between

ectoparasites and helminths were also evident, which, due to the niche partitioning of these two spatially disparate groups within their hosts, were likely to be mediated by a systematic response of the immune system (Lello *et al.*, 2004; Graham, 2008; Lass *et al.*, 2013). The interactive associations between the parasite community suggests that changes that directly affect the intensity of one species could have knock-on effects for the rest of the community.

The parasite community analysis carried out in Chapter 5 was via statistical models, and it is harder to assess interactions in these observational studies compared to experimental ones (Fenton *et al.*, 2010, 2014). As such, future work that perturbs the parasite community by removing one parasite at a time (see Ferrari *et al.*, 2004; Pedersen and Antonovics, 2013), could be employed in combination with the addition of eco-immunology or 'wild immunology', to provide insight into the role of host immunity in mediating interactions observed (Abolins *et al.*, 2011; Jackson *et al.*, 2011; Pedersen and Babayan, 2011). Until recently, immunology has focused on laboratory animals due to a lack of workable immune assays in wild mice (Abolins *et al.*, 2011). Considerable advances have been made in developing immune assays, specifically for wild rodents (Jackson *et al.*, 2009, 2011, 2014; Abolins *et al.*, 2011; Brown *et al.*, 2013; Turner and Paterson, 2013). Future work could test whether these interactions are indeed immune-mediated by examining the expression of cytokine coding genes changes in hosts. Taking the interaction between *H. polygyrus* intensity and *Syphacia* spp. presence/absence as an example, hosts infected with just *H. polygyrus* could be compared with coinfected hosts by carrying out quantitative PCR (qPCR) of gene expression on tissue removed from mice (following Murphy *et al.*, 2013). A significant difference in gene expression of hosts single-infected with *H. polygyrus* compared with those single-infected with *Syphacia* spp. and coinfected hosts could confirm or reject this hypothesis.

Chapter 5 constitutes the first study to investigate the interactions that occur between multiple ectoparasite and helminth species, and the number of interactions occurring cross-boundary between ectoparasites and helminths suggests that these two sectors of the community are highly interactive. Although beyond the scope of the current work, future studies should include the microparasite community. Previously, Telfer *et al.* (2010) examined the microparasite community of wild rodents and found the community to be highly interactive. In addition, it is known that the helminth community interacts with the microparasite community (Graham, 2008; Lass *et al.*,

2013; Nunn *et al.*, 2014). The importance of including microparasites in a whole parasite community analysis is of particular importance, as rodents are well known indicators of zoonotic disease (Schmaljohn and Hjelle, 1997; Mills, 2006; Luis *et al.*, 2013; Baráková *et al.*, 2014). Indeed, Trentino, the location of the field sites for this study, is a known hotspot for tick-borne diseases (Hudson *et al.*, 2001; Carpi *et al.*, 2009), with *Borrelia burgdorferi* sensu lato, Apicomplexa, *Ehrlichia* spp. and Tick-borne Encephalitis being prevalent in ticks (Beninati *et al.*, 2002; Perkins *et al.*, 2003; Kallio-Kokko *et al.*, 2006; Rizzoli *et al.*, 2009; Chapter 6). Many of these infections are zoonotic, and determining how the microparasite community interacts with the ectoparasite and helminth community could provide data that are important for human health.

Whilst interactions between coinfecting parasite species were detected in Chapter 5, it should be noted that the parasites themselves are infected with hyperparasites, which may potentially interact with each other and the host (Childs and Paddock, 2002; Bremer *et al.*, 2005; Lo *et al.*, 2006; Klyachko *et al.*, 2007; Duron *et al.*, 2008; Clay *et al.*, 2008; Carpi *et al.*, 2011; Ahantarig *et al.*, 2013; Rynkiewicz *et al.*, 2015). As such, the final experimental chapter examined the role of tick-borne endosymbionts on the behaviour of a common parasite infecting yellow-necked mice, the sheep tick *Ixodes ricinus* (Chapter 6). It is assumed that ticks do not emigrate quickly from their host once it has died, suggesting that tick counts from dead hosts accurately estimate the infection load from the live host (e.g. McCay and Durden, 1996; Sherrard-Smith *et al.*, 2012). Infection with endosymbionts, however, can alter tick behaviour, causing them to be more active and increase the likelihood that they will find a new host and transmission can continue (Azad and Beard, 1998; Alekseev *et al.*, 2000; Roy *et al.*, 2006; Zhong *et al.*, 2007; Herrmann and Gern, 2012; Belova *et al.*, 2012). Thus, Chapter 6 quantified how many ticks left a host within 24 hours of its death, and established whether ticks that emigrated were more likely to be infected with endosymbionts, which here constitutes both non-pathogenic and pathogenic species (Chapter 7). Ticks were first recorded leaving their hosts within 2 hours of death, and within 24 hours, 54.7% of ticks had emigrated from the host. This suggests that studies that leave dead hosts in the environment for more than two hours are under-representing tick burdens of living hosts. The presence of endosymbionts was associated with accelerated emigration of ticks from dead hosts compared to uninfected hosts, suggesting a behavioural adaptation of endosymbionts to increase the likelihood of the infected tick finding a new host.

All ticks and mice that were used in Chapter 6 came from low altitude sites, as these mice harboured the greatest intensity of ticks (Chapter 5). If tick-borne pathogens became more prevalent at low altitude, this suggests that climate change would result in increased risk of humans contracting a tick-borne pathogen from a tick bite. Furthermore, as ticks can be coinfected with a range of pathogens (Randolph *et al.*, 1996, 1999; Azad and Beard, 1998; Perkins *et al.*, 2003), it may also be of interest to determine whether the prevalence of tick-borne coinfections increases at low altitude. If there was a greater prevalence of coinfected ticks under warmer temperatures, the chances of becoming infected with more than one pathogen from a single tick bite would increase (Swanson *et al.*, 2006), and this would have important health implications for humans in a future, warmer climate.

Altitudinal gradients are used to study the effects of climate change on ecosystems when it is not possible to conduct long-term experiments (Pickett, 1989; Hodkinson, 2005; Körner, 2007; Gilbert, 2010). Whilst these gradients have proved effective at measuring a range of communities under different climatic conditions, there are potentially confounding factors, which may influence results. Vegetation differs between the two altitudes; broadleaf woodlands at low altitude tend to change to a mixture of broadleaf woodlands and pine forests at high altitude (Batllori *et al.*, 2009, Gillingham pers. obs.). The effect of vegetation on parasites of rodents in this Alpine environment, however, has been shown to be less important than host characteristics (Ferrari, 2005). Regardless, to reduce any potential confounding effects of differences in vegetation that may have knock-on effects on the parasite community assemblage, field sites were carefully selected in broadleaf woodlands at both altitudes. Atmospheric pressure also decreases with increasing altitude. Atmospheric pressure is a little considered effect on parasites, but one study has simulated high altitude pressure (over 5,000 m) and found no significant effect on the survival of arthropod vectors or disease transmission (Williams, 1959).

The density of yellow-necked mice was not different between altitudes, but there were changes in the small mammal community. Specifically, bank voles (*Myodes glareolus*) were found at high altitude, but were absent at low altitude. Yellow-necked mice are found up to 1800 m (Reutter *et al.*, 2003), whereas bank voles have only been found above 800 m in a range of locations in Trentino (Gillingham, pers. obs., Konečny *et al.*, unpublished). The parasite community of these rodent species is different: in particular, bank voles are infected with the gastrointestinal nematode *Heligmosomoides glareoli*

(see Haukisalmi and Henttonen, 1993a; b; Behnke *et al.*, 2001b); and yellow-necked mice with *H. polygyrus* (see Ferrari *et al.*, 2004; Ferrari, 2005). *H. polygyrus* does not establish in bank voles (Quinnell *et al.*, 1991) and *H. glareoli* has not been recorded infecting yellow-necked mice (Behnke, pers. comm.). Whilst spill-over of parasites from one species is known to affect the health of another species (e.g. Tompkins *et al.*, 2003), the parasite community of bank voles in Trentino has not been previously investigated. Parasite species spill-over from one host to another is rare and requires circumstances that not only allow the species to establish in the novel host, but to be successfully transmitted between hosts (Kuiken *et al.*, 2006). As such, parasite spill-over form other small mammals was unlikely to be a confounding factor.

To conclude, this study has identified the direct effect that temperature perturbations have on parasite life-history traits (Chapter 2). Host sex, body mass and breeding condition affect parasite infection levels (Chapter 3, 5), and coinfecting parasites may alter the entire parasite community (Chapter 4, 5). Finally, this study has suggested that hyperparasitism alters vector behaviour, which may have important implications for disease transmission (Chapter 6). Future work should focus on free-living systems and ensure that the interplay between climate, host biology and parasite infection continues to be considered, instead of treating them as single, independent factors to understand the impact of a changing global climate on host-parasite interactions.

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Appendix A.1: Supplementary tables of data presented in Chapter 2

Table A.1.1 Table of studies used in a meta-analysis investigating the effect of a temperature perturbation on parasite abundance, fecundity and development time. The parasite species and the location of where the parasite strain originally came from is given, along with the 'ambient' temperature and the range of temperatures used in the study

Study	Parasite species	Ambient temp. (°C)	Temp. range (°C)
Al-Habbib and Grainger 1983	*Fasciola hepatica*	10	10-30
Arene 1986	*Ascaris suum*	16	16-34
Azam *et al.* 2012	*Toxocara canis*	22	15-35
Bayoh and Lindsay 2003	*Anopheles gambiae*	26	16-34
Bayoh and Lindsay 2004	*Anopheles gambiae*	26	10-40
Bentley and Burgner 2011	*Triaenophorus crassus*	12.75	11.5-14.0
Beveridge *et al.* 1989	*Trichostrongylus colubriformis*	20	4-30
Beveridge *et al.* 1989	*Trichostrongylus rugatus*	20	4-30
Beveridge *et al.* 1989	*Trichostrongylus vitrinus*	20	4-30
Binni *et al.* 2010	*Amblyomma lepidum*	27	27-35
Boag and Thomas 1985	*Cooperia curticei*	20	5-30
Boag and Thomas 1985	*Cooperia oncophora*	20	5-30
Boag and Thomas 1985	*Dictyocaulus oncophora*	20	5-30
Boag and Thomas 1985	*Haemonchus contortus*	20	5-30
Boag and Thomas 1985	*Nematodirus battus*	20	5-30
Boag and Thomas 1985	*Nematodirus filicollis*	20	5-30
Boag and Thomas 1985	*Nematodirus spathiger*	20	5-30
Boag and Thomas 1985	*Ostertagia circumcincta*	20	5-30
Boag and Thomas 1985	*Ostertagia ostertagi*	20	5-30
Boag and Thomas 1985	*Trichostrongylus axei*	20	5-30

Table A.1.2 (cont.)

Study	**Parasite species**	**Ambient temp. (°C)**	**Temp. range (°C)**
Boag and Thomas 1985	*Trichostrongylus colubriformis*	20	5-30
Boag and Thomas 1985	*Trichostrongylus retortaeformis*	20	5-30
Chilton and Bull 1994	*Amblyomma limbatum*	22	21-36
Chilton and Bull 1994	*Amblyomma hydrosauri*	22	21-36
Chilton *et al.* 2000	*Amblyomma limbatum*	22	13-34
Chilton *et al.* 2000	*Amblyomma hydrosauri*	22	13-34
Cordi and Otto 1934	*Strongyloides stercoralis*	24	5-40
Ernst et al. 2005	*Benedenia seriolae*	24.5	14-28
Gibson 1981	*Ostertagia ostertagi*	10	5-35
Griffin 1988	*Oswaldocruzia filiformis*	9	5-35
Guglielmone 1992	*Amblyomma triguttatum triguttatum*	30	15-40
Gupta 1961	*Trichostrongylus retortaeformis*	24.5	5-35
Heath 1979	*Haemaphysalis longicornis*	28	18-44
Heath 1979	*Ixodes holocyclus*	28	18-44
Heath 1979	*Rhipicephalus sanguineus*	28	18-44
Heath 1981	*Haemaphysalis longicornis*	28	18-44
Heath 1981	*Ixodes holocyclus*	28	18-44
Heath 1981	*Rhipicephalus sanguineus*	28	18-44
Hernandez *et al.* 2013	*Graphidium strigosum*	9	5-25
Hernandez *et al.* 2013	*Trichostrongylus retortaeformis*	9	5-25
Koprivnikar and Poulin 2009	*Maritrema novaezealandenis*	15	15-25
Krasnov *et al.* 2001	*Xenopsylla conformis*	25	25-28
Krasnov *et al.* 2001	*Xenopsylla ramesis*	25	25-28

Table A.2 (cont.)

Study	Parasite species	Ambient temp. (°C)	Temp. range (°C)
Lightner 1975	*Schistosomatium douthitti*	22.5	8-36
Lightner and Ulmer 1974	*Trichinella spiralis*	22.5	8-36
Macnab and Barber, 2012	*Schistocephalus solidus*	18	15-20
Maroli and Pozio 2000	*Trichinella spiralis*	26	8-26
Martínez-Ibarra *et al.* 2008	*Triatoma mexicana*	27	25-30
Morley *et al.* 2010	*Echinoparyphium recurvatum*	20	10-29
Ogden *et al.* 2004	*Ixodes scapularis*	20	-10-32
Paull *et al*. 2012	*Ribeiroia ondatrae*	17	17-26
Rueda *et al.* 1990	*Aedes aegypti*	27	15-34
Rueda *et al.* 1990	*Culex quinquefasciatus*	27	15-34
Salih and Grainger 1982	*Ostertagia circumcincta*	10	5-35
Shoukry *et al.* 2000	*Hyalomma schulzei*	29	21-34
Studer *et al.* 2010	*Maritrema novaezealandenis*	7-16	16-30
Tsutsumi *et al.* 2002	*Neoheterobothrium hirame*	20	10-25
Tubbs *et al.* 2005	*Benedenia seriolae*	17-19	13-21
Tubbs *et al.* 2005	*Zeuxapta seriolae*	17-19	13-21

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Table A.1.2 Meta-analysis of published studies reporting temperature perturbation impacts on parasite life-history traits. The numbers of studies and data points included in the meta-analysis are given for each category investigated

Experimental variables		**Number of studies**	**Number of data points**
Parasite life-history traits	Abundance	10	67
	Fecundity	9	58
	Development time	18	247
Parasite type	Ectoparasites	17	286
	Endoparasites	22	157
Parasite taxa	Apicomplexa	1	3
	Arthropods	14	266
	Nematodes	10	110
	Platyhelminthes	12	64
Host type	Ectotherms	15	150
	Endotherms	22	503
Host taxa	Amphibians	2	18
	Fish	8	43
	Invertebrates	5	34
	Mammals	22	293
	Reptiles	2	55
Experiment location	Laboratory	35	425
	Field	1	18
Total studies used in the meta-analysis		36	443

Appendix A.2: Supplementary table of data presented in Chapter 5

Table A.2.1 Interaction terms excluded from each of the GLMM/GLMs due to there being insufficient data. All other second order interactions were included (see text for details). Interactions between terms is represented with a colon (:)

Dependent variable	Independent continuous/categorical variables
Log *Heligmosomoides polygyrus* intensity	Host breeding condition : *R. straminea* presence/absence
	R. straminea presence/absence X tick presence/absence
Log *Rodentolepis straminea* intensity	Total ticks and all second order interactions
	Host sex : altitude
	Host sex : breeding condition
	Host sex : log (x+1) *Laelaps agilis*
	Host sex : *L. europaeum* presence/absence
	Host sex : lice spp. presence/absence
	Host breeding condition interactions
	Host body mass : altitude
	Body mass : log(x+1) *L. agilis*
	H. polygyrus presence/absence : total flea presence/absence
	H. polygyrus presence/absence : *L. europaeum* presence/absence
	Syphacia spp. presence/absence : *L. europaeum* presence/absence
	Syphacia spp. presence/absence : Lice spp. presence/absence
	Total flea presence/absence : *L. europaeum* presence/absence
Log *Syphacia* spp. intensity	Host breeding condition : body mass
	Breeding condition : *R. straminea* presence/absence
	R. straminea presence/absence : total ticks presence/absence
	Laelaps agilis presence/absence : lice spp. presence/absence
Log total tick intensity	Host breeding condition : *R. straminea* presence/absence
Log total flea intensity	Altitude : *R. straminea* presence/absence
	Altitude : total tick presence/absence
Log total flea intensity	Breeding condition : *R. straminea* presence/absence
	Breeding condition : *Syphacia* spp. presence/absence
	Heligmosomoides polygyrus presence/absence : *R. straminea* presence/absence

Table A.2.1 (continued)

Dependent variable	Independent continuous/categorical variables
Log total flea intensity (continued)	*H. polygyrus* presence/absence : total tick presence/absence
	R. straminea presence/absence : total tick presence/absence
	R. straminea presence/absence : *L. europaeum* presence/absence
	Syphacia spp. presence/absence : total tick presence/absence
	Breeding condition : *R. straminea* presence/absence
	R. straminea presence/absence : total tick presence/absence
	Breeding condition : *R. straminea* presence/absence
	H. polygyrus presence/absence : total tick presence/absence
	R. straminea presence/absence : total tick presence/absence
	Laelaps agilis and all interactions
Log *Laelaps agilis* mite intensity	Altitude : *R. straminea* presence/absence
	Altitude : total tick presence/absence
Log *Leptotrombidium europaeum* mite intensity	Altitude : *L. europaeum* presence/absence
	Host sex : *R. straminea* presence/absence
	Breeding condition : *R. straminea* presence/absence
Log lice spp. intensity	Breeding condition : *Syphacia* spp.
	H. polygyrus presence/absence : total tick presence/absence
	R. straminea presence/absence : *Syphacia* spp. presence/absence
	R. straminea presence/absence : total tick presence/absence
	R. straminea presence/absence : *L. europaeum* presence/absence
	Syphacia spp. presence/absence : total tick presence/absence