



Predators, parasites, and the social behaviour of the guppy *Poecilia reticulata*

Jessica Frances Stephenson

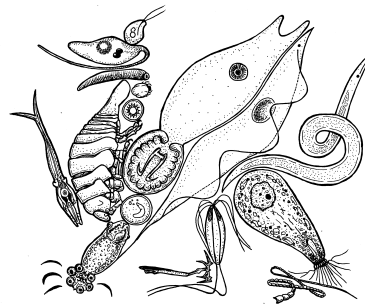
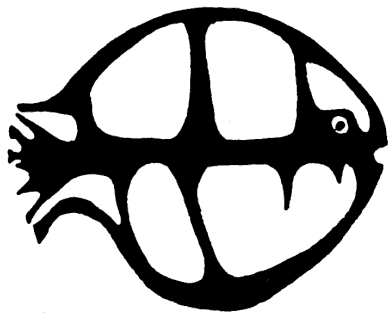
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for the degree of Doctor of Philosophy*

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Cardiff Research into Infection and Parasites in Ecological Systems

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Abstract

Predators can have both direct and indirect effects on how their prey interact with parasites. This thesis explores these effects using observational and experimental approaches. A behavioural experiment suggested that the direct effects of predators are size- and sex-biased, with small and male guppies, *Poecilia reticulata* Peters 1859, more prone to *Gyrodactylus turnbulli* Harris 1986 parasite-induced vulnerability to predation (Chapter 2). Trait-mediated indirect effects of predators also appear important to this host-parasite interaction, as revealed by surveys of natural Trinidadian populations under different predation regimes. First, predator-driven life history evolution predicts an apparent population divergence in parasite tolerance (Chapter 3). Similar divergence in a second trait, social behaviour, may drive sex- and age-biased parasitism: the guppies most liable to shoal have the highest infection probability (Chapter 4).

Social behaviour is thus an important driver of parasite transmission, but how parasites affect social interactions remains poorly understood. The second part of this thesis investigates how guppies may use sensory information to mitigate this cost of sociality. Many fishes rely on chemical and visual information and the interaction between sensory modalities to behave appropriately; for example, chemical cues change how guppies respond to visual cues (Chapter 5). In a social context, guppies use both chemical and visual cues to detect infection in conspecifics, but only avoid those in the later stages of infection (Chapter 6). Infection avoidance behaviour is not innate, but likely results from juvenile guppies imprinting on cues of conspecifics, and associating with these cues in adulthood (Chapter 7). This imprinting-mediated avoidance appears to be adaptive: a transmission experiment showed that the onset of avoidance behaviour coincides with the stage of infection at which conspecifics are most infectious (Chapter 8). The sensory ecology of the host and the community in which it lives therefore have important implications for disease dynamics.

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Chapter 1

General introduction: the effects of predators on the parasites of their prey, and sensory ecology as a key defence mechanism

1.1 Natural enemy ecology: the interacting effects of predators and parasites

The nascent field of ‘enemy ecology’ unites the traditionally distinct host-parasite ecology and predator-prey ecology to explore how these fields can be mutually informative (Raffel *et al.*, 2008; Daly and Johnson, 2011). There are inherent similarities between the threats posed by predators and parasites despite differences in their size, relationship intimacy, and resource consumption: both enemies can induce costly defensive responses in the prey or host and can be lethal (Werner and Peacor, 2003; Raffel *et al.*, 2008, 2010; Preston *et al.*, 2014). Partly as a result of the recognition of their inherent parallels with predators, parasites are increasingly being incorporated into food webs, community structure, and ecosystem energetics (Lafferty *et al.*, 2008). As reviewed below, investigations of the interacting effects of predators and parasites support the suggestion that the study of either enemy in isolation is unrealistic.

Studies of a wide range of taxa have demonstrated that parasites commonly increase the vulnerability of their hosts to predation. In some systems, the parasite depends on this trophic transmission to complete its complex life cycle, and these studies may therefore be reporting host manipulation by the parasite (Lafferty and Kuris, 2002; Poulin, 2010),

for example: *Trichostrongylus tenius*-infected red grouse, *Lagopus lagopus scoticus* (see Hudson *et al.*, 1992); moose, *Alces alces* infected with *Echinococcus granulosus* (see Joly and Messier, 2004); stickleback, *Gasterosteus aculeatus* infected with *Schistocephalus solidus* (see Barber *et al.*, 2004; Blake *et al.*, 2006); and Iberian hares, *Lepus granatensis*, infected with a range parasites (Alzaga *et al.*, 2008). However, parasite-induced vulnerability to predation also occurs in systems where predation of its host usually leads to the death of the parasite, such as *Polycaryum laeve* infections of *Daphnia* (see Johnson *et al.*, 2006). This effect of infection on host escape response in the absence of manipulation could be due to the activation of a costly immune response (Lochmiller and Deerenberg, 2000): Eraud *et al.* (2009) and Janssens and Stocks (2014) found that stimulation of the immune response using non-pathogenic methods increased the vulnerability to predation of Eurasian collared doves (*Streptopelia decaocto*; using lipopolysaccharide injection) and larval damselfly (*Coenagrion puella*; using non-pathogenic bacteria) respectively.

Broadly comparable to parasites affecting how their hosts interact with predators, predators affect how their prey interact with parasites. For example predation, particularly if it is selective of parasitised prey, may result in the population of hosts as a whole being less parasitised (Packer *et al.*, 2003). That this effect may be density-mediated has been well-studied (e.g. Anderson and May, 1981, Holt and Roy, 2007): predators should reduce parasite transmission because predation can reduce prey density, thereby decreasing contact rates among hosts (Packer *et al.*, 2003). However, the reduction in host density may increase the probability of any one individual host becoming infected (Côté and Poulin, 1995), and host social behaviour may cause non-linearities between host density and contact rate (e.g. Johnson *et al.*, 2011). Further, recent work suggests that the trait-mediated effects of predation can be at least as important as those mediated by density (Werner and Peacor, 2003; Hatcher *et al.*, 2006). Trait-mediated indirect effects arise when a change in the phenotype of individuals of one species, caused by the presence of another, alters how the reacting species interacts with others in the community (Werner and Peacor, 2003). Predators affect the morphology, physiology, life history and behaviour of surviving prey, each of which is likely to change how the prey interacts with its parasites. For example, *Daphnia dentifera* attain a relatively large body size in the presence of chemical cues of an invertebrate predator, and consequently upon death release more spores of a virulent yeast parasite (Duffy *et al.*, 2011). Additionally, Parris and Beaudoin (2004) found that fungal pathogens reduce tadpole development rates only when predators are present, and they hypothesised that this is due to the higher level of predator-induced physiological stress. Predators may also evoke behavioural changes: negatively phototactic *D. magna* clones avoid visually hunting predators, and as a result suffer increased exposure to parasite spores in pond sediment (Decaestecker *et al.*, 2002). Similarly, tadpoles reduce activity to avoid being

detected by predators, and are consequently unable to employ behavioural avoidance of trematode infection (Daly and Johnson, 2011; Johnson and Hoverman, 2014).

Although there are therefore clear parallels between these two enemies, the strength of selection wrought by each is a key distinction: while an individual may only experience a small fitness reduction due to parasitic disease, the reduction in fitness as a result of predation is commonly absolute. This distinction is unsurprisingly reflected in the responses of a number of taxa to these two forces, for example tadpoles of two species show a dramatic behavioural response to the presence of a predator, but none to the cues of parasitised conspecifics, or the infectious stages of virulent trematode parasites (Preston *et al.*, 2014). Evolutionary responses to predation pressure are also stronger than those to the pressure exerted by parasites. A field survey of the fast-evolving Trinidadian guppy *Poecilia reticulata*, the study organism of this thesis, revealed that population divergence in guppy traits was far better explained by the level of predation pressure rather than that of parasitism faced by the guppies at each site (Gotanda *et al.*, 2013). Further, although a handful of studies implicate parasite-mediated selection in population divergence and speciation (e.g. Blais *et al.*, 2007; Eizaguirre and Lenz, 2010; reviewed by Karvonen and Seehausen, 2012), none has convincingly demonstrated the causative role of parasites (Karvonen and Seehausen, 2012). By contrast, evidence for divergence mediated by predation pressure is substantial and growing (reviewed by Rundle and Nosil, 2005; Nosil, 2012).

Because of the intuitive and well-supported fact that predation pressure has stronger ecological and evolutionary effects on species traits than parasitism when both enemies are present, in this thesis I take the approach of investigating how predation pressure, and prey response to it, affect host-parasite interactions. The study organism I used, the Trinidadian guppy, has been instrumental to our understanding of the role predation plays in shaping the evolution of species (Magurran, 2005), and therefore provides a unique opportunity to evaluate how this force may affect how prey interact with a second enemy. The guppy's appeal stems from the topography of its habitat; waterfalls that represent significant upstream migration barriers, both to guppies and crucially to their major aquatic predators, bisect the streams they inhabit, creating replicated upper and lower course populations. Lower course populations experience more intense predation pressure than those in upper courses, and this variation in predation pressure has driven the evolution of population-specific differences in a number of guppy traits, such as body size; male coloration; mate choice behaviour; anti-predator behaviour; and life history (Haskins *et al.*, 1961; Seghers, 1974; Endler, 1978, 1980; Reznick *et al.*, 1997; reviewed by Houde, 1997; Magurran, 2005). Conclusively, these trait differences evolve rapidly during transplant experiments when lower course population guppies are

introduced into upper courses, or when voracious predators are introduced into upper course populations (Endler, 1980; Reznick *et al.*, 1997; Gordon *et al.*, 2009).

In contrast to predation, parasitism is poorly characterised in Trinidadian guppy populations. The dominant multicellular guppy parasites, *Gyrodactylus turnbulli*, *G. bul-latarudis* and *G. poeciliae* (see Xavier *et al.*, 2015) have been the almost exclusive focus of disease ecology research in this system. *Gyrodactylus* spp. are ectoparasitic monogenean platyhelminths that feed on fish mucous and epithelium, and are directly transmitted between hosts during social contact (Richards *et al.*, 2010; Johnson *et al.*, 2011). They impact guppy swimming ability (Hockley *et al.*, 2013), foraging behaviour (Kolluru *et al.*, 2006), reproductive fitness (Kennedy *et al.*, 1987) and survival (van Oosterhout *et al.*, 2007). Guppies show marked variation in their ability to defend against these parasites (Madhavi and Anderson, 1985; van Oosterhout *et al.*, 2003; Cable and van Oosterhout, 2007a,b), which, as well as genetics (Madhavi and Anderson, 1985), can be related to temperature (Scott and Nokes, 1984), guppy initial body condition (Tadiri *et al.*, 2012), and the availability of food, especially carotenoids, during the infection (Kolluru *et al.*, 2006; Kolluru *et al.*, 2009; Tadiri *et al.*, 2012). Guppies are able to mount both effective innate and acquired immune responses to the parasite (Bakke *et al.*, 2007; Cable and van Oosterhout, 2007b), and there is evidence to suggest the role of Major Histocompatibility Complex (MHC) class IIB genes in this response (Fraser and Neff, 2010; Fraser *et al.*, 2010).

Predator-driven divergence between upper and lower course guppies is particularly well-supported in two host traits, life history (Reznick and Bryga, 1987; Reznick *et al.*, 1990; Magurran, 2005; Gordon *et al.*, 2009) and social behaviour (Seghers, 1974; Magurran and Seghers, 1990; Magurran *et al.*, 1995; Magurran, 2005), and both of these traits are known to have important implications for other host-parasite interactions. Social behaviour dictates the rate at which an individual contacts others, and hence the number of transmission events it is involved in; contact rate is highly heterogeneous in natural populations, with some individuals having much greater contact rates than others (May and Anderson, 1987; Woolhouse *et al.*, 1997; Lloyd-Smith *et al.*, 2004, 2005; Bansal *et al.*, 2007; Clay *et al.*, 2009). These highly connected individuals may give rise to ‘super-spreading’ events that theory predicts can perpetuate epidemics (Galvani and May, 2005; Lloyd-Smith *et al.*, 2005; Small *et al.*, 2006; Stein, 2011). While social behaviour may determine the spread of parasites through populations, life history affects the response of hosts to infection: theory (van Boven and Weissing, 2004; Miller *et al.*, 2007) and empirical data (e.g. Sparkman and Palacios, 2009; Johnson *et al.*, 2012) have linked the evolution of a faster life history to a decrease in the allocation of resources to defence against pathogens: the ‘pace of life’ hypothesis (Ricklefs and Wikelski, 2002). Defence against pathogens encompasses both ‘resistance’, or the ability of hosts to limit

the extent of infection and ‘tolerance’, or the ability of hosts to limit the fitness cost of a given infection (Roy and Kirchner, 2000; Råberg *et al.*, 2009). Many interspecific comparisons have used immunological measures as a proxy for resistance, and have found that it decreases with increasing pace of life (Tieleman *et al.*, 2005; Martin *et al.*, 2007; Lee *et al.*, 2008; Previtalli *et al.*, 2012; Pap *et al.*, 2014). Only one study, to my knowledge, has investigated how the pathology sustained by host species as a result of infection, a measure of tolerance, increases with pace of life (Johnson *et al.*, 2012). Compared to these interspecific comparisons, far fewer investigations have tested the pace of life hypothesis of pathogen defence allocation within species, but they do support theoretical predictions: populations of garter snake *Thamnophis elegans* (see Sparkman and Palacios, 2009), and individual tropical house wrens *Troglodytes aedon* (see Tieleman *et al.*, 2005) with faster paces of life have reduced resistance, again quantified by immune function.

In this thesis I test the importance of predator-driven divergence in both life history and social behaviour in driving population level differences in guppy-gyrodactylid interactions. Our understanding of the ecology of guppy populations under different predation regimes, and of the guppy-gyrodactylid host-parasite interaction provides a tantalising opportunity to address these outstanding questions about the enemy ecology of natural populations. For example, predators indirectly affect transmission by driving a greater shoaling tendency in lower course populations (Seghers, 1974; Endler, 1978; Magurran and Seghers, 1994b). The resultant increased frequency of contact between hosts facilitates *Gyrodactylus* spp. transmission (Richards *et al.*, 2010; Johnson *et al.*, 2011), and hence *Gyrodactylus* spp. prevalence (the proportion of hosts infected, *sensu* Bush *et al.*, 1997) tends to be greater in lower course populations (Martin and Johnsen, 2007; Fraser and Neff, 2010; Gotanda *et al.*, 2013). This observation is by no means consistent between studies, however: some field studies report higher levels of *Gyrodactylus* spp. parasite prevalence in upper course populations (van Oosterhout *et al.*, 2006), and others in lower course populations (Martin and Johnsen, 2007; Fraser and Neff, 2010; Fraser *et al.*, 2010; Gotanda *et al.*, 2013). Additionally, several studies state that although they report differences in overall parasite prevalence between predation regimes, this pattern was not well-supported (Martin and Johnsen, 2007; Fraser and Neff, 2010; Fraser *et al.*, 2010). As well as these observational studies, researchers have used experimental infections of guppies from populations experiencing different predation regimes to test for differences between them in their defence against parasites. According to the ‘pace of life’ hypothesis, one would predict that the longer-lived, upper course guppies would be more resistant, but the data are inconclusive: studies have found that lower course guppies (Cable and van Oosterhout, 2007b), upper course (Dargent *et al.*, 2013), or neither (Pérez-Jvostov *et al.*, 2012) may be more resistant to the parasite.

In the first part of this thesis I examine the enemy ecology of this important model fish species (Fig. 1.1). I first describe a behavioural experiment testing for the potential role of *Gyrodactylus* spp. parasite-induced vulnerability to predation in driving the distribution of these parasites among natural guppy populations (Chapter 2). I then use a dataset collected over an unprecedented spatial and temporal scale to address the inconsistencies and outstanding questions in the existing literature. I illustrate how predator-driven divergence in life history evolution may determine how the host responds to its parasites (Chapter 3), and how divergence in social behaviour may affect parasite transmission across predation regimes (Chapter 4). Despite this cost, social behaviour confers many benefits and individuals bear potentially more severe costs, such as increased predation risk, reduced mating opportunities and less efficient foraging, by avoiding conspecifics (reviewed by Krause and Ruxton, 2002). However, the choice of with which individuals to associate may enable social animals to mitigate some of the increased risk of disease transmission posed by group living, while continuing to enjoy the benefits. In the second part of this thesis I investigate how sensory ecology, through enabling the avoidance of infectious individuals, may be a key defence mechanism against directly transmitted parasites. In the next section of this introduction, I describe fish sensory ecology, our understanding of the sensory ecology of disease, and identify the outstanding questions in this field that I address in the main body of the thesis.

1.2 Sensory ecology: a key defence mechanism?

Aquatic habitats are highly variable in their chemical composition and light environment (Lythgoe, 1980; Hara, 1994). As well as this broad scale habitat variation, fish also face within-habitat variation in their sensory environment. This range and changeability of environments they inhabit, their diversity in behaviour, morphology and physiology, and their experimental tractability mean that fish provide excellent study systems with which to investigate multimodal sensory ecology. Research in this field has largely focused on the olfactory and visual sensory systems. The relative importance of information from each of these senses to fish from different habitats varies dramatically. Many species demonstrate dynamic investment in the use of these two senses; it is intuitive that they should influence each other to maximise the information received (Rowe, 1999; McLennan, 2003). Indeed, that the sensitivity of fish vision is affected by olfactory stimulation has been demonstrated using electrophysiological and behavioural trials (Maaswinkel and Li, 2003; Stephenson *et al.*, 2011, 2012). In order to understand the interaction between these two senses it is important to understand each one in isolation: here fish vision is described first, followed by olfaction.

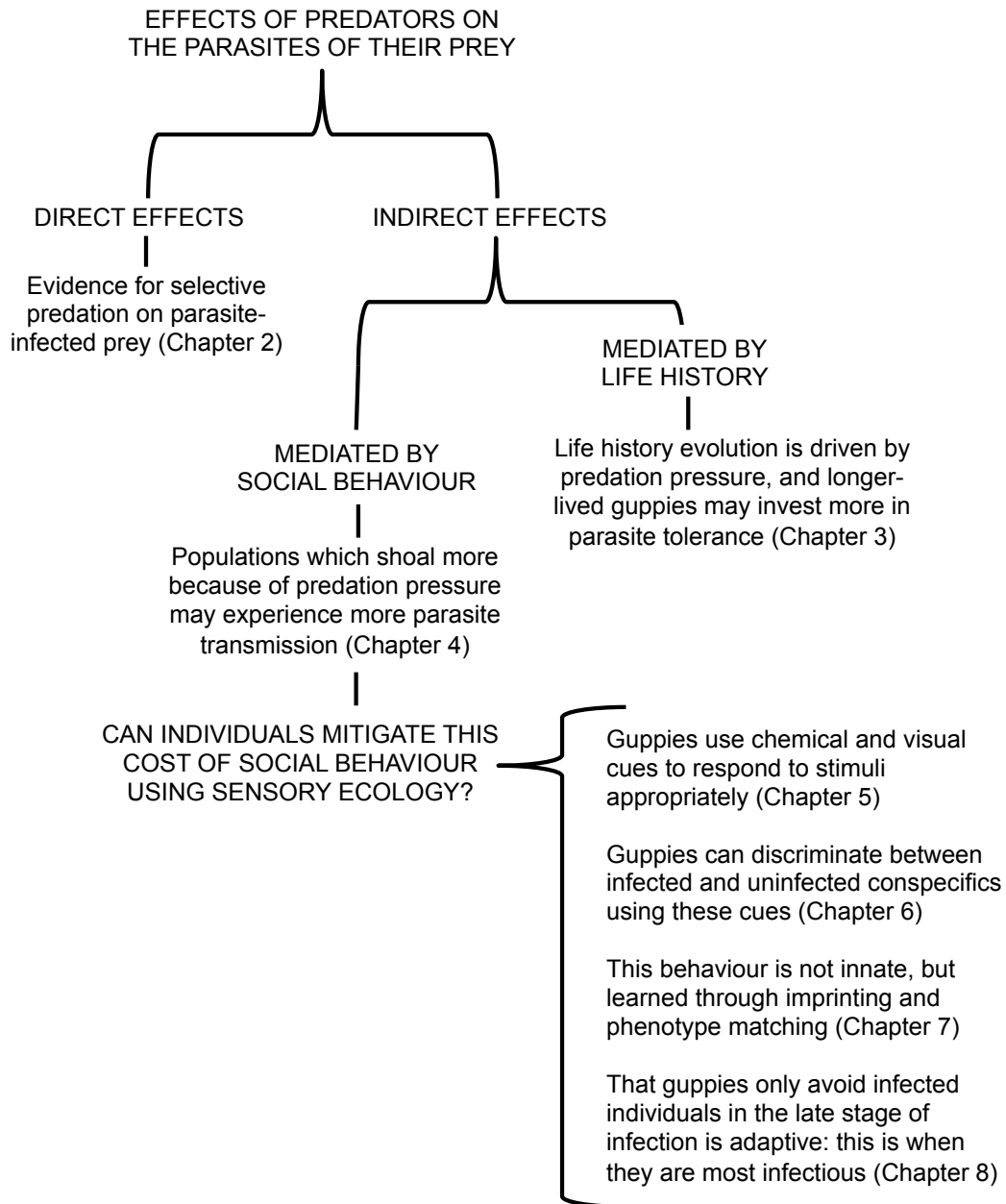


FIGURE 1.1: This flowchart illustrates how the thesis explores the effects of predators on the parasites of their prey, and how prey may mitigate the costs of this enemy ecology.

In fish, as in most vertebrates, the retina contains two types of visual photoreceptors: rods for use in low light (scotopic) and cones for use in bright light (photopic). In most vertebrates, discrimination between light of different wavelengths, that is colour vision, is achieved by comparing the response from two or more types of cones (Bowmaker, 2008). The spectral sensitivity of cones depends on the visual pigments they contain. Visual pigments are composed of a chromophore (an aldehyde of vitamin A) embedded in a protein moiety, opsin (Bowmaker, 2008). Interactions between the chromophore and the opsin of visual pigments determine the exact wavelength to which the visual pigment is most sensitive (λ_{\max}). λ_{\max} can be altered through amino acid substitutions in the opsin protein, the locations of which determine the magnitude of the change in λ_{\max} (between 2 and 35 nm; Yokoyama, 2000). Allelic variation in opsin genes can therefore be directly linked to differences in spectral sensitivity, and has been implicated in intra- and interspecific variation in male nuptial colouration and female preferences in Lake Victoria cichlids (Terai *et al.*, 2002, 2006). Differences in opsin gene expression can also cause large variations in spectral sensitivity: the cichlids *Dimidiochromis compressiceps* and *Metriaclima zebra* share identical opsin genes, but the spectral sensitivities of their three visual pigments differ by 79, 48 and 36 nm (Carleton and Kocher, 2001). Ontogenetic changes in opsin gene expression have been linked to changes in habitat turbidity between different age classes (Carleton and Kocher, 2001; Terai *et al.*, 2006). This has led to the idea that these environmentally-directed changes in spectral sensitivity could drive and maintain population or species divergence in male colouration and female mate choice preferences. This hypothesis is so far supported by empirical data from cichlids (Maan *et al.*, 2006) and guppies (Sandkam *et al.*, 2015), but studies on other taxa are needed.

The study organism I used in the work described in this thesis, the guppy, possesses nine opsin genes, compared to three in humans (Hoffmann *et al.*, 2007; Ward *et al.*, 2008; Sandkam *et al.*, 2015). Each gene is named for the range of light it detects: SWS1 (SWS1, short wavelength-sensitive) detects ultraviolet; SWS2A and SWS2B (SWS2, short wavelength-sensitive 2) detect blues and purples; RH2-1 and RH2-2 (RH2, rhodopsin-like) detect greens; and LWS-1, LWS-2, LWS-3 and LWS-R (LWS, long wavelength-sensitive) detect reds and oranges (Ward *et al.*, 2008; Sandkam *et al.*, 2015). Studies of retinal absorption suggest the resulting visual pigments have λ_{\max} of 359 nm (ultraviolet), 408 nm (violet/blue), 464 nm (green) and an unusually broad peak of 520-580 nm (red/orange; Hoffmann *et al.*, 2007). This broad peak of long wavelength sensitivity (LWS) can be explained by the existence of the four LWS opsin genes which display considerable sequence variation both within and between populations (Weadick and Chang, 2007; Ward *et al.*, 2008; Tezuka *et al.*, 2014), with some sequence differences being directly linked to opsin function and λ_{\max} (Yokoyama, 2000; Yokoyama and Radlwimmer, 2001). As well as sequence variation, there is a similar level of variation

in opsin gene expression between individuals of the same sex (Rennison *et al.*, 2011), between different age classes and sexes (with adults expressing more LWS opsins than juveniles, and females more than males; Laver and Taylor, 2011), and between populations (Sandkam *et al.*, 2015). Behavioural experiments complement this work by revealing that individuals differ in their sensitivity to these long wavelengths (Archer *et al.*, 1987; Archer and Lythgoe, 1990; Endler *et al.*, 2001). This differentiation in LWS opsin gene expression additionally correlates with the reported variation in female mate choice preferences for orange colouration in males between populations (Brooks and Endler, 2001; Sandkam *et al.*, 2015). In summary, species of the *Poecilia* genus appear to have a larger repertoire of LWS visual pigments than any other fish taxon (Ward *et al.*, 2008), and although there is substantial variation, guppies have excellent vision overall (Archer *et al.*, 1987; Sandkam *et al.*, 2015).

Olfaction appears to be less variable within and between fish species than vision. Despite variations in the gross morphology of the olfactory organ in fish, the epithelium ultrastructure is remarkably conserved across fish species (Hansen and Zielinski, 2005; Hara, 2006). Hamdani and Doving (2007) describe the olfactory system as divided into three sections, each of which is primarily associated with one type of olfactory receptor neurone (ORN; crypt, microvillous and ciliated cells). These, in turn, detect a different category of olfactory stimulus (amino acids, bile salts or pheromones), although there is some overlap (Hansen *et al.*, 2003; Derby and Sorensen, 2008; Vielma *et al.*, 2008). Each of these stimuli can be linked to distinct behaviours: feeding, alarm responses and reproduction, respectively (Hamdani and Doving, 2007). The three ORN types are distributed at random across the epithelium but their axons project to three discrete regions of the olfactory bulb, and output to the telencephalon is again in three separate sections of the olfactory tract (Hamdani and Doving, 2007). Electrical stimulation of each induces the behaviour appropriate to each class of olfactory stimulus: stimulation of the lateral olfactory tract (LOT) induces feeding behaviour, the medial part of the medial olfactory tract (mMOT) alarm, and the lateral part of the medial olfactory tract (lMOT) reproductive behaviour (Doving and Selset, 1980). Sectioning the LOT of the pintado catfish, *Pseudoplatystoma corruscans*, results in a latency to feed response similar to that which occurs following sectioning of the entire olfactory bulb. If the MOT is sectioned instead, however, latency is significantly shorter (Giaquinto and Hoffman, 2010).

The olfactory capabilities of the guppy appear to fall far below those of its visual sensory system. Guppies have an unusually low number of ORNs (Bettini *et al.*, 2009), and some behavioural studies have found that they lack responses to chemical cues (e.g. Archard *et al.*, 2008; Guevara-Fiore *et al.*, 2010). Others have found however, that olfaction is important to juveniles in discriminating between kin and non-kin (Griffiths

and Magurran, 1999), adult females in detecting alarm cue (Brown *et al.*, 1999), and both females (Brask *et al.*, 2012) and males (Crow and Liley, 1979) in discerning female reproductive status. As well as potential issues with experimental context (Magurran, 2005; Archard *et al.*, 2008), these contradictory findings may reflect bias in studies of guppy sensory ecology: there are far fewer studies investigating the olfactory abilities of this fish than its vision. Additionally, rearing environment may play an important role in determining the sensory capabilities of this species. Guppies show developmental plasticity in the performance of the visual and olfactory sensory systems; those raised in low light levels respond more strongly to food olfactory cues, and less strongly to visual cues, than those raised in brighter light (Chapman *et al.*, 2010). Although light levels in nature are higher than either of those used by Chapman *et al.* (2010), they are highly variable (Reznick *et al.*, 2001), which suggests that sensory compensation during development may explain some of the variation in the importance of olfaction indicated by previous studies. In the second part of this thesis I build on this work by investigating how guppies use olfactory and visual cues, and how this sensory ecology may enable them to mitigate the transmission risk inherent in social behaviour.

There has been much debate about the semantics of sensory ecology, and to avoid confusion I here define the terms important to this thesis. A ‘signal’ is a trait that has evolved specifically because it changes the behaviour of the receiver in a way that benefits the signaller. A ‘cue’, on the other hand, exclusively benefits the receiver (Maynard Smith and Harper, 2003). The work I present here uses cues, and not signals. Additionally, it is most likely that the chemical cues I investigate are detected and discriminated between by the glomerular olfactory system, although other chemical senses may also be stimulated (Wyatt, 2010). For the majority of fishes, evidence suggests that in the majority of contexts olfaction is the main sense by which chemicals are detected and discriminated between (Wyatt, 2010). Even in the control of feeding behaviour, where gustation is intuitively most likely to be involved, it appears that olfaction is the more important sense (Valentinčič and Caprio, 1994; Valentinčič *et al.*, 1994; Valentinčič and Caprio, 1997; Hara, 2006). Although the cues I use here can therefore be considered olfactory cues, I refer to them throughout the thesis as chemical cues to avoid ambiguity and facilitate comparisons with other taxa in which the sensory modality involved in detection is unclear.

Previous studies have investigated the effect of both chemical and visual cues of infection in determining animal social behaviour. Associating with individuals infected with directly transmitted diseases increases transmission risk (Poulin, 1999) and there is much evidence that animals across taxa avoid doing so (Goodall, 1986; Kiesecker *et al.*, 1999; Kavaliers *et al.*, 2003; Behringer *et al.*, 2006; Croft *et al.*, 2011; Schaller, 2011). These ‘social barriers’ to disease transmission may be as important as immunological or physical

ones (Loehle, 1995; Daly and Johnson, 2011; Schaller, 2011; Zylberberg *et al.*, 2012; Johnson and Hoverman, 2014). Avoidance behaviour at the individual level can influence disease dynamics at the population level (Gudelj and White, 2004), but the mechanisms involved have been little investigated. Empirical studies of infection avoidance behaviour suggest that it is more commonly based on chemical, rather than visual cues (Kiesecker *et al.*, 1999; Behringer *et al.*, 2006; Arakawa *et al.*, 2012). Although many species avoid individuals with visually obvious symptoms of disease, these are not necessarily cues of infectiousness. For example, fishes avoid conspecifics infected with indirectly transmitted parasites that change their visual appearance (Barber and Huntingford, 1995; Krause *et al.*, 1996; Barber *et al.*, 1998; Tobler and Schlupp, 2007). However, these fish are putatively avoiding the oddity effect in the absence of infectious disease (Landeau and Terborgh, 1986; Barber *et al.*, 1998). Visible attributes that can be affected by parasites may often serve as indirect cues of infection, such as colour (Hamilton and Zuk, 1982; Milinski and Bakker, 1990; Houde and Torio, 1992) or behaviour (Kennedy *et al.*, 1987). However, infected individuals may be able to tolerate parasites with no change in these cues (Folstad and Karter, 1992; Medzhitov *et al.*, 2012), actively disguise them (Aubert *et al.*, 1997; Lopes *et al.*, 2012), or there may be alternative explanations for these changes (e.g. dietary history and colouration; Grether, 2000). Conversely, changes in these attributes may make infected individuals more attractive social partners (e.g. Bouwman and Hawley, 2010).

For many systems, therefore, chemical cues may provide more reliable, quantitative information about an individual's infection, including how infectious it is. For example, chemical cues change through the course of infection in *Plasmodium chaubadi* malaria-infected mice, *Mus musculus*, making the mouse a more attractive blood meal for the mosquito vector when the parasite reaches the infectious stage of its lifecycle (De Moraes *et al.*, 2014). The infectiousness of hosts of directly transmitted parasites likely also varies through time: the number of infecting parasites, or 'infection load', changes through the course of infection for most directly transmitted parasites (Scott and Anderson, 1984; Poulin, 2007; Schmid-Hempel, 2011), and may be an important determinant of infectiousness (Lloyd-Smith *et al.*, 2006; Matthews *et al.*, 2006). This temporal change in the infectiousness of hosts infected with directly transmitted parasites may be linked to changes in the cues they emit, which could provide conspecifics with the opportunity to exhibit avoidance of only the most infectious individuals. This threat-sensitive avoidance behaviour would reduce the cost of avoidance behaviour, while maximising its benefit.

The guppy *Poecilia reticulata*-*Gyrodactylus turnbulli* host-parasite system presents an exceptional opportunity to test the hypothesis that individuals show threat-sensitive avoidance of the most infectious conspecifics. *G. turnbulli* has a generation time of 24-48 hours at 25°C (Cable, 2011), so guppy infection load, and therefore potentially

infectiousness (Lloyd-Smith *et al.*, 2006; Matthews *et al.*, 2006), can change rapidly through time. Because of its mode of transmission and the fitness costs of infection, the ability to recognise and avoid infected individuals is likely to be under strong selection and there is some evidence that avoidance occurs: the presence of infected conspecifics reduces shoal cohesion in semi-natural conditions (Croft *et al.*, 2011). However, this reduction in cohesion will in turn reduce the efficacy of shoaling as an antipredator defence (Chivers *et al.*, 1995). Guppies should therefore balance their avoidance behaviour with an assessment of the infectiousness of individual conspecifics. Specific chemical cues of gyrodactylid infection are likely to include hormones such as cortisol (Stoltze and Buchmann, 2001), and chemical changes in mucous (Buchmann and Lindenstrøm, 2002) that increase in concentration during the course of infection. Similarly, the visual cues of infection, such as the parasites themselves and the resultant host pathology and sickness behaviour, will become more obvious as the infection progresses. At later stages, and particularly at high *Gyrodactylus* spp. infection loads, guppies typically display clamped fins, paleness, and difficulty swimming (Cable, 2011). Guppies are able to monitor other temporally variable physiological characteristics in conspecifics using chemical cues (reproductive status; Brask *et al.*, 2012), and they also have excellent vision (Anstis *et al.*, 1998): it is highly likely therefore, that these fish could discriminate between potential social partners based on their infectiousness.

In the second part of this thesis, I follow on from the observation that populations which shoal more may experience more parasite transmission (Chapter 4) by investigating how individuals can potentially mitigate this cost of social behaviour (Fig. 1.1). I first demonstrate that the guppy, typically considered a highly visual animal, uses chemical cues to respond to ambiguous visual cues appropriately (Chapter 5). I build on this finding by demonstrating that guppies avoid the chemical and visual cues of infected conspecifics, but only in the later stages of their infection (Chapter 6). I further investigate the mechanism behind this avoidance behaviour by testing whether the avoidance of the cues of infection is innate, or the result of imprinting during development (Chapter 7). The final experiment described in this thesis demonstrates that ‘infection load’, or the number of parasites an individual is infected with, is an important determinant of infectiousness in this system, reaching a ‘peak of infectiousness’ when the number of *G. turnbulli* infecting a host surpasses *ca.* 40. This peak of infectiousness coincides with the onset of avoidance behaviour observed in Chapters 6 & 7, demonstrating that guppies use sensory ecology as a key defence mechanism against socially transmitted parasites (Loehle, 1995). Finally, in Chapter 9 I highlight future research questions based on the salient findings of this thesis.

1.3 Ethical note

The work presented in this thesis investigates the factors affecting the dynamics of infectious disease. As such, it has potentially important applications, and implications for future research. However, a necessary cost of this work is the use of live animals, both captive and wild, and the exposure of these animals to disease. This work was conducted under the UK Home Office license (PPL 30/2876) with approval by the Cardiff University Animal Ethics Committee and throughout I adopted the principle that ‘the best animal welfare is a prerequisite for the best science’, following the ”Guidelines for the treatment of animals in behavioural research and teaching” recommended by The Association for the Study of Animal Behaviour (2012).

In order to collect the experimental data presented in Chapters 2, 5, 6 & 7 I imported and maintained a stock of wild guppies under laboratory conditions. These fish were collected from a population in the lower Caura River, Trinidad (UTM: 20 P 67952.77 m E, 118037.64 m N; elevation 112m). This is a very large and healthy population; we were able to collect 600 fish from less than 10 m of river. Although some smaller populations of this species would have been severely affected by this collection, the population we chose would have been little affected by the removal of this number of fish. We were equally careful in the packaging and transport of these fish, which resulted in less than 1% mortality. Their maintenance under laboratory conditions was to a similarly high standard: guppies were maintained at low densities to maximise welfare and water quality, both of which were monitored regularly.

The experimental work presented in this thesis involved Home Office regulated procedures and was planned carefully to minimise the harm caused by these procedures and the number of animals used. I used data from previous studies using this system to identify the duration of infection with *Gyrodactylus turnbulli* that increases guppy risk of morbidity or mortality beyond a level justifiable by the research outcomes. For this reason, most of the fish used in Chapters 2, 6 & 7 were not infected for longer than 9-10 days. For all experiments, particularly those in which longer infections were necessary (Chapters 6, 7 & 8), I used power analyses of previously collected data to minimise the sample size required. As well as the studies presented in this thesis, these guppies were used in an ongoing breeding experiment; it was of paramount importance to maintain them under optimum conditions at all times and minimise the harm caused by the experimental procedures. Additionally, all behavioural trials were recorded; these recordings can be used for future studies and for training purposes to promote a reduction in the number of animals used, and refinement of the procedures. I discuss the specific ethical considerations of each experiment in the individual chapters.

Chapters 3 & 4 present analyses of data from a large number of guppies (4715) collected from natural populations. Although this is a considerable number of wild animals, they were collected from healthy populations over the course of seven years (2003 - 2009; see Appendix A: Table A1 for details); the impact is therefore likely to have been negligible. Any impact is also arguably outweighed by the large number of important findings to which these fish have contributed to date: molecular studies of the population structure of guppies (Barson *et al.*, 2009; Willing *et al.*, 2010) and gyrodactylids (Xavier *et al.*, 2015); characterisation of adaptive variation and drift in the Major Histocompatibility Complex (MHC; Llaurens *et al.*, 2012); research into the genetic basis of melanin pigmentation (Tezuka *et al.*, 2011); comparisons of the allelic variation in opsin genes across populations experiencing divergent environmental conditions (Tezuka *et al.*, 2014); evidence for parasite-mediated selection (van Oosterhout *et al.*, 2007); and observations of the role of biotic (Cable *et al.*, 2013) and abiotic (Schelkle *et al.*, 2012) factors in *Gyrodactylus* spp. infections in natural populations of guppies. By further using these existing data I aimed to maximise the value of these previous collections, and was able to address outstanding questions about the ecology of this host-parasite interaction without the need to remove more guppies from their natural habitat. I have also published the dataset in its raw form for other researchers to use (Stephenson *et al.*, 2015), *in lieu* of further field collections.

1.4 Contribution to and position within the thesis

In this chapter I have introduced and reviewed the key themes and identified the outstanding questions that this thesis addresses. The next chapter investigates the potential role of the direct effects of predators on parasites by testing how infection with *Gyrodactylus turnbulli* may affect the vulnerability of guppies to predation, and hence provides an important basis upon which the subsequent chapters build.

Chapter 2

A further cost for the sicker sex: male-biased parasite-induced vulnerability to predation

2.1 Abstract

Males are typically the sicker sex: they are more likely to be infected with parasites and less ‘tolerant’, or less able to mitigate the fitness costs of a given infection, than females across taxa. One such cost of infection for many animals is an increased probability of being captured by a predator. A clear, hitherto untested, prediction is therefore that this parasite-induced vulnerability to predation is more pronounced among males than females. We tested this prediction in the sexually size dimorphic guppy *Poecilia reticulata*. A behavioural experiment demonstrated that infection with *Gyrodactylus turnbulli* reduced the distance covered during the escape response of small adults (commonly male) by 20.1%, whereas that of larger (commonly female) fish was unaffected. Furthermore, the distance covered by infected males, but not females, was negatively correlated with their infection load. These results indicate that parasite-induced vulnerability to predation is indeed male-biased in natural populations due to differences in the size and parasite tolerance of the sexes. We discuss the causes and consequences of sex-biased enemy ecology for disease transmission in natural populations.

2.2 Introduction

Male animals are widely considered the sicker sex: typically they are more likely to be infected with parasites, and less able to mitigate the fitness costs of a given infection (less ‘tolerant’; Roy and Kirchner, 2000; Råberg *et al.*, 2009) than females across taxa (Zuk and McKean, 1996). Although there are exceptions, a striking number of host-parasite interactions conforms to this pattern (reviewed by Schmid-Hempel, 2011). Ultimately, this difference is due to sexual selection driving divergent life history strategies between the sexes (due to Bateman’s Principle; Zuk and McKean, 1996; Schmid-Hempel, 2011). Females maximise fitness by lengthening lifespan and producing more broods; in order to do so they must invest more in self-maintenance systems, such as defence against pathogens. Males, by contrast, maximise fitness by mating with as many females as possible; they therefore invest more in male-male competition and reproduction, and generally have shorter lifespans than females (Clutton-Brock and Isvaran, 2007; Zuk, 2009). Empirical evidence supports the role of proximate factors such as sex hormones in the resulting sex-difference in pathogen defence (Schmid-Hempel, 2011). For example, the male hormone testosterone is immunosuppressive (Folstad and Karter, 1992; Schmid-Hempel, 2011), and additionally influences male behaviour in such a way that increases the probability of becoming infected (Gear *et al.*, 2009).

In theory, the Trinidadian guppy, *Poecilia reticulata*, should provide an excellent example of female-biased defence against infection. Females can continue to use stored sperm to fertilise broods for up to 10 months after mating; males can therefore theoretically sire offspring long after death (Lopez-Sepulcre *et al.*, 2013). By contrast, females give birth to broods of live young, the sizes of which increase with body size and, as female growth is indeterminate, age (Reznick *et al.*, 1990). The reproductive fitness of female, but not male, guppies is therefore intrinsically linked to survival and longevity. Consequently, female lifespan is estimated to be 1.65 times longer than that of males (Arendt *et al.*, 2014), which leads to generally female biased sex ratios in populations across rivers in Trinidad (Seghers, 1974; McKellar *et al.*, 2009; Arendt *et al.*, 2014). The dichotomy in their life histories is manifest in many of the behavioural differences between the sexes (Magurran and Seghers, 1994b; Magurran and Macías Garcia, 2000; Magurran, 2005). For example, males spend more time harassing females in a frenetic attempt to maximise mating, whereas females spend the majority of their time foraging (Magurran and Seghers, 1994b). The extent of this sexual conflict is perhaps clearest under increased predation risk: males intensify reproductive effort, attempting more sneaky matings, while the risk-averse females employ anti-predator behaviours (Magurran and Nowak, 1991).

Despite the divergence in their life histories, which predicts a divergence in their defence against pathogens (Chapter 3; Ricklefs and Wikelski, 2002), evidence for a sex bias in resistance, or the ability of hosts to limit an infection (Roy and Kirchner, 2000; Råberg *et al.*, 2009) in this species is equivocal. Previous studies using experimental infections with *Gyrodactylus* spp. and quantifying resistance as the number of parasites infecting individual hosts have found no consistent difference between the sexes (e.g. van Oosterhout *et al.*, 2003; Cable and van Oosterhout, 2007b; Pérez-Jvostov *et al.*, 2012). Likewise, recent field studies have indicated either no sex bias (Martin and Johnsen, 2007; Fraser *et al.*, 2010), or, exclusively in populations experiencing high levels of predation pressure, female-biased infection with *Gyrodactylus* spp. (Chapter 4: Fig. 4.1; Appendix A: Fig. A.1; Gotanda *et al.*, 2013). Only one study has reported male-biased *Gyrodactylus* spp. infection, and this was limited to populations experiencing low predation pressure (Gotanda *et al.*, 2013). These patterns indicate a role for predators in driving patterns in the parasitism of natural guppy populations, but the mechanisms involved remain unclear. For example, predator-driven shoaling behaviour, which facilitates parasite transmission, is more pronounced among females and has been implicated in driving the observed female-biased *Gyrodactylus* spp. infection in lower course populations (Chapter 4). An additional, non-mutually exclusive explanation is that parasite infection differentially affects the mortality of male and female guppies. Indeed, males appear to lose more body condition than females as a result of *Gyrodactylus* spp. infection (Chapter 3): a strong indication of a sex difference in tolerance (Råberg *et al.*, 2009). Van Oosterhout *et al.* (2007) provide one source of differential mortality due to this difference in tolerance between the sexes: *Gyrodactylus* spp. infection makes male guppies, but not females, more likely to be swept downstream during flood events. We here investigate whether male-biased *Gyrodactylus* spp.-induced vulnerability to predation could be another.

It is intuitive and well-established that parasite infection increases the vulnerability of the host to predation. This effect could be due to manipulation by the parasite to facilitate trophic transmission, or simply a byproduct of the cost of infection (Johnson *et al.*, 2010). Regardless of the mechanism, there is growing evidence for the role of parasite-induced vulnerability to predation in a variety of ecological systems, for example: parasite diversity as well as infection load reduces the ability of Iberian hares, *Lepus granatensis*, to evade capture; *Echinococcus granulosus* infection increases the vulnerability of moose, *Alces alces*, to predation by wolves, *Canis lupus* (see Joly and Messier, 2004); and fish preferentially feed upon *Polycaryum laeve*-infected *Daphnia* (see Johnson *et al.*, 2006). In fish, an important component of predation avoidance is the escape response: a short (< 1 s), anaerobic burst of high velocity movement (Domenici and Blake, 1997). Several studies have indicated that parasite infection can reduce fish

escape responses (Barber *et al.*, 2004; Östlund-Nilsson *et al.*, 2005; Blake *et al.*, 2006; Binning *et al.*, 2013), though the parasites involved are commonly so large that they alter fish morphology (e.g. *Schistocephalus solidus*, see Barber *et al.*, 2004; Blake *et al.*, 2006) or drag coefficient (e.g. isopods, Östlund-Nilsson *et al.*, 2005; Binning *et al.*, 2013). It is likely, though previously untested, that the considerably smaller *Gyrodactylus* spp. (< 1 mm) also reduce escape response purely through reducing host condition: previous studies have noted that guppies display reduced activity (López, 1998; van Oosterhout *et al.*, 2003; Kolluru *et al.*, 2009) and swimming ability (Hockley *et al.*, 2013) during *G. turnbulli* infection. Here we use a behavioural experiment to test whether *G. turnbulli* infection has a male-biased effect on guppy escape response. The results indicate that male-biased parasite-induced vulnerability to predation could contribute to an explanation for the apparently counterintuitive finding of female-biased infection reported from natural populations experiencing high levels of predation (Chapter 4; Appendix A; Gotanda *et al.*, 2013).

2.3 Materials and Methods

2.3.1 Fish origin and maintenance

The guppies used to experimentally test the effect of infection with *Gyrodactylus turnbulli* on escape response were second-generation laboratory-bred descendants of wild guppies from the Caura River, Trinidad (UTM: 20 P 67952.77 m E, 118037.64 m N; elevation 112m). Wild guppies ($n = ca. 600$) were transported to Cardiff University in June 2012 (Cefas APB authorisation number CW054-D-187A), where they were prophylactically treated for infection using Binox® (Nitrofurazone; Jungle Laboratories Corporation®, Cibolo, Texas). Fish were housed in 70 L mixed sex stock tanks on a 12h light: 12h dark lighting regime (overhead fluorescent lighting) at $24 \pm 1^\circ\text{C}$ and fed daily with Aquarian® tropical fish flakes supplemented with *Artemia* and bloodworm. Fry were removed from the breeding tanks soon after birth, reared in one of four juvenile tanks until sex determination was possible (*ca.* 6-8 weeks), and then separated by sex. All fish used in the experiment were therefore virgins, controlling for the fact that reproductive status influences the escape response of females (Ghalambor *et al.*, 2004).

2.3.2 Experimental infections

We used 38 guppies (19 females, 19 males) as our ‘infected’ treatment group, and 30 guppies (19 females, 11 males) as the ‘uninfected’ treatment group. These sample sizes were dictated by the numbers of fish available due to an additional experiment that

was running simultaneously. The weight and standard length of all individuals was recorded immediately prior to infection. To help discriminate between the effects of inherent differences between the sexes and those due purely to their different sizes, we sized-matched males and females (difference in mean standard length: 0.25 mm; $t(66) = -0.51$; $p = 0.61$), as well as the infected and uninfected fish (difference in mean standard length: 0.42 mm; $t(66) = -0.88$; $p = 0.38$). Fish (overall mean standard length, $\text{mm} \pm \text{SEM}$: 16.4 ± 0.3 ; mean weight, $\text{mg} \pm \text{SEM}$: 73.4 ± 3.9) were held individually in 1 L containers throughout the experiment. The *Gt3* strain of *G. turnbulli*, originally isolated from guppies in a petshop in Nottingham, UK in 1997, was used to infect experimental fish. Fish to be infected were randomly assigned to batch 1 (12 females, 8 males) or batch 2 (7 females, 11 males) allowing all behavioural trials to be conducted at an equivalent stage of infection (Day 8: $n=10$; or 9: $n=28$). Fish were infected under anaesthetic (0.02% tricaine methanesulfonate; MS222; PHARMAQ Ltd., UK) on day 0 through the transfer of 2.3 ± 0.1 (mean \pm SEM) parasites from a heavily infected, recently killed donor, as observed using a dissecting microscope and fibre optic illumination. We subsequently monitored infections under anaesthetic (0.02% MS222) on days 1, 3, 5, 7 and one day post-trial (Day 9: $n=10$; or 10: $n=28$).

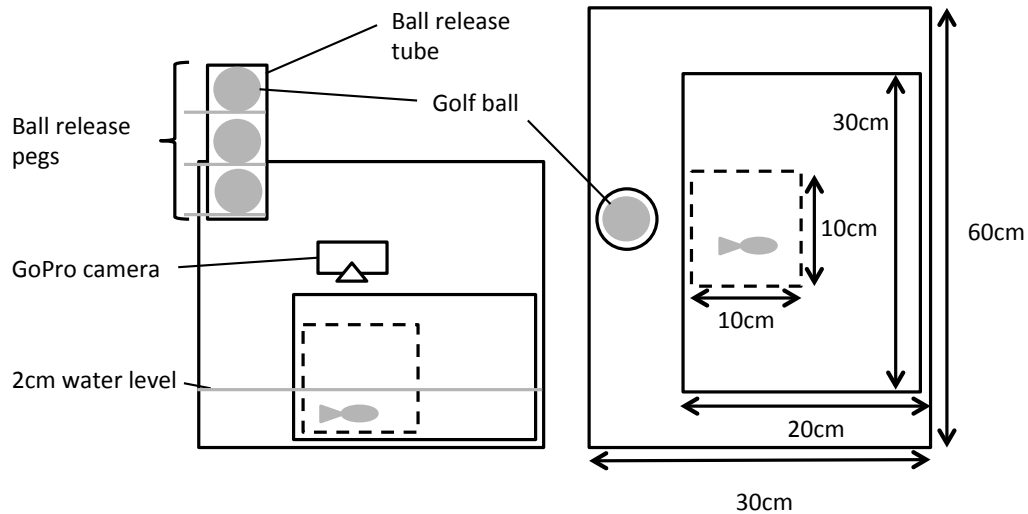


FIGURE 2.1: The apparatus used to test for the effect of infection with *Gyrodactylus turnbulli* on guppy escape response. The left panel gives a side view, the right an overhead view.

2.3.3 Experimental set-up and design

To assess their escape response, guppies were individually exposed to multiple startling stimuli within a trial arena. The experimental setup consisted of a 30×60 cm outer

tank with a 20×30 cm inner tank, both filled with dechlorinated water at 25-26°C to a depth of 2 cm (Fig. 2.1). An arena constructed from plastic mesh ($10 \times 10 \times 16$ cm; Darice® Inc. USA), was placed in the smaller tank on a waterproof white background. The side length was selected to approximate five body-lengths of the test fish (Ghalambor *et al.*, 2004). A remotely operated ball release was situated above the gap between the inner and outer tanks, containing three golf balls at 35, 40 and 45 cm height, each with a separate release mechanism. No variation in the escape response was attributable to this difference in ball drop height ($p > 0.05$, included as a factor in the general linear mixed model described below), which is unsurprising given that escape responses are a threshold ‘all-or-nothing’ behaviour (Bergstrom, 2002). At the start of each trial, a fish was placed in the arena and left for a 10 min acclimatisation period. The first ball was released after acclimatisation, followed by the remaining two at 3 min intervals. A wirelessly operated camera (HERO3+ White Edition; GoPro Inc., USA), set to film at 60 frames per second, was suspended 20 cm above the arena and activated for approximately 15 s during the release of each ball. Between the trials of individual fish, the inner tank was emptied and cleaned with 70% ethanol in order to remove any chemical cues or dislodged parasites. The 68 fish were tested over the course of six days. We used EthoVision XT (Noldus) to record the distance the fish travelled (mm) in the 60 ms immediately following the initiation of the escape response for each video (3 videos per fish: $n = 204$). We also recorded whether the fish was in the half of the arena next to the ball, or the half away from the ball (position in the test arena), and its orientation (facing towards the ball, away from the ball, or neutral) when the ball dropped. If multiple escape responses occurred following a single ball drop only the first was analysed.

2.3.4 Ethical note

This work was conducted under the UK Home Office license (PPL 30/2876) with approval by the Cardiff University Animal Ethics Committee. As described above, during the course of this experiment guppies were subjected to social isolation, and some were additionally experimentally infected with parasites. Although social isolation is likely to have temporarily elevated their stress levels, previous work using this method indicates that guppies show no sign of having suffered lasting harm. To further mitigate the effects of this isolation, fish were held in close proximity in transparent tanks; they were therefore in visual contact with conspecifics throughout. Among the infected fish, none were infected for longer than 10 days, which is the point at which mortality increases (Dargent *et al.*, 2013). We monitored infected fish carefully and none exhibited severe pathology at any point during the experiment (fused fin rays, difficulty swimming, loss

Response variable	Error family	Link function	Main effects	Two-way interactions	Random effect
Distance covered in 60 ms (mm)	Gaussian	Identity	Infection status (c)* Infection load (n) Sex (c) Position (c) Orientation (c) Drop (o) No. responses (n) Weight (n)*	Infection status \times Sex Infection status \times Weight*	Fish identity

TABLE 2.1: The starting model used to test the hypothesis that infection with *Gyrodactylus turnbulli* affects guppy escape response. This starting model was simplified using backwards stepwise deletion of non-significant fixed effects to minimise the Akaike’s Information Criterion (AIC); those that remained in the final model are denoted with an asterisk. See the main text for an explanation of the fixed effects, which were included as categorical (c), numeric (n), or ordinal (o) variables.

of appetite Cable, 2011). Throughout the experiment, mortality was low (<5%), and infected fish recovered fully with treatment.

2.3.5 Data analysis

We used a general linear mixed model (Gaussian error family with an identity link function) in the *lme4* package in R (3.0.2; R Core Team, 2013; Bates *et al.*, 2014) to test whether infection with *G. turnbulli* affected the escape response of guppies (Table 2.1). We used the distance (mm) the fish moved in the 60 ms immediately following the initiation of the escape response as the response variable, and included fish identity as a random effect because we recorded three escape responses per fish. Fixed effects included: infection status (infected or uninfected); infection load (how many *G. turnbulli* the fish was carrying when tested); fish weight (as a measure of size); fish sex; fish position in the test arena; fish orientation when the ball was dropped; drop number (1, 2 or 3); total number of responses to each ball; two way interactions between infection status and sex, and infection status and fish weight. We simplified the model by sequentially removing non-significant fixed effects from the full model to minimise Akaike’s Information Criterion (AIC). The analysis was conducted using weight as a continuous variable, but for Figure 2.2 we divided the fish into ‘small’ and ‘large’ categories (divided at the median value of 72 mg) for clarity.

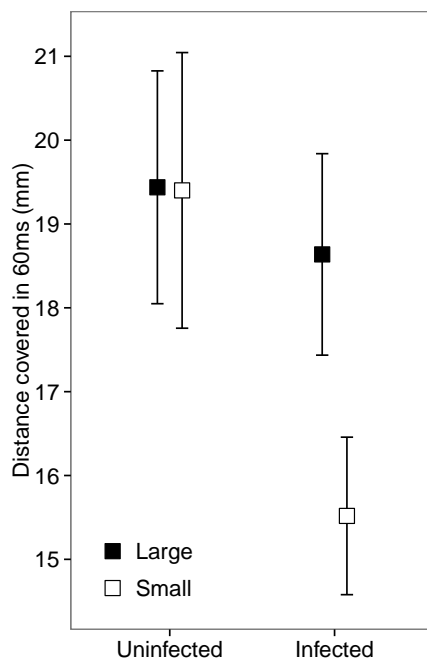


FIGURE 2.2: Infection with *Gyrodactylus turnbulli* reduced the distance that small fish (up to 72 mg; white squares) covered during the escape response by an average of 20.1%. There was no effect of infection on the escape response of large fish (over 72 mg; black squares). The error bars are the standard error of the mean. The analysis was conducted using weight as a continuous variable, but for the figure we divided the fish into ‘small’ and ‘large’ categories (divided at the median value of 72 mg) for clarity.

2.4 Results

How infection with *Gyrodactylus turnbulli* affected guppy escape response depended on fish size: small infected fish covered an average of 20.1% less distance in the first 60 ms of the escape response than small uninfected fish, but infection did not affect the distance covered by large fish (Fig. 2.2; infection status \times fish weight interaction: $F_{1, 56.2} = 5.67$, $p = 0.021$). Data from natural populations, the collection of which is described elsewhere (Chapters 3 & 4), demonstrated that females were significantly larger than males across all sampled populations (Fig. 4.2; weight (mg) \pm SEM females: 148.69 ± 2.71 ; males: 79.34 ± 0.75 ; $t(2016.4) = 24.64$, $p < 0.0001$). A further indication of sex-biased parasite-induced vulnerability to predation is that the distance that males covered decreased by 0.05 mm *per* additional *G. turnbulli* (Fig. 2.3; $F_{1, 20.9} = 3.80$, $p = 0.047$), but no such relationship existed among females (Fig. 2.3; $p > 0.10$).

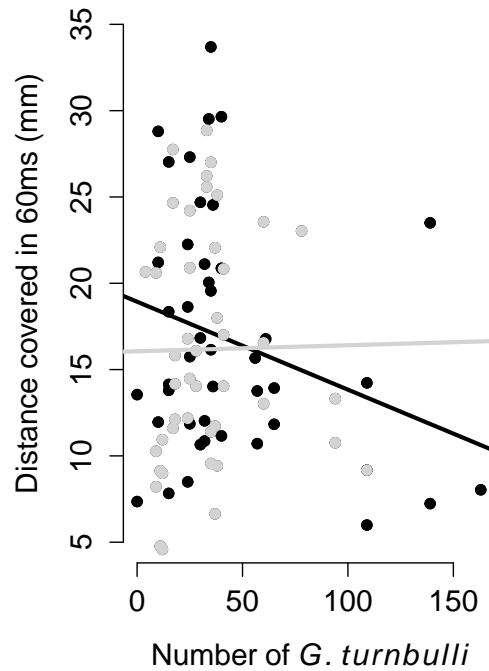


FIGURE 2.3: There was a sex difference in how the number of *Gyrodactylus turnbulli* parasites infecting a guppy (its ‘infection load’) affected its escape response: males (black circles) covered 0.05 mm less distance during the escape response *per* additional parasite. The escape response of females (grey circles) was unaffected by their infection load. The lines are the linear regressions of male (black) and female (grey) infection load on the distance covered during the first 60 ms of the escape response.

2.5 Discussion

Infection with *Gyrodactylus turnbulli* reduced the distance covered by small adult guppies during the escape response by an average of 20.1%, whereas the escape response of larger fish was unaffected (Fig. 2.2). Wild guppy females are significantly larger than males (Fig. 4.2), so this result provides evidence that parasite-induced vulnerability to predation is sex-biased in natural populations. As well as a size effect, there appears to be an inherent sex difference: the distance covered by infected males, but not females, was negatively correlated with infection load (Fig. 2.3). The weaker escape response of infected males relative to infected females supports our hypothesis that, rather than being an exception to the rule, guppies reveal a further cost for the sicker sex in the form of male-biased parasite-induced vulnerability to predation. The resulting higher mortality among infected males, potentially correlated with their infection load, explains why observational studies record female-biased *Gyrodactylus* spp. infection in high predation populations (Chapter 4; Gotanda *et al.*, 2013).

Infection with *G. turnbulli* reduced the distance covered by small adult guppies during the escape response, indicating that these parasites increase guppy vulnerability to predation (Domenici and Blake, 1997; Bergstrom, 2002; Ghalambor *et al.*, 2004). Parasites can affect fish swimming performance in a number of ways (reviewed by Barber *et al.*, 2000). *Gyrodactylus* spp. infection causes changes in the guppy epidermis (Gheorghiu *et al.*, 2012), reduced feeding and activity levels (van Oosterhout *et al.*, 2003; Kolluru *et al.*, 2009), and at higher infection loads the pathology includes clamped fins (Cable *et al.*, 2002) with obvious negative impacts on swimming (Hockley *et al.*, 2013). The effect of infection on host escape response could also be due to activation of a costly immune response (Lochmiller and Deerenberg, 2000), independent of the effects of the parasite: Eraud *et al.* (2009) and Janssens and Stocks (2014) found that stimulation of the immune response using non-pathogenic methods increased the vulnerability to predation of Eurasian collared doves (*Streptopelia decaocto*; using lipopolysaccharide injection) and larval damselfly (*Coenagrion puella*; using non-pathogenic bacteria) respectively. The immune response of our test fish would have been activated: we tested the guppies on days 8 and 9 post infection and at this stage the growth of the parasite infrapopulation on many hosts slows, and may become negative (Fig. 6.1), indicative of an active immune response (Bakke *et al.*, 2007).

We found that the effect of infection on guppy escape response was strongest among small adults, and that the distance covered by males was negatively correlated with infection load. We suggest this size- and sex-dependence is due to smaller and male fish being less tolerant of infection than larger and female fish. Positive correlations between body size and resistance or tolerance of parasitism have been recorded in a number of species, for example Soay sheep, *Ovis aries* (see Coltman *et al.*, 2001) and garter snakes, *Thamnophis elegans* (see Sparkman and Palacios, 2009). Although we failed to detect a size- or sex-dependent change in body condition (a common measure of tolerance; Råberg *et al.*, 2009) in our experimental fish over the nine days of their infection, data from wild populations indicate that *Gyrodactylus* spp. tolerance is lower among male than female guppies (Chapter 3). Additionally, *Gyrodactylus* spp. infections are energetically costly (van Oosterhout *et al.*, 2003; Bakke *et al.*, 2007; Kolluru *et al.*, 2009), and it is likely that differences in tolerance between these groups of fish would be manifest in other physiological costs that explain the decrease in escape response (Barber *et al.*, 2000; Lochmiller and Deerenberg, 2000). In support of this hypothesis, smaller and male guppies select habitats with lower velocity, less turbulent flow, particularly when infected with *G. turnbulli*, suggesting they are more energetically limited generally (Hockley *et al.*, 2013), as has been found for smaller sticklebacks (Krause *et al.*, 1998), and are more affected by infection (Hockley *et al.*, 2013). As males are smaller than females (Fig. 4.2), this size effect, in addition to inherent sex differences in *Gyrodactylus* spp.

tolerance (Chapter 3) and swimming ability (Hockley *et al.*, 2013), will likely translate into male-biased parasite-induced vulnerability to predation in wild populations.

The sex- and size-biased parasite-induced vulnerability to predation indicated by our findings will have implications for the evolutionary ecology of this host-parasite interaction. For example, our results suggest that *Gyrodactylus* spp. parasites select for larger body size in the host, in opposition to the selection imposed by predators, which select for smaller body size (Reznick *et al.*, 1990). While previous work has indicated that smaller fish may be more resistant to *Gyrodactylus* spp. in that they develop smaller infection loads (van Oosterhout *et al.*, 2003, 2008), the present study suggests that infection load is less important than the size of the fish in determining the effect of infection on escape response. Parasitism could therefore be an important force in driving the evolution of larger body sizes in upper course, low predation populations. Further, removal of the most heavily infected males will have implications for the epidemiology of the disease. Due to their low shoal fidelity and high contact rates resulting from the search for mating opportunities (Croft *et al.*, 2003a,b), males are likely to be key in both intra- and inter-shoal transmission of the parasite. Consequently, selective predation on males, particularly if it is correlated with their infection load, may well reduce parasite prevalence and intensity in natural populations (Packer *et al.*, 2003). It is also likely that this process will exacerbate the male-biased predation already implicated in driving female biased sex ratios in this species (McKellar *et al.*, 2009; McKellar and Hendry, 2011).

We have demonstrated the importance of the direct (present study) and indirect (Chapter 4) effects of predators on the distribution of parasites among host populations. In the guppy-gyrodactylid system at least it is likely that the relative importance of these effects changes through time: guppy population densities, and therefore the presence of predators at a given site is highly temporally variable (Magurran, 2005). Because the indirect effect of predators is mediated by an evolved guppy trait, shoaling, that differs between high and low predation populations even after several generations in the laboratory (Huizinga *et al.*, 2009), it will drive constantly higher transmission rates among lower course guppies (Chapter 4). By contrast, patterns driven by selective predation on infected guppies will only be evident when predators are present. It is clear from Fig. 4.2 that both these processes are important. Relative to low predation sites, small infected guppies are underrepresented in high predation sites, probably due to the direct effect of predators: these are the fish most prone to parasite-induced vulnerability to predation (present study). Large uninfected guppies are similarly relatively scarce in high predation sites, probably due to the indirect effects of predators: increased transmission due to increased shoaling results in higher re-infection rates (Chapter 4). In summary, these findings clearly demonstrate that the community in which the host and

parasite interact can have profound implications for that interaction: we support recent calls for further investigation of the impact of community interactions, particularly predation, on disease ecology (Packer *et al.*, 2003; Hatcher *et al.*, 2006; Lafferty *et al.*, 2008; Raffel *et al.*, 2008, 2010).

2.6 Authorship Acknowledgement

The manuscript resulting from this chapter is authored by:

Jessica F. Stephenson, Cormac Kinsella (Cardiff University), Joanne Cable (Cardiff University) and Cock van Oosterhout (University of East Anglia).

2.7 Contribution to and position within the thesis

In this chapter I provide evidence from a behavioural experiment that the direct effects of predators on guppy parasites, that is selective predation of *Gyrodactylus* spp.-infected guppies, is likely to play an important role in the dynamics of this disease. The non-consumptive effects of predators may also be important, however: the next two chapters use data from large-scale field surveys of wild guppy populations under different predation regimes to investigate how predators, through driving population divergence in host traits, may indirectly affect this host-parasite interaction.

Chapter 3

Predator-induced life history evolution in Trinidadian guppies predicts sex-specific decrease in parasite tolerance

3.1 Abstract

A common evolutionary response to predation pressure is increased investment in reproduction, ultimately resulting in a fast life history. Theory and comparative studies suggest that such short-lived organisms invest less in defence against pathogens than those that are longer-lived. Combining these tenets of evolutionary theory leads to the specific prediction that populations experiencing high levels of predation pressure invest less in pathogen defence than those experiencing low levels. We tested this prediction in the Trinidadian guppy, *Poecilia reticulata*. Guppy populations in the lower courses of rivers experience higher levels of predation pressure, and have evolved faster life histories, than those in the upper courses. Additionally, sexual selection has driven divergent life histories between the sexes; female guppies live longer than males. Data from a large-scale field survey showed that fish infected with *Gyrodactylus* spp. parasites were of a lower body condition (quantified using the scaled mass index) than uninfected fish, but only in lower course populations. Also in these populations, infected males were of a lower condition than infected females. Upper course and female guppies therefore appear to sustain lower fitness costs of infection, indicating increased investment in parasite tolerance. Our results thus provide support for the ‘pace of life’ theory of pathogen defence allocation, and clear evidence of a trade-off in responses to two different enemies.

3.2 Introduction

That predation pressure can drive the evolution of faster life histories has been demonstrated both theoretically (Gadgil and Bossert, 1970; Law, 1979; Michod, 1979) and empirically (Reznick *et al.*, 1990, 1996, 2001). More recently, theory (van Boven and Weissing, 2004; Miller *et al.*, 2007) and empirical data (e.g. Sparkman and Palacios, 2009; Johnson *et al.*, 2012) have linked the evolution of a faster life history to a decrease in the allocation of resources to defence against pathogens: the ‘pace of life’ hypothesis (Ricklefs and Wikelski, 2002). Throughout this study we consider defence against pathogens to encompass both ‘resistance’, or the ability of hosts to limit the extent of infection and ‘tolerance’, or the ability of hosts to limit the fitness cost of a given infection (Roy and Kirchner, 2000; Råberg *et al.*, 2009). Many interspecific comparisons have used immunological measures as a proxy for resistance, and have found that it decreases with increasing pace of life (Tieleman *et al.*, 2005; Martin *et al.*, 2007; Lee *et al.*, 2008; Previtalli *et al.*, 2012; Pap *et al.*, 2014). Only one study, to our knowledge, has investigated how the pathology sustained by host species as a result of infection, a measure of tolerance, increases with pace of life (Johnson *et al.*, 2012). Compared to these interspecific comparisons, far fewer investigations have tested the pace of life hypothesis of pathogen defence allocation within species, but they do support theoretical predictions: populations of garter snake *Thamnophis elegans* (see Sparkman and Palacios, 2009), and individual tropical house wrens *Troglodytes aedon* (see Tieleman *et al.*, 2005) with faster paces of life have reduced resistance, again quantified by immune function. These studies did not look at host tolerance, however, which would provide a more complete picture of how life history variation may affect disease ecology in natural systems (Johnson *et al.*, 2012). The present study is therefore the first to investigate how intraspecific life history divergence, driven by predation regime, correlates with variation in host tolerance of infection.

As well as natural selection driving life history divergence between populations, sexual selection can drive divergent life history strategies between the sexes (due to Bateman’s Principle). This divergence has a similar predicted effect on their relative investment in pathogen defence (Zuk and McKean, 1996; Schmid-Hempel, 2011). Females maximise fitness by lengthening lifespan and producing more broods; in order to do so they must invest more in self-maintenance systems, such as pathogen defence. Males, by contrast, maximise fitness by mating with as many females as possible; they therefore invest more in male-male competition and reproduction, and generally have shorter lifespans than females (Clutton-Brock and Isvaran, 2007; Zuk, 2009). Empirical evidence supports the role of proximate factors such as sex hormones in the resulting sex-difference in

pathogen defence (Schmid-Hempel, 2011). For example, the male hormone testosterone is immunosuppressive (Folstad and Karter, 1992; Schmid-Hempel, 2011), and additionally influences male behaviour in such a way that increases the probability of becoming infected (Gear *et al.*, 2009). Even without the assumption that longevity is more important for female than male fitness, models predict that under intense sexual selection, males invest less in pathogen defence (Stoehr and Kokko, 2006). As a direct result, male animals are typically more likely to be infected, and less tolerant of their infections than females across taxa (Zuk and McKean, 1996).

The Trinidadian guppy, *Poecilia reticulata*, provides an unprecedented opportunity to test the specific predictions of these tenets of evolutionary theory. The guppy's appeal as a model system stems from the topography of its habitat; waterfalls that represent significant upstream migration barriers, both to guppies and crucially to their major aquatic predators, bisect these streams creating several replicated upper and lower course populations. Lower course populations experience more intense predation pressure than those in upper courses, and this variation in predation pressure drives changes in a number of traits (Haskins *et al.*, 1961; Endler, 1978; Houde, 1997; Reznick *et al.*, 1997; Magurran, 2005). Conclusively, these trait differences evolve rapidly during transplant experiments when lower course guppies are introduced into upper courses, or when voracious predators are introduced into upper course populations (Endler, 1980; Reznick *et al.*, 1997; Gordon *et al.*, 2009). One of the most striking predator-driven trait changes is life history, with lower course guppies having evolved faster life histories than those in the upper courses (Reznick *et al.*, 1990, 1996, 2001). Additionally, across upper and lower course populations, sexual selection has driven a similar magnitude of divergence in life history between male and female guppies. Females can continue to use stored sperm to fertilise broods for up to 10 months after mating; males can therefore sire offspring long after death (Lopez-Sepulcre *et al.*, 2013). By contrast, females give birth to broods of live young, the sizes of which increase with body size and, as female growth is indeterminate, age (Reznick *et al.*, 1990). Female, but not male, reproductive fitness is therefore intrinsically linked to survival and longevity. Consequently, female lifespan is estimated to be 1.65 times longer than that of males (Arendt *et al.*, 2014).

Despite clear differences in life history between guppy populations and sexes, previous studies have failed to find strong evidence supporting the predicted correlated differences in pathogen defence (van Oosterhout *et al.*, 2006; Cable and van Oosterhout, 2007b; Martin and Johnsen, 2007; Fraser and Neff, 2010; Fraser *et al.*, 2010; Pérez-Jvostov *et al.*, 2012; Dargent *et al.*, 2013; Gotanda *et al.*, 2013). In fact, recent field surveys revealed a counterintuitive female-biased infection with *Gyrodactylus* spp. parasites in populations experiencing high levels of predation pressure (Chapter 4: Fig. 4.1; Appendix A: Fig. A.1; Gotanda *et al.*, 2013). We propose that the metric of resistance, as used by these

previous studies and quantified using infection loads, is an inappropriate metric with which to investigate population- and sex-differences in pathogen defence in this system. Such data are easily confounded by differences in exposure to parasites between upper and lower courses, and between male and female guppies (Johnson *et al.*, 2012). Due to the unidirectional flow of rivers, lower course guppy populations will be exposed to a higher number and diversity of parasites than will upper course populations (Blasco-Costa *et al.*, 2013). Additionally, female-biased exposure to and transmission of directly transmitted *Gyrodactylus* spp. likely explains the observed female-biased infection in lower courses (Chapter 4). By contrast, tolerance can be considered ‘the integrated product of parasite exposure and host defences over time and across space’ (Johnson *et al.*, 2012). Indeed, the few studies of this system that have used a measure of tolerance rather than resistance have found evidence to support the correlation between pace of life and investment in defence against parasites, at least between the sexes. Males suffer greater parasite-induced mortality, a clear measure of tolerance: infection with *Gyrodactylus* spp. makes male guppies more likely to be swept downstream during flood events (van Oosterhout *et al.*, 2007), and eaten by predators (Chapter 2) than females.

Here, we present observational data from natural guppy populations that indicate a sex-specific decrease in parasite tolerance in natural populations experiencing high levels of predation pressure. As such, these data conform to the pattern in parasite defence predicted by the well-studied predator-induced life history evolution in this system. We have previously illustrated that predator-driven changes in a behavioural trait of prey, shoaling, may indirectly effect the guppy-*Gyrodactylus* spp. host-parasite interaction (Chapter 4; Stephenson *et al.*, 2015). We here use data from a large-scale field survey to suggest that prey life history could be a second trait mediating the indirect effects of predators on the parasites of their prey (Werner and Peacor, 2003). The trade-off that faces prey species in allocating resources to either reproduction (to counterbalance the effects of predators) or defence against pathogens may provide an empirical example of how defence effectiveness declines with enemy diversity (Jokela *et al.*, 2000; Rigby and Jokela, 2000). We discuss reasons for the apparently predator-dependent effect of parasites suggested by our data, and call for further investigation of the impact of community interactions on disease ecology.

3.3 Materials and Methods

3.3.1 Data collection

We used data from a total of 2998 sexually mature adult guppies collected at 23 upper course and 28 lower course sites of 24 rivers in Trinidad between 2003 and 2009. The fieldwork data collection methods have been describe elsewhere (Chapter 4; Stephenson *et al.*, 2015). Briefly, whole shoals were enclosed in the river by a seine net, and individual fish were scooped out of the water using small buckets to avoid dislodging any ectosymbionts. Fish standard length, weight and sex were recorded. The fish were killed on site with an overdose of 0.02% tricaine methanesulfonate (MS222; PHARMAQ Ltd., UK) and preserved individually in 90% molecular grade ethanol. All fish, and the ethanol in which they had been transported, were then examined using a dissection microscope with fibre optic illumination for externally visible symbionts. The prevalence of species other than *Gyrodactylus* spp. in our sample was low (*Trichodina* spp. = 0.061; *Ichthyophthirius* spp. = 0.010; *Apiosoma* spp. = 0.004; digenean metacercariae = 0.006; *Camallanus* spp. = 0.0004; fungal infection = 0.007), and we therefore only report results for *Gyrodactylus* spp. infections. We did not identify *Gyrodactylus* spp. to species level, and acknowledge that there are at least three species complexes that commonly infect guppies (*G. turnbulli*, *G. bullatarudis* and *G. poeciliae*; see Xavier *et al.*, 2015).

3.3.2 Ethical note

The data presented in this chapter are the result of the collection of a large number of guppies from natural populations (2998). Although this is a considerable number of wild animals, they were collected from healthy populations over the course of seven years (see Appendix A: Table A1 for details); the impact is therefore likely to have been negligible. Any impact is also arguably outweighed by the large number of important findings to which these fish have contributed to date: molecular studies of the population structure of guppies (Barson *et al.*, 2009; Willing *et al.*, 2010) and gyrodactylids (Xavier *et al.*, 2015); characterisation of adaptive variation and drift in the Major Histocompatibility Complex (MHC; Llaurens *et al.*, 2012); research into the genetic basis of melanin pigmentation (Tezuka *et al.*, 2011); comparisons of the allelic variation in opsin genes across populations experiencing divergent environmental conditions (Tezuka *et al.*, 2014); evidence for parasite-mediated selection (van Oosterhout *et al.*, 2007); and observations of the role of biotic (Cable *et al.*, 2013) and abiotic (Schelkle *et al.*, 2012) factors in *Gyrodactylus* spp. infections in natural populations of guppies. By further using these data, both here and in Chapter 4, we were able to address outstanding

Response variable	Error family	Link function	Main effects	Two-way interactions	Random effect
Standardised scaled mass index	Gaussian	Identity	Weight (n)*	Course × Sex	Sample site
			Sex (c)*	Course × <i>Gyrodactylus</i> *	
			<i>Gyrodactylus</i> (c)*	<i>Gyrodactylus</i> × Sex*	
			<i>Gyrodactylus</i> (n)	<i>Gyrodactylus</i> × Weight*	
			Course (c)*	Sex × Weight	

TABLE 3.1: The starting model used to test the hypothesis that upper and lower course guppies differ in their tolerance of *Gyrodactylus* spp. infection. This starting model was simplified using backwards stepwise deletion of non-significant fixed effects to minimise the Akaike’s Information Criteria (AIC); those that remained in the final model are denoted with an asterisk. Fixed effects were included as categorical (c) or numeric (n) variables. ‘*Gyrodactylus*’ refers to presence (c) or count (n) of *Gyrodactylus* spp. parasites on a fish.

questions about the ecology of this host-parasite interaction without the need to remove more guppies from their natural habitat. We have also published the dataset in its raw form for other researchers to use (Stephenson *et al.*, 2015), *in lieu* of further field collections. This work was conducted under the UK Home Office license (PPL 30/2876) with approval by the Cardiff University Animal Ethics Committee.

3.3.3 Data analysis

All data analyses were performed in R (3.0.2; R Core Team, 2013), and we used course (upper *vs.* lower) as a proxy for the predation regime faced by the guppies at each site, as is common practice in this system (Chapter 4; Seghers, 1974; Reznick and Endler, 1982; Magurran *et al.*, 1994; Reznick *et al.*, 1997). In order to investigate how guppies differed in their tolerance of *Gyrodactylus* spp. infection in natural populations, we calculated the scaled mass index (SMI; Peig and Green, 2009) for each fish using the *lmodel2* package (Legendre, 2014) and the equation provided by Peig and Green (2009), standardised such that within upper and lower courses both males and females had a mean SMI of 0 to account for differences in body shape between the sexes, and between populations experiencing different predation regimes (Ghalambor *et al.*, 2004; Magurran, 2005). We used this standardised SMI as the response variable in a general linear mixed model in the *lme4* package (Bates *et al.*, 2014; Gaussian error family and identity link function; Table 3.1). We included sample site as a random term to account for the fact that we sampled more than one fish from each site. Fixed terms included: presence and number of *Gyrodactylus* spp. parasites; fish sex; course (upper *vs.* lower) and fish weight (as a measure of size). We also included the two-way interactions between these variables. In all analyses, model simplification involved removing non-significant fixed effects from the full model to minimise the Akaike’s Information Criterion (AIC).

3.4 Results

Infected guppies were of a lower condition (lower scaled mass index) than uninfected guppies, but this difference was only evident in lower course, high predation, populations (Fig. 3.1; course \times infection status interaction: $F_{1, 2986.4} = 21.49$, $p < 0.0001$). In these populations, this apparent effect of infection on fish condition was greater among males than among females (Fig. 3.1; sex \times infection status interaction: $F_{1, 2958.5} = 6.32$, $p = 0.012$).

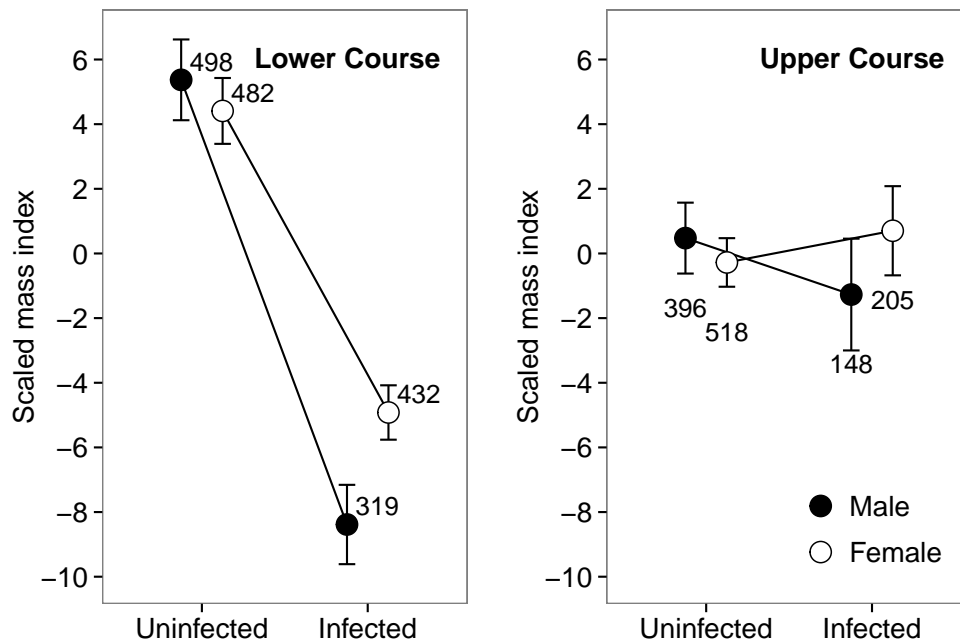


FIGURE 3.1: In lower course, high predation populations, infected guppies were of a lower body condition than uninfected guppies, and this effect was more pronounced in males (black circles) than in females (white circles; left-hand graph). In contrast, there was no effect of *Gyrodactylus* spp. infection on guppy body condition in the upper course, low predation populations (right-hand graph). We used the scaled mass index (SMI; Peig and Green, 2009) standardised such that within upper and lower courses both males and females had a mean SMI of 0 to account for differences in body shape between the sexes, and between populations experiencing different predation regimes. The scale can be interpreted as the difference in milligrams from the mean weight for a fixed length. Error bars are the standard error of the mean and the numbers give the total number of fish that contributed to each data point.

3.5 Discussion

Guppies infected with *Gyrodactylus* spp. were of a lower condition than uninfected guppies, but this difference was only evident in lower course, high predation, populations

(Fig. 3.1). In these populations, this apparent effect of infection on fish condition was greater among males than among females (Fig. 3.1). Guppies from lower course, high predation populations, particularly males, thus appear less tolerant of *Gyrodactylus* spp. infection than those from low predation populations. As lower course guppies and males are faster-lived than upper course and female guppies, our results provide support for the pace of life theory of pathogen defence allocation (Ricklefs and Wikelski, 2002). While the evidence for a difference in resistance to *Gyrodactylus* spp. between males and females, and between guppy populations from these two predation regimes is inconclusive (Chapter 4; Cable and van Oosterhout, 2007b; Fraser and Neff, 2010; Pérez-Jvostov *et al.*, 2012; Dargent *et al.*, 2013; Gotanda *et al.*, 2013), our results strongly indicate a difference between them in their tolerance of infection.

As *G. turnbulli* infection increases guppy vulnerability to predation (Chapter 2), predation pressure should select for increased defence against these parasites (Rantala *et al.*, 2011); however, *Gyrodactylus* spp. infection appeared to have a more negative impact on body condition in populations additionally experiencing high predation pressure. This observation provides support for the existence of a trade-off between defence against these two classes of enemy (Jokela *et al.*, 2000). Lower course guppies have evolved a faster life history in response to predation pressure, and as a result have a less effective defence against *Gyrodactylus* spp. parasites than those in the upper course. The existence of this trade-off is additionally supported by the findings of Dargent *et al.* (2013) that upon release from predation pressure (*via* translocation), guppies evolved increased resistance, and perhaps tolerance, to *G. turnbulli* (but see Stephenson, 2014 for other methodological and biological explanations of this result). An intriguing alternative is that parasites act in concert with predators in driving the evolution of shorter life histories in lower course guppy populations, as has been found in *Arabidopsis thaliana* (see Pagán *et al.*, 2008). Lower course guppies are additionally exposed to more, and a higher diversity of parasites than those in the upper courses (Chapter 4; Appendix A: Table A.1) because of the nature of rivers (Blasco-Costa *et al.*, 2013). This diversity likely further reduces the effectiveness of the defence that lower course guppies can mount to any one of their enemies (Jokela *et al.*, 2000).

Predator-induced stress could drive the difference in tolerance we observed between upper and lower course guppies: it has severe physiological costs in other systems (Boonstra *et al.*, 1998), which can decrease the efficiency of pathogen defence (Rigby and Jokela, 2000; van Der Veen, 2005; but see Coors and De Meester, 2010; Haislip *et al.*, 2012). This effect is often mediated by elevated levels of glucocorticoids such as cortisol (Kerby *et al.*, 2011). For example, individual salmonids with artificially raised cortisol levels were less resistant to *Gyrodactylus salaris* infection (Harris *et al.*, 2000). However, male guppies from high predation populations have lower cortisol levels

than those from low predation populations (Fischer *et al.*, 2014), which suggests that if predator-induced physiological stress is important in guppy response to parasitism, it is not mediated by the cortisol pathway. Alternatively, as suggested by their elevated cortisol levels, upper course guppies could actually experience greater mortality as a result of infection. Our data could support this explanation if mortality occurred before symptoms such as a loss of condition become evident, for example due to loss downstream in spate conditions (van Oosterhout *et al.*, 2007). We consider this scenario unlikely because spate conditions will affect both lower and upper course guppy populations. The observations of van Oosterhout *et al.* (2007) that infection increased the likelihood of being swept downstream likely also apply to, and may be stronger among, lower course guppies: in the absence of infection, upper course guppies are less likely to be swept downstream than lower course guppies (Mohammed *et al.*, 2012).

Stressors other than predators, both biotic and abiotic, differ between upper and lower courses and may affect guppy tolerance of *Gyrodactylus* spp. infection. Lower course guppies are more likely to be co-infected by multiple strains and species of parasite (Chapter 4; Blasco-Costa *et al.*, 2013). The synergistic effects of multiple infections, if they commonly occur with *Gyrodactylus* spp., may explain the lower body condition of *Gyrodactylus* spp.-infected guppies in the lower courses (Johnson and Hoverman, 2012). Additionally, their low shoal fidelity (Croft *et al.*, 2003a; Magurran, 2005) and resulting higher number of contacts mean males may be more likely to become co-infected than females. We screened guppies for multicellular ectoparasites only and, as prevalence of those other than *Gyrodactylus* spp. was very low, do not show the data (see Appendix A, Table A.1 for more information). Experimental work or further field surveys including bacterial or viral pathogens may reveal the importance of co-infections for the sex- or predation regime-dependent effect of *Gyrodactylus* spp. on guppy fitness (Johnson and Hoverman, 2012). Additionally, the lower courses of the rivers we sampled are subject to more anthropogenic disturbance than are the upper courses (Magurran, 2005). Reduced canopy cover due to deforestation in these areas (Magurran, 2005) is likely to have increased water temperatures, potentially increasing parasite fitness to the detriment of the host (Scott and Nokes, 1984). Increased pollution in the lower courses relative to the upper may also diminish guppy tolerance of *Gyrodactylus* spp. infection (Marcogliese and Pietroock, 2011; Gheorghiu *et al.*, 2012; Sala-Bozano *et al.*, 2012). Controlled experimental work, as well as detailed surveys of the differences in these variables between upper and lower course sites would help elucidate the role of environmental conditions in driving the population differences in tolerance that our data suggest.

A further potentially confounding factor is the difference in *Gyrodactylus* spp. exposure between upper and lower course guppy populations and between males and females

(Johnson *et al.*, 2012). Increased exposure to parasites in the lower courses could lead to more frequent infections, which could explain the reduced guppy condition in these populations. Although plausible, we consider it unlikely that this process is driving our result. First, increased exposure does not explain the apparent sex difference in tolerance that we observe: females in these populations are more often exposed to *Gyrodactylus* spp. parasites due to their more pronounced shoaling behaviour (Chapter 4; Richards *et al.*, 2010), and yet the present study shows that they have a higher tolerance of their infection than males. Second, increased exposure would have to have affected only those fish that were infected when they were sampled - the uninfected fish from these supposedly overexposed lower populations were of a significantly higher condition than those that were infected.

An important consideration is that these populations of guppies do not differ in their tolerance of infection, but instead differ in their resistance. If, for example, lower course guppies were less resistant to *Gyrodactylus* spp. than upper course guppies they would, by definition, develop heavier infection loads, and conceivably bear greater fitness costs as a result. Although we cannot rule it out, we consider this explanation relatively unlikely. We included the number of *Gyrodactylus* spp. parasites infecting each fish in the starting statistical model (Table 3.1), but it was not important in explaining the variation in guppy body condition. This result corroborates previous findings: the evidence for a difference in resistance, and therefore infection load, between males and females and between predation regimes from both experimental infections and from field observations is inconclusive (Chapter 4; Cable and van Oosterhout, 2007b; Martin and Johnsen, 2007; Fraser and Neff, 2010; Pérez-Jvostov *et al.*, 2012; Dargent *et al.*, 2013; Gotanda *et al.*, 2013).

Predator-driven changes in host traits may indirectly affect parasite traits; our results may reflect differences in the parasites between upper and lower course populations, rather than exclusively differences between the guppies. We previously reported that predator-driven changes in the shoaling behaviour of the guppy facilitates *Gyrodactylus* spp. transmission (Chapter 4). Increases in transmission opportunities are predicted to increase the virulence of parasites (Schmid-Hempel, 2011), which potentially explains why we observed a greater loss in body condition among guppies infected with lower course parasites than those infected with upper course parasites. This explanation is not mutually exclusive with differences between the hosts in these populations, indeed it is very likely that traits of both host and parasite differ between predation regimes.

Parasite evolution may also contribute to the sex difference in tolerance; the difference may be due to the extent to which the parasite has co-evolved with each sex. The sex ratios of many guppy populations (Pettersson *et al.*, 2004; Arendt *et al.*, 2014) and

particularly shoals (Magurran and Macías Garcia, 2000; Croft *et al.*, 2006) are female-biased, and females are more likely to survive to transmit their infection (Chapter 2; van Oosterhout *et al.*, 2007), so *Gyrodactylus* spp. parasites may be more adapted to the female host environment (Fellous and Koella, 2009; Duneau and Ebert, 2012). In the lower courses, predators also facilitate segregation between the sexes (Croft *et al.*, 2006), which could in turn facilitate this adaptation; that females in these populations are more often and more heavily infected (Chapter 4; Appendix A: Fig. A.1), and are more tolerant than males (present study and Chapter 2), could be indicative of sex-specific coevolution with *Gyrodactylus* spp. (Little *et al.*, 2010; Cousineau and Alizon, 2014). Differences in infected host condition between the sexes and populations due to differences in their tolerance will likely have implications for the epidemiology of the disease in these populations: higher condition hosts tend to initiate faster-spreading and longer-lasting epidemics of *Gyrodactylus* spp. in laboratory settings (Tadiri *et al.*, 2012).

In conclusion, while our result is correlational, we suggest that the striking and well-established predator-driven divergence in life history strategy between upper and lower course guppies, and between males and females, is the best-supported explanation for the observed divergence in their tolerance of *Gyrodactylus* spp. parasites. Many studies have investigated the role of parasites in affecting predator-prey relationships (e.g. Chapter 2; Hudson *et al.*, 1992; Johnson *et al.*, 2006), but this is among the first to have investigated the potentially equally important role of predators in affecting host-parasite relationships. Our data therefore lend support to recent calls for a unification of predator and parasite ecology into the field of ‘enemy ecology’ (Raffel *et al.*, 2008, 2010).

3.6 Authorship Acknowledgement

The manuscript resulting from this chapter is authored by:

Jessica F. Stephenson, Cock van Oosterhout (University of East Anglia) and Joanne Cable (Cardiff University).

3.7 Contribution to and position within the thesis

In this chapter I illustrate how predator-driven life history evolution predicts the patterns observed in the *Gyrodactylus* spp. parasite tolerance of wild guppies. I next illustrate how predators, through driving changes in a second trait, guppy shoaling behaviour, indirectly affect the distribution of parasites in natural populations.

Chapter 4

Parasites of Trinidadian guppies: evidence for sex- and age-specific trait-mediated indirect effects of predators

4.1 Abstract

Predation pressure can alter the morphology, physiology, life history and behaviour of prey; each of these in turn can change how surviving prey interact with parasites. These trait-mediated indirect effects may change in direction or intensity during growth or, in sexually dimorphic species, between the sexes. The Trinidadian guppy *Poecilia reticulata* presents a unique opportunity to examine these interactions; its behavioural ecology has been intensively studied in wild populations with well-characterised predator faunas. Predation pressure is known to have driven the evolution of many guppy traits; for example, in high predation sites, females (but not males) tend to shoal, and this anti-predator behaviour facilitates parasite transmission. To test for evidence of predator-driven differences in infection in natural populations, we collected 4715 guppies from 62 sites across Trinidad between 2003 and 2009 and screened them for ectosymbionts including *Gyrodactylus* spp. A novel model-averaging analysis revealed that females were more likely to be infected with *Gyrodactylus* spp. parasites than males, but only in populations with both high predation pressure and high infection prevalence. We propose that the difference in shoaling tendency between the sexes could explain the observed difference in infection prevalence between males and females in high predation sites. The infection rate of juveniles did not vary with predation regime, probably

because juveniles face constant predation pressure from conspecific adults and therefore tend to shoal in both high and low predation sites. This represents the first evidence for age- and sex-specific trait-mediated indirect effects of predators on the probability of infection in their prey.

4.2 Introduction

Predators affect the density and traits of their prey, and each of these can have important implications for infectious disease dynamics in prey populations. Density-mediated effects are well-studied (e.g. Anderson and May, 1981, Holt and Roy, 2007), and make the specific prediction that predators should reduce parasite transmission because predation can reduce prey density, thereby decreasing contact rates among hosts (Packer *et al.*, 2003). However, recent work suggests that trait-mediated effects can be at least as important as those mediated by density (Werner and Peacor, 2003, Hatcher *et al.*, 2006). Trait-mediated indirect effects arise when a change in the phenotype of individuals of one species, caused by the presence of another, alters how the reacting species interacts with others in the community (Werner and Peacor, 2003). Predators affect prey morphology, physiology, life history and behaviour, each of which is likely to change how the prey interacts with its parasites. For example, *Daphnia dentifera* attain a relatively large body size in the presence of chemical cues of an invertebrate predator, and consequently upon death release more spores of a virulent yeast parasite (Duffy *et al.*, 2011). Additionally, Parris and Beaudoin (2004) found that fungal pathogens reduce tadpole development rates only when predators are present, and they hypothesised that this is due to the higher level of predator-induced physiological stress. Predators may also evoke behavioural changes: negatively phototactic *D. magna* clones avoid visually hunting predators, and as a result suffer increased exposure to parasite spores in pond sediment (Decaestecker *et al.*, 2002). Here, we investigated the net effect of predation on disease transmission by comparing parasite prevalence among several wild guppy populations.

The sexes of many species are dimorphic in a variety of traits and it is logical, if unexplored, that trait-mediated indirect effects act differently upon males and females. This may be due to intrinsic differences; for example, male crayfish possess larger claws than females and are therefore less vulnerable to predation. Males consequently show a less dramatic behavioural response to the presence of a predator (Stein and Magnuson, 1976). Response to parasites is also known to differ markedly between the sexes in vertebrates; males have a higher parasite prevalence (the percentage of potential hosts infected, Bush *et al.*, 1997) than females in a wide range of taxa (Zuk and McKean 1996).

Reasons for this bias include sex differences in traits that alter exposure or susceptibility to parasites, or both (Zuk and McKean, 1996). Predator-driven sex-specific changes in these traits may well contribute to sex-biased parasitism.

Ontogenetic development often leads to dramatic changes in size, morphology, physiology and habitat use; trait-mediated indirect effects are likely to act differently on individuals as they age (Werner and Peacor, 2003). The effects of the presence or cues of predators on various traits have been shown to differ between the size and age classes of a number of species. For example, smaller, more vulnerable tadpoles reduce activity by up to 98% more than large tadpoles in the presence of a predator (McCoy and Bolker, 2008). Predator-driven trait changes may therefore also contribute to ontogenetic differences in parasitism.

Guppies inhabiting the streams of Trinidad provide an unprecedented opportunity to test for the importance of age- and sex-specific trait-mediated indirect effects on parasitism in a natural system. The guppy (*Poecilia reticulata*) system has been instrumental to our understanding of the role predation plays in shaping the evolution of species (Magurran, 2005). Its appeal as a model system stems from the topography of its habitat; waterfalls that represent significant upstream migration barriers, both to guppies and crucially to their predators, bisect these streams creating several replicated upper and lower course populations. It is well recognised that lower course populations experience more intense predation pressure than those in upper courses, and that this variation in predation pressure drives sex-specific trait changes (Haskins *et al.*, 1961; Endler, 1978; Houde, 1997; Reznick *et al.*, 1997; Magurran, 2005). Conclusively, these trait differences evolve rapidly during transplant experiments when lower course population guppies are introduced into upper courses, or when voracious predators are introduced into upper course populations (Endler, 1980; Reznick *et al.*, 1997; Gordon *et al.*, 2009). Trait changes between upper and lower course guppies that have been attributed to predation pressure include: body size; male colouration; mate choice behaviour; life history and life expectancy (Haskins *et al.*, 1961; Seghers, 1974; Endler, 1978, 1980; Reznick *et al.*, 1997; reviewed by Houde, 1997; Magurran, 2005). Anti-predator behaviour is among the most striking of these trait changes; females from lower course populations show greater shoal cohesion (Seghers, 1974; Endler, 1978) and spend more time shoaling than those from upper courses (Magurran and Seghers, 1994b). Conversely, juvenile guppies from upper and lower courses show an equally strong shoaling tendency (Magurran and Seghers 1990); this is likely to be because juveniles face a similar threat of predation from adult guppies across populations.

In contrast to predation, parasitism is poorly characterised in Trinidadian guppy populations, but small-scale studies provide us with clear predictions to test on a larger scale.

The dominant guppy parasites, which include *Gyrodactylus turnbulli*, *G. bullatarudis* and *G. poeciliae* (Xavier *et al.*, 2015; hereafter *Gyrodactylus*) are directly transmitted ectoparasitic monogeneans that impact guppy swimming ability (Cable and Harris, 2002), reproductive fitness (e.g. Kennedy *et al.*, 1987) and survival (Chapter 2; van Oosterhout *et al.*, 2007). Shoaling is an important anti-predator behavioural trait in guppies that facilitates *Gyrodactylus* transmission (Richards *et al.*, 2010; Croft *et al.*, 2011; Johnson *et al.*, 2011), and females are more likely to become infected than males because of their higher shoaling tendency (Richards *et al.*, 2010; Johnson *et al.*, 2011). Notably, Johnson *et al.* (2011) found that *Gyrodactylus* transmission remained high in aquaria with reduced guppy density, indicating that social interactions were more important than density in maintaining transmission rates. The few field studies describing this parasite-host-predator system have also indicated its suitability for investigating sex-specific trait-mediated indirect effects. As we would predict from the small-scale studies, *Gyrodactylus* prevalence tends to be greater in populations in the lower courses of rivers in Trinidad, where guppies shoal more, although this is not always the case (Martin and Johnsen, 2007; Fraser and Neff, 2010; Gotanda *et al.*, 2013). Additionally, prevalence may differ between males and females depending on the course of the river (Gotanda *et al.*, 2013), but some studies have found no sex difference in infection (Martin and Johnsen, 2007; Fraser and Neff, 2010), despite sex differences in shoaling behaviour.

Rather than asking how parasites contribute to patterns already recognised as driven by predators in this system (e.g. body size and colouration: Gotanda *et al.*, 2013), we build on previous work by asking how this predator-driven spatial pattern in guppy traits indirectly affects *Gyrodactylus* parasite prevalence. Specifically, we test the prediction that populations that experience higher predation pressure will show greater parasite prevalence. Furthermore, we expect prevalence among females to be higher than among males, especially in populations that experience higher predation pressure, given that they shoal more than males. Because juveniles face similar levels of predation pressure across populations, we predict parasitism among juveniles to be relatively consistent. We additionally test whether the level of inter-site variation could explain the apparently contradictory results in previous guppy-gyrodactylid field surveys, and test for temporal patterns in parasite prevalence. We use extensive field sampling to test these predictions. A multi-model inference and model averaging approach to data analysis suits datasets like ours that contain a large number of explanatory variables, because it takes into account model uncertainty and can provide better estimates of model parameters than more traditional null hypothesis significance tests (Bolker *et al.*, 2009; Grueber *et al.*, 2011).

4.3 Materials and Methods

4.3.1 Data collection

We collected 4715 guppies from 62 sites in Trinidad between 2003 and 2009 (Appendix A: Table A.1). All work using these fish was conducted in accordance with the UK Home Office regulations (PPL 30/2876) with approval by the Cardiff University Animal Ethics Committee. Whole shoals were enclosed in the river by a net, and individual fish were scooped out of the water using small buckets to avoid dislodging ectoparasites. Fish standard length, weight and class (female, male or juvenile) were recorded. We attempted to obtain equal numbers of each sex at each site. The fish were killed on site with an overdose of 0.02% tricaine methanesulfonate (MS222; PHARMAQ UK, Ltd.) and preserved individually in 90% molecular grade ethanol. All fish, and the ethanol in which they had been transported, were then examined using a stereo-microscope with fibre optic illumination for any externally visible symbionts. It is unknown whether organisms such as *Trichodina* and *Apiosoma* cause any harm to their hosts, and we therefore collectively refer to these and other potential guppy parasites as ‘symbionts’. *Gyrodactylus* spp., *Trichodina* spp., *Ichthyophthirius* spp., *Apiosoma* spp., digenean metacercariae, *Camallanus* spp. and fungal infections were recorded; no other taxa were found. For *Gyrodactylus* spp. (hereafter *Gyrodactylus*), we did not identify the parasites to species level, and acknowledge that there are three species complexes that infect guppies (*G. turnbulli*, *G. bullatarudis* and *G. poecilae*; Xavier *et al.*, 2015). We recorded the number of *Gyrodactylus* parasites present but only the presence of other symbionts because of difficulties in quantification. Throughout this paper we define prevalence as the proportion of fish that was infected, *Gyrodactylus* mean intensity as the average number of parasites carried by the infected fish in a population, and mean abundance as the average number of parasites carried by all fish in a population (Bush *et al.*, 1997).

4.3.2 Ethical note

The data presented in this chapter are the result of the collection of a large number of guppies from natural populations (4715). Although this is a considerable number of wild animals, they were collected from healthy populations over the course of seven years (see Appendix A: Table A1 for details); the impact is therefore likely to have been negligible. Any impact is also arguably outweighed by the large number of important findings to which these fish have contributed to date: molecular studies of the population structure of guppies (Barson *et al.*, 2009; Willing *et al.*, 2010) and gyrodactylids

(Xavier *et al.*, 2015); characterisation of adaptive variation and drift in the Major Histocompatibility Complex (MHC; Llaurens *et al.*, 2012); research into the genetic basis of melanin pigmentation (Tezuka *et al.*, 2011); comparisons of the allelic variation in opsin genes across populations experiencing divergent environmental conditions (Tezuka *et al.*, 2014); evidence for parasite-mediated selection (van Oosterhout *et al.*, 2007); and observations of the role of biotic (Cable *et al.*, 2013) and abiotic (Schelkle *et al.*, 2012) factors in *Gyrodactylus* spp. infections in natural populations of guppies. By further using these data, both here and in Chapter 3, we were able to address outstanding questions about the ecology of this host-parasite interaction without the need to remove more guppies from their natural habitat. We have also published the dataset in its raw form for other researchers to use (Stephenson *et al.*, 2015), *in lieu* of further field collections. This work was conducted under the UK Home Office license (PPL 30/2876) with approval by the Cardiff University Animal Ethics Committee.

4.3.3 Data analysis

To test for the factors important in *Gyrodactylus* infection, we constructed models using both *Gyrodactylus* presence (0 or 1) and *Gyrodactylus* count (number of parasites) on each fish as the response variables using the methods described below. Note that *Gyrodactylus* presence is used to estimate prevalence, whereas the count is used to calculate mean abundance (*sensu* Bush *et al.*, 1997). Here we describe the presence model (i.e. prevalence) because it shows the best fit to the data. This discrepancy in our ability to explain prevalence and abundance in these populations is unsurprising; parasite abundance is more dependent than prevalence on host infection history, susceptibility and immune response, all of which are beyond the scope of our data. We do, however, give the results from the *Gyrodactylus* count model (i.e. abundance) in Appendix A (Tables A.2 and A.3 and Figs. A.1 and A.2). All statistical analyses were conducted using R statistical software (3.0.2; R Core Team, 2013).

Throughout the analyses we use the watercourse, ‘course’, the fish were sampled from (i.e. lower, mid, upper sections of the river or Pitch Lake [hereafter ‘lake’]) as a proxy for the predation regime faced by guppies at each site. The lake should also be considered separately from the rivers because of the unique properties of water from Pitch Lake; this has been found to protect guppies from *Gyrodactylus* infection (Schelkle *et al.*, 2012). During sampling, we recorded the presence of fish species known to prey upon guppies, including *Anablepsoides hartii*, *Aequidens pulcher*, *Cichlasoma taenia*, *Polycentrus schomburgkii*, *Crenichla alta*, *Hoplias malabaricus* and *Gobiomorus dormitor*. Presence of predatory species is a recognised measure of predation pressure in this system (Seghers, 1974; Reznick and Endler, 1982; Magurran and Seghers, 1994a;

Reznick *et al.*, 1997). To confirm that our ‘course’ variable, which reflected the location of the site within the rivers or lake, was correlated with predator fauna, we calculated the species richness of the major guppy predators (*P. schomburgkii*, *C. alta*, *H. malabaricus* and *G. dormitor*) at each site. Each of these species has a trophic level of greater than 3.5 (Fishbase; www.fish-base.org), indicating that they are generally ichthyophagous (Magurran, 2005). We used ANOVA to test for differences in major guppy predator species richness between sites in each of our four categories. Sites categorised as ‘upper’ had significantly lower mean predatory species richness than those categorised as ‘mid’, ‘lower’ or ‘lake’ (Appendix A: Figure A.3; $F_{3, 54} = 9.14$, $p < 0.001$). Overall, therefore, the levels of our course variable differed significantly in mean predator species richness, but there was variation between sites within each course. Consequently, we used the ‘course’ variable in further models rather than predator species richness because the latter is less accurate; we may have missed the predators at some sites, and they may move between sites within course. Additionally, the ‘course’ variable reflects the presence of waterfall migration barriers and, hence, which sites these predators could access. Guppy traits driven by predation pressure such as shoaling change over evolutionary time (Endler, 1980; Reznick *et al.*, 1997; Gordon *et al.*, 2009), and are therefore not dependent on the presence of predators on our sampling day. In summary, the overall differences in predation pressure between courses, confirmed by our own predator species richness score, more accurately represents the predator-driven traits of the guppies in our sample.

We used the presence or absence of *Gyrodactylus* parasites on guppies as the categorical response variable in a binomial Generalised Linear Mixed Model (GLMM) in the *lme4* package. The starting model included: year; class (female, male or juvenile guppy); course (upper, mid, lower, or lake) and fish weight as fixed main effects. The following two-way interactions between these factors were included: course \times class; course \times weight; class \times weight. The prevalence of other symbionts in our sample was low (*Trichodina* spp. = 0.061; *Ichthyophthirius* spp. = 0.010; *Apiosoma* spp. = 0.004; digenean metacercariae = 0.006; *Camallanus* spp. = 0.0004; fungal infection = 0.007). We therefore only included the presence of *Trichodina* spp., the most common symbiont after *Gyrodactylus* in our sample, and its interaction with host class, as explanatory variables in the *Gyrodactylus* presence model. We sampled only 14 sites on more than one occasion (Appendix A: Table A.1.1, Fig. A.4), and as the full model contained site as part of the random effect, it was not possible to include the year \times course interaction in this full model.

During simplification of the starting model, it became clear that there were several, equally well supported models based on comparisons of Aikake’s Information Criterion (AIC) and R^2 (calculated using the method given by Nakagawa and Schielzeth, 2013).

In order to take into account model uncertainty, and to increase the robustness of the parameter estimates and assess their relative importance, we employed an information theoretic approach to multi-model inference (Burnham and Anderson, 2002; Grueber *et al.*, 2011).

Following Grueber *et al.* (2011), we constructed a global model using the *lme4* package (Bates *et al.*, 2014) in R with all fixed terms included about which we had *a priori* hypotheses, namely all of those in the starting model given above. We then standardised the model parameters to a mean of 0 and a standard deviation of 0.5 using the *arm* package (Gelman and Su, 2014). We used the ‘zero method’ of parameter weighting because we are interested in an estimate of the effect that each of our parameters had on prevalence, rather than the effect of one particular parameter (Burnham and Anderson, 2002; Nakagawa and Freckleton, 2011). Using the ‘dredge’ function in the *MuMIn* package (Bartoń, 2014) we created a set of models, and from these selected those within the top 4 delta AIC ($n = 4$). We used AIC, rather than AIC_c , because the number of observations was more than 40 times the number of explanatory variables in our starting model (Burnham and Anderson, 2002). The ‘model.avg’ function then produced averaged parameter estimates from this top set of models, and the relative importance of these parameters. The relative importance of each parameter was calculated by summing the Akaike weights across all the models in which the parameter occurred (Burnham and Anderson, 2002). We had some issues with non-convergence of the model (Grueber *et al.*, 2011): the confidence intervals for the estimates for 2008 and 2009 were very large, probably because of the relatively small sample sizes for these years ($n = 192$ and 111 respectively). Re-running the model excluding these two years improved the convergence and did not change the conclusions (remaining $n = 4412$).

The sampling was conducted over different spatial scales. This was incorporated into the model as a hierarchical random factor; sample site was nested within course, nested within river, nested within drainage. During model simplification we used Likelihood Ratio Tests to examine the importance of each level of this nested term. We included these factors as random terms because we wanted to be able to generalise these results to other sites across other rivers.

4.4 Results

4.4.1 *Gyrodactylus* prevalence: Fixed effects

The second-order interactions between course, year, host class and weight proved to be important predictors of *Gyrodactylus* infection (Tables 4.1 and 4.2). Our data confirmed

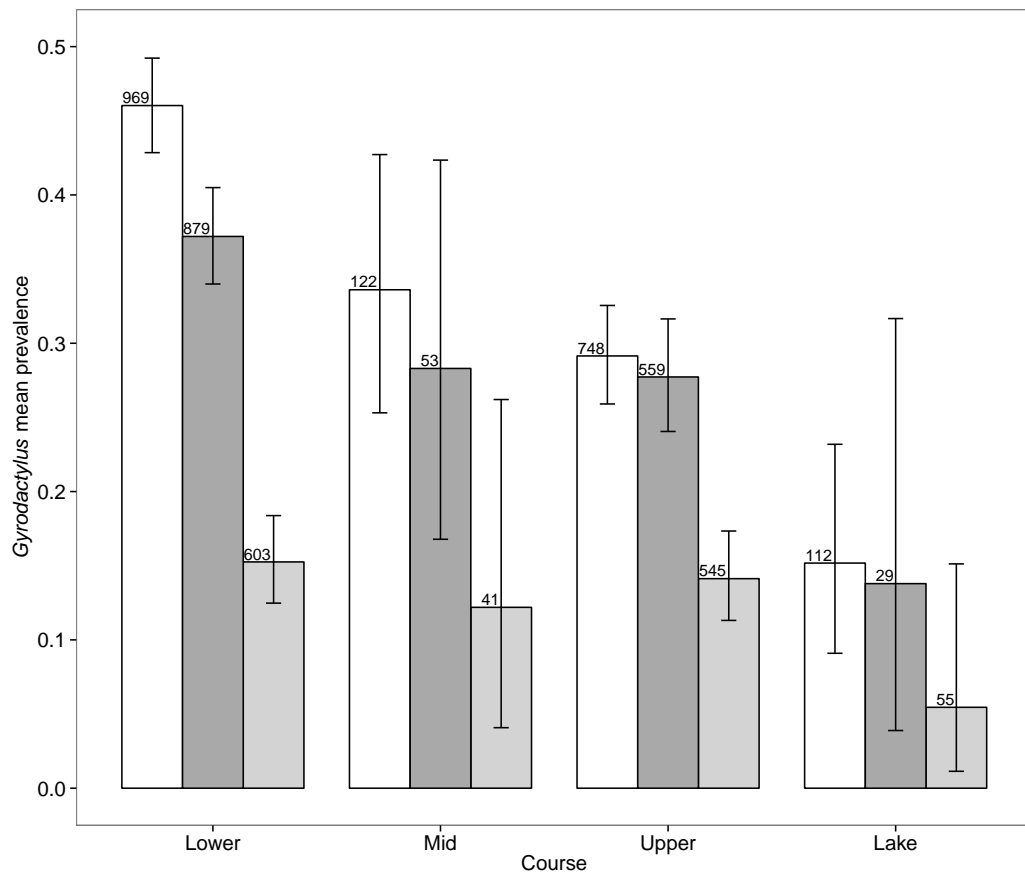


FIGURE 4.1: *Gyrodactylus* spp. mean prevalence in fish from the lower, mid and upper courses and the Pitch Lake ('Lake'). White bars are data from females, dark grey from males, and light grey from juveniles. Error bars are the 95% confidence intervals; these were calculated following the Clopper-Pearson or 'exact' method for binomial distributions. The numbers give the total number of fish that contributed to each bar.

the pattern that *Gyrodactylus* prevalence is higher in the lower courses of rivers in this system (course, Table 4.1; Martin and Johnsen, 2007; Gotanda *et al.*, 2013), and demonstrated a difference in this pattern between males, females and juveniles (course \times class; Fig. 4.1). Prevalence among juveniles did not change between courses of the river. For females and males, however, there was a marked difference in prevalence between the courses. Prevalence was higher in females than in males in the lower course, but this difference became less and less evident through courses with decreasing overall prevalence. These patterns were not evident in the *Gyrodactylus* abundance data (Appendix A; Fig. A.1).

In upper course guppies, prevalence of *Gyrodactylus* increased with weight for juvenile but not adult guppies; whereas in lower course guppies, weight was an important predictor of *Gyrodactylus* prevalence across all three guppy classes (course \times weight and class \times weight, Table 4.1; Fig. 4.2).

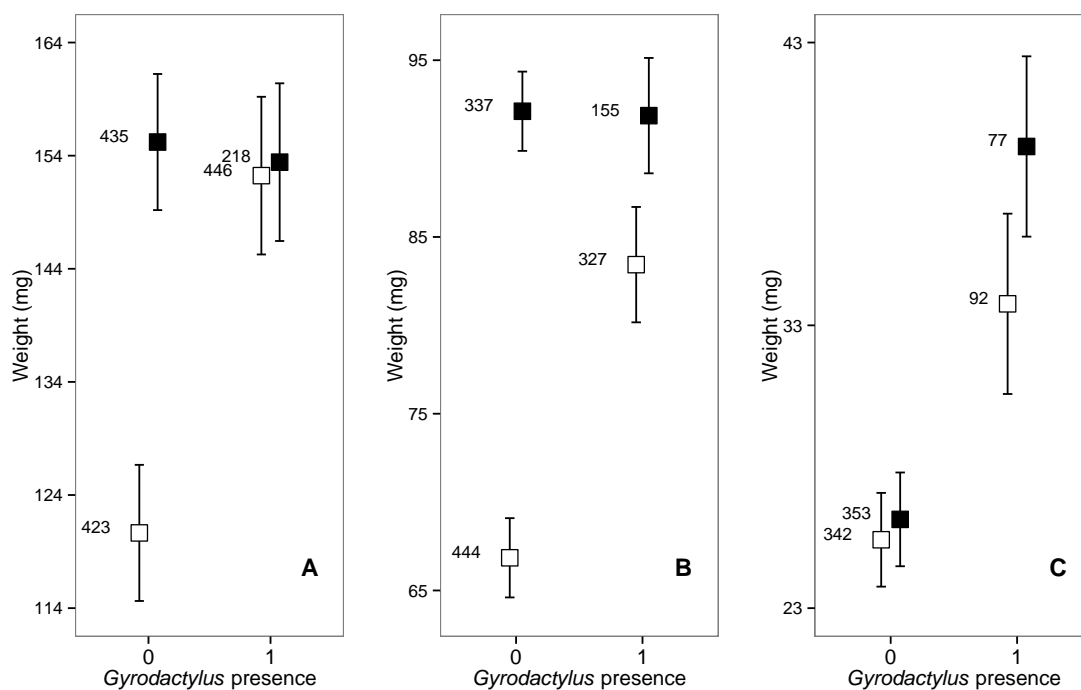


FIGURE 4.2: The relationship between fish weight and *Gyrodactylus* spp. presence in females (A), males (B) and juveniles (C) across the lower and upper courses. These data are from sites where at least one fish was infected with *Gyrodactylus* spp. The white squares represent data from lower course, the black upper course sites. Error bars are the 95% confidence intervals, and the numbers give the total number of fish that contributed to each data point.

Our data revealed a temporal change in the prevalence of *Gyrodactylus* parasites (year, Table 4.2; Fig. 4.3). To test whether this was an artefact of the sites sampled in these years, and therefore represented a spatial rather than temporal change in prevalence, we plotted the change in prevalence between the 1st and 2nd visit to each site that was visited more than once (Appendix A: Fig. A.4). The pattern observed in the overall data (Fig. 4.3) was also present, though non-significant, in the lower sites in this subset ($n = 7$; mean change in prevalence [95% confidence limits] = 0.174 [-0.10, 0.45]).

We used a subset of the data (data from lower and upper course populations only) to run two additional GLMs including the year \times course interaction as *post-hoc* tests. The response variable for one model was the mean prevalence from each sample site (using a quasipoisson error family and a log link function), and for the other we used data from the individual fish (binomial error family). These tests revealed that, although there was a strong year effect across both site and individual level analyses (site: $F_{3, 64} = 3.06$, $p = 0.034$; individual: Deviance $_{3,4177} = 151.25$, $p < 0.001$), the year \times course interaction was only significant in the individual level analysis (Deviance $_{7, 4177} = 183.27$, $p < 0.001$;

Fig. 4.3). From our data, therefore, we can say that *Gyrodactylus* prevalence increased through time, and that there was a suggestion that this increase happened faster at lower than upper course sites.

4.4.2 *Gyrodactylus* prevalence: Random effects

The prevalence of *Gyrodactylus* was highly spatially variable and most variation (61%) occurred between sample sites, i.e. on the smallest spatial scale. In contrast, differences between drainages, between rivers within drainages, and between courses within rivers did not significantly contribute to differences in prevalence (Appendix A: Fig. A.5) and these factors were all removed from the random model (following Bolker *et al.*, 2009). Comparison of the AIC values between models with and without each of these nested random terms confirmed that the model containing just sample site as a random term was superior: it had the lowest AIC. The global model for the model averaging therefore included the simplified random term (site).

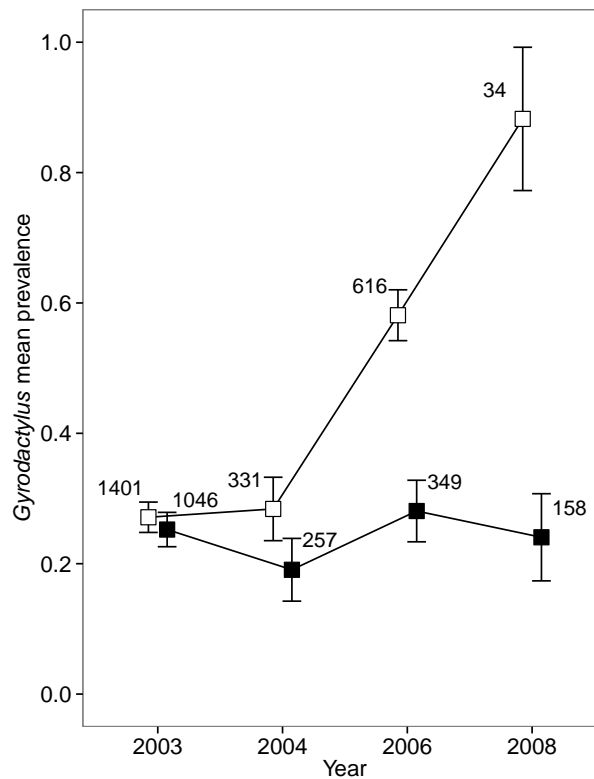


FIGURE 4.3: Change in *Gyrodactylus* spp. prevalence over the years sampled in this study. White squares represent data from fish from lower course sites and black squares fish from the upper. The data are the means from all fish sampled and the error bars are the 95% confidence intervals around those means. These were calculated following the Clopper-Pearson or ‘exact’ method for binomial distributions. The numbers on the plot give the overall number of fish contributing to each data point.

Model	Degrees of Freedom	Log Likelihood Ratio	AIC	Δ AIC	AIC weight
2/3/4/5/7/8/9	21	-1870.15	3782.30	0.00	0.54
1/2/3/4/5/7/8/9	22	-1870.15	3784.30	2.00	0.20
2/3/4/5/8/9	15	-1877.25	3784.49	2.10	0.18
1/2/3/4/5/6/7/8/9	24	-1869.02	3786.04	3.74	0.08

TABLE 4.1: The top-ranked candidate models explaining variation in *Gyrodactylus* spp. infection of guppies. AIC = Akaike information criteria; the model terms are coded as follows: 1: Presence of *Trichodina* spp.; 2: Course; 3: Class, 4: Year, 5: Weight, 6: Presence of *Trichodina* \times Class, 7: Course \times Class; 8: Course \times Weight; 9: Class \times Weight.

Predictor	Standardised coefficient	Unconditional SE	95% CI		Relative importance of overall predictor
			2.5%	97.5%	
*(Intercept)	-4.303	1.896	-8.021	-0.587	
Course-lower	2.274	1.968	-1.583	6.132	Course=1
Course-mid	0.424	2.173	-3.835	4.684	
Course-upper	1.543	1.990	-2.358	5.444	
Class-j	2.204	1.477	-0.691	5.099	Class=1
Class-m	0.161	0.632	-1.078	1.399	
Year-2004	0.177	0.144	-0.106	0.460	Year=1
*Year-2006	1.903	0.169	1.572	2.234	
Weight	1.640	0.853	-0.033	3.312	Weight=1
*lower:j	-2.887	1.045	-4.935	-0.838	Course:Class=1
*mid:j	-4.719	1.373	-7.409	-2.028	
*upper:j	-2.967	1.050	-5.024	-0.910	
lower:m	-0.266	0.691	-1.620	1.089	
mid:m	-0.763	0.910	-2.546	1.020	
upper:m	-0.539	0.698	-1.908	0.830	
lower:weight	-0.848	0.862	-2.537	0.841	Course:Weight=1
mid:weight	-1.310	1.094	-3.453	0.834	
upper:weight	-1.603	0.879	-3.327	0.120	
*j:weight	2.812	0.629	1.580	4.044	Class:Weight=0.82
m:weight	0.759	0.395	-0.015	1.532	
trichodina	-0.014	0.278	-0.559	0.531	Trichodina=0.28
j:trichodina	-1.246	1.077	-3.357	0.865	Class:Trichodina=0.08
m:trichodina	0.244	0.387	-0.515	1.003	

TABLE 4.2: Model-averaged standardised coefficients, unconditional standard error, and 95% confidence intervals of predictors for guppy infection with *Gyrodactylus* spp. Predictors for which the confidence intervals do not include zero are denoted with an *; ‘Class’ refers to host class: female, male or juvenile; ‘trichodina’ refers to the presence of *Trichodina* spp.

4.5 Discussion

Our results show that *Gyrodactylus* infection in guppies is age- and sex-specific, and that there is significant spatiotemporal variation between Trinidadian populations. *Gyrodactylus* prevalence was higher among females than males, but only in fish from the lower courses (i.e. high predation sites) of the 26 rivers sampled (Fig. 4.1). There was no difference in prevalence among juveniles from different courses (lower, mid, upper or lake; Fig. 4.1). These findings support the role of age- and sex-specific trait-mediated indirect effects of predators on infection prevalence in their guppy prey. Although our results are correlational, we interpret them in light of a vast number of experimental studies on this system to draw conclusions about the factors driving parasite prevalence on guppies in the natural environment in Trinidad.

Gyrodactylus prevalence was higher in females than in males but only in the lower courses of the rivers; there are several, non-mutually exclusive explanations for this pattern. Host shoaling behaviour is important for parasite transmission (Richards *et al.*, 2010; Croft *et al.*, 2011), and females tend to shoal more than males because males trade off the advantages of schooling against the search for mating opportunities (Magurran and Seghers, 1994a; Griffiths and Magurran, 1998). This sex difference is due to the fact that female reproductive fitness is dependent on survival and longevity; male guppies, by contrast, can sire offspring up to 10 months *post mortem* (Lopez-Sepulcre *et al.*, 2013) and consequently spend more time harassing females than engaging in anti-predator behaviour (Magurran and Seghers, 1994a). The relatively higher parasite prevalence of females observed only in the lower courses is consistent with the fact that shoaling is more common in downstream populations due to increased predation pressure (Seghers, 1974; Endler, 1978). Laboratory studies also confirm that the frequency of social contact governs *Gyrodactylus* epidemics and that the more gregarious female guppies contract infections sooner than more solitary males (Johnson *et al.*, 2011).

Further support for the role of predator-driven trait-mediated indirect effects on parasite prevalence comes from the unchanging prevalence among juveniles across the lower, mid and upper courses. Juvenile guppies from upper and lower course populations face equal predation pressure from adult guppies and show equally strong shoaling tendencies in both habitats (Magurran and Seghers, 1990). This suggests that there is no difference in the rate of parasite transmission among juveniles of the upper, mid and lower course populations, which would explain why they show similar parasite prevalence across habitats.

Our results indicate the importance of exposure, rather than susceptibility, to parasites in this system. Two lines of evidence suggest that guppies from low predation populations

are less resistant to *Gyrodactylus* infection than those from high predation populations. First, laboratory infections reveal that upper Aripo (low predation) guppies have lower innate resistance to *Gyrodactylus* than lower Aripo guppies (high predation; Cable and van Oosterhout, 2007b). Secondly, guppies from low predation populations have higher cortisol levels than those from high predation populations (Fischer *et al.*, 2014), and higher cortisol levels are associated with lower *Gyrodactylus* resistance in salmonids (Harris *et al.*, 2000). Despite guppies from high predation populations therefore likely being more resistant, *Gyrodactylus* prevalence was higher among them; this is most probably due to the increased transmission potential at these sites.

The Pitch Lake provides an interesting exception to the pattern we observed elsewhere; although guppies here experience relatively high levels of predation pressure, *Gyrodactylus* prevalence was low and uniform across males, females and juveniles. A previous study has, however, described the anthelmintic properties of Pitch Lake water (Schelkle *et al.*, 2012), and this might bring *Gyrodactylus* prevalence in the lake below the threshold necessary for it to be noticeably affected by guppy response to predation pressure.

Infection probability was positively correlated with the size of guppies, but this correlation was only observed in the lower courses (Fig. 4.2). Laboratory experiments and computational modelling show that parasite load and the duration of infection increases with increased host size (van Oosterhout *et al.*, 2003; Cable and van Oosterhout, 2007a; van Oosterhout *et al.*, 2008), and this may result in the positive correlation between body size and infection incidence observed in the lower courses. Catch bias may also be important; larger fish are likely to be able to support parasites with less of a decrease in condition than smaller fish (Krause *et al.*, 1998), which in turn will improve their chance of survival (and of being observed). However, the pattern of infected fish being larger than uninfected fish does not hold for adults in the upper course populations. Parasite infection will not increase predation risk in these upper courses; infected fish are more likely to survive and clear their infection. Furthermore, given that shoaling increases the probability of contracting an infection, lower course guppies are more likely to become re-infected as they grow. Upper course guppies, by contrast, are less likely to become re-infected once they clear an infection because of their more solitary lifestyle and the lower overall prevalence in these populations. The correlation between size and infection probability is the same across habitats for juveniles. Because juveniles show a similar shoaling behaviour in the upper and lower courses, the probability of acquiring an infection increases with increased size (and age) at similar rates across habitats.

Age- and sex-specific predator-driven trait-mediated indirect effects provide the most parsimonious and well-supported explanation for our results, but the patterns may be partially explained by other processes. First, parasite infection may lead to increased

predation risk (Chapter 2; Hatcher *et al.*, 2006; Johnson *et al.*, 2006). It is likely that *Gyrodactylus*-infected fish are predated more frequently, and that this parasite-induced vulnerability to predation is more severe on males than on females (Chapter 2). These direct effects of predators may therefore have an important role in driving the pattern we observe here. In addition to the effects of male-biased parasite-induced vulnerability to predation, it is possible that lower course males are simply less tolerant of *Gyrodactylus* spp. infection, and therefore experience a higher level of parasite-induced mortality, than lower course females. This explanation of the current result is supported by the data presented in Chapter 3. Further work is required to elucidate the relative importance of these mechanisms in driving the observed patterns in sex-biased parasite distribution among natural guppy populations.

Another role for predators in driving this pattern could be as paratenic hosts for the parasites. *Gyrodactylus* infections were found on a number of *Anablepsoides hartii* from sites sampled in this study (Cable *et al.*, 2013). Experimental infections demonstrate that the parasites can survive on *A. hartii* and transfer from them to guppies in semi-natural conditions (Cable *et al.*, 2013). However, whether *Gyrodactylus* can use other predatory species in the same manner is unknown. Given that the population size of guppies tends to be considerably higher than that of their predators, we do not think that presence of other paratenic hosts will have important implications for guppy-*Gyrodactylus* dynamics.

Differences in guppy density or sex ratio between sites could influence the patterns we describe. One of the challenges in this field is to ascribe observed patterns to trait- rather than density-mediated indirect effects (Raffel *et al.*, 2010). Extensive work on this system has shown, however, that guppy populations do not differ consistently in density or sex ratio, neither spatially nor temporally (Pettersson *et al.*, 2004; Magurran, 2005). Coupled with the ‘boom and bust’ infection trajectories on fish in the laboratory (Cable and van Oosterhout, 2007a), these factors may dramatically alter parasite prevalence and intensity rapidly and across small spatial scales. Any density-mediated indirect effects on parasitism are therefore likely to be transient and would not contribute to the large-scale pattern we observe. Differences in predation level, on the other hand, are consistent both spatially and temporally and drive adaptive evolutionary responses in the host, such as shoaling (Houde, 1997; Reznick *et al.*, 1997; Magurran, 2005). Additionally, the rate of *Gyrodactylus* transmission is not density dependent, but is governed by the frequency of social contacts, i.e. shoaling (Johnson *et al.*, 2011). The consistent differences in parasite prevalence between populations that experience different predation pressure are thus more likely to be trait- than density-mediated.

Density-mediated effects might be important, however, on a small spatial scale. We found that variation in the prevalence and abundance of *Gyrodactylus* infection was greatest at the smallest scale, i.e. between the 62 individual sample sites, and this variation exceeded the differences that existed between courses, rivers and drainages (Appendix A: Fig. A.5). The typical guppy researcher's methodology of taking one lower course and one upper course sample per river (van Oosterhout *et al.*, 2006; Martin and Johnsen, 2007; Fraser and Neff, 2010; Gotanda *et al.*, 2013) is therefore likely to miss important sources of variation. This variability also explains previous findings from smaller scale field surveys that contradict the present study, such as the apparent absence of sex differences in *Gyrodactylus* infection in wild guppies (Martin and Johnsen, 2007; Fraser and Neff, 2010 *cf.* Gotanda *et al.*, 2013).

The probability of gyrodactylid infection in guppies increased over the seven years sampled and this increase appears to have happened more quickly in the lower than upper courses of the rivers (Fig. 4.3; Appendix A: Fig. A.2). Other studies of temporal change in this system only sampled across two consecutive years (Fraser *et al.*, 2010; Gotanda *et al.*, 2013), and therefore could not detect this pattern. Clearly, many of the factors important in determining spatial variation also apply to the temporal pattern, and because we resampled so few sites ($n = 14$), our ability to discriminate between temporal and spatial explanations for this pattern is limited. Because the temporal effect was so important in our models, however, we feel obliged to suggest a potential explanation for this pattern. Although air temperatures recorded at Trinidad's Piarco Airport do not show an increase over the study period (<http://www.tutiempo.net/>), river temperature is closely linked to canopy cover. Due to Trinidad's rapid urbanisation (Magurran, 2005), canopy cover in the lowland sites is likely to have decreased, which in turn may have raised water temperatures. Moderate increases in temperature lead to faster parasite population growth rate in laboratory studies (Scott and Nokes, 1984), which could have contributed to the observed temporal pattern in the downstream populations.

Trait-mediated indirect effects are unlikely to act homogeneously across all individuals in a population due to sex-specific differences and allometric variation between life stages. The most parsimonious explanation of our results is that predators induce age- and sex-specific trait-mediated indirect effects on the probability of parasitism by *Gyrodactylus*. Although the importance of trait-mediated indirect effects in community structure and predator-prey interactions is gaining appreciation (Raffel *et al.*, 2010), that these effects can act differently on individuals within the same species has not previously been demonstrated. The sex-specific action of these effects is perhaps particularly relevant to parasite ecology because of the well-established sex difference in parasitism and transmission rates (Zuk and McKean, 1996). The difference we observe in parasite prevalence between males, females and juveniles in populations subjected to

different predation pressures is, we believe, the first example of age- and sex-specific trait-mediated effects of predation on parasite prevalence in a natural system.

4.6 Authorship Acknowledgement

The manuscript resulting from this chapter is authored by:

Jessica F. Stephenson, Cock van Oosterhout (University of East Anglia), Ryan S. Mohammed (University of the West Indies) and Joanne Cable (Cardiff University).

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4.7 Contribution to and position within the thesis

In this chapter I describe evidence that predators, through driving changes in guppy shoaling behaviour, indirectly affect the distribution of parasites in natural populations. Transmission of directly transmitted parasites such as *Gyrodactylus* spp. is therefore likely facilitated by hosts to coming into close proximity, as happens during social behaviours such as shoaling. For guppies, shoaling is a key defence against predators, but this increased risk of disease transmission is a clear cost. The subsequent chapters of this thesis investigate how guppies may use sensory information to mitigate this cost of social behaviour. The next chapter investigates how guppies use chemical and visual cues to behave appropriately, and hence provides an important basis upon which the subsequent chapters build.

Chapter 5

Keeping eyes peeled: guppies exposed to chemical alarm cue are more responsive to ambiguous visual cues

5.1 Abstract

Information received from the visual and chemical senses is qualitatively different. For prey species in aquatic environments, visual cues are spatially and temporally reliable but risky as the prey and predator must often be in close proximity. Chemical cues, by contrast, can be distorted by currents or linger and thus provide less reliable spatial and temporal information, but can be detected from a safe distance. It is likely, therefore, that prey assimilate information from different senses to behave in an appropriate way. Here I tested the hypothesis that the context prey assign to, and therefore their response to, an ambiguous disturbance and subsequent visual cues depends on the chemical cues to which they are exposed. Guppies, *Poecilia reticulata*, used chemical cues to respond to water disturbance in a threat-sensitive manner. Those exposed to concentrated conspecific alarm cue reduced activity, but not responsiveness to visual cues, relative to those exposed to dilute alarm cue, cues of unharmed conspecifics, or a water control. Together, these results indicate that guppies are more responsive to disturbance and visual cues when in receipt of threatening chemical cues. Guppies exposed to chemical cues of unharmed conspecifics were least affected by disturbance, and females recovered faster than males. By using chemical cues to assign context to otherwise ambiguous cues, guppies show adaptive interpretation of the cues received by multiple sensory modalities.

5.2 Introduction

Information received from the visual and chemical senses is qualitatively different. For prey species in aquatic environments, visual cues of predation are spatially and temporally reliable but risky as the prey and predator often have to be in close proximity due to short visualisation distances (Lythgoe, 1979). Chemical cues, by contrast, can be distorted by currents or linger and thus provide less reliable spatial and temporal information, but can be detected from a safe distance or while in hiding (Brown and Magnavacca, 2003). For this reason, chemical cues can be considered long-distance cues in moving water (Dusenberry, 1992), and may often be the first cue an animal receives (McLennan, 2003). Previous studies indicate that the first cue received alerts the recipient to the potential presence of a second cue, enhancing the detectability and discriminability of the second cue and therefore reducing the chance of overlooking vital information (Rowe, 1999; Rowe and Guildford, 1999). This effect may be particularly strong when the cues are detected by different sensory modalities ('multimodal'; Rowe, 1999). In humans, for example, sounds change how moving objects are perceived (Sekuler *et al.*, 1997). Because prey response to threatening cues is vital for survival, but responding to non-threatening cues as threatening is a waste of resources (Helfman, 1989; Lima and Dill, 1990), prey ability to discriminate between these cue types is likely to be under strong selection. Here I test whether chemical cues determine the context (i.e. threatening or non-threatening) that prey fish assign to water disturbance and visual cues.

Chemical cues released by predators or threatened conspecifics alert individuals to predation risk (Chivers and Smith, 1998; Brown, 2003). One such chemical cue, 'alarm cue', is released from fish skin damaged during predation events and, if detected, provides reliable information about predation risk in the immediate environment regardless of predator identity (Brown, 2003). Behavioural responses to alarm cue were first demonstrated by von Frisch (1938, 1941) in European minnows, *Phoxinus phoxinus*, and have since been documented in a wide range of species: ostariophysans, salmonids, gobies, poecilids, gasterosteids, percids, cottids, cichlids and centrarchids (Chivers and Smith, 1998). In many fishes the innate response to alarm cue is sufficiently strong that a single associative conditioning event can enhance existing antipredator behaviour (Brown and Smith, 1998; Berejikian *et al.*, 1999; Mirza and Chivers, 2000; Berejikian *et al.*, 2003; Vilhunen, 2006), and condition a response to the odours of novel predators (Ferrari *et al.*, 2005), non-predatory fish (Larson and McCormick, 2005), sound (Wisenden *et al.*, 2008), areas of habitat in the wild (Kim *et al.*, 2011) and non-biological visual cues (Hall and Suboski, 1995; Yunker *et al.*, 1999). Additionally, the concentration of chemical alarm cue can indicate the strength of predation risk on a temporal or spatial scale;

several species use cue concentration to change their behaviour in a ‘threat-sensitive’ way, i.e. in proportion to the perceived threat (Helfman, 1989; e.g. ambon damselfish, *Pomacentrus amboinensis*, see Lönnstedt and McCormick, 2011; Trinidadian guppies, *Poecilia reticulata*, see Brown *et al.*, 2009; Atlantic salmon, *Salmo salar*, see Hawkins *et al.*, 2007). At concentrations below the ‘minimum behavioural response threshold’ (Mirza and Chivers, 2003), overt antipredator behaviours are not elicited, and prey instead exhibit covert responses, such as changes in foraging posture (Foam *et al.*, 2005), or the acquisition of novel predator cues (Ferrari *et al.*, 2005).

Observations from ecologically relevant situations indicate that visual and chemical cues act synergistically to determine fish response to predation threat. The response of blacknose shiners, *Notropis heterolepis*, and glowlight tetras, *Hemigrammus erythrozonus* to visual cues of a predator is greater when fish are pre-exposed to alarm cue than the response to either the visual or chemical cue in isolation (Wisenden *et al.*, 2004). Pre-exposure to concentrations of alarm cue too low to elicit overt behavioural responses still enhance the response of glowlight tetras to threatening visual cues (Brown *et al.*, 2004). Conversely, alarm cue might only elicit responses from fish that are already wary (Magurran *et al.*, 1996), potentially due to visual cues, or lack thereof in turbidity or darkness; fish in these conditions typically show stronger responses to threatening chemical cues (Hartman and Abrahams, 2000; Leduc *et al.*, 2010; Leahy *et al.*, 2011). Although there is now a modest body of work indicating that threatening cues from different sensory modalities interact in determining fish behaviour, no study has yet investigated how threatening cues detected by one modality affect an individual’s response to ambiguous cues in a different modality.

Here I used the guppy to test the novel hypothesis that chemical cues allow fish to assign a context (i.e. threatening or non-threatening) to an ambiguous disturbance in their vicinity, and use this context to respond to it and subsequent visual cues in an adaptive manner. Wild populations of guppies experiencing high levels of predation have evolved graded responses to alarm cue, i.e. a response proportional to the concentration of cue presented (Brown *et al.*, 2009). The provenance of the cue is also important: guppies respond most strongly to alarm cue from fish from their own population (Brown *et al.*, 2010). Guppies are additionally able to use chemical cues to assess the sex (Shohet and Watt, 2004), reproductive status (Brask *et al.*, 2012), and health (Chapter 6) of unharmed conspecifics. In this experiment, I exposed guppies to either one of two concentrations of conspecific chemical alarm cue (100% or 10%), the chemical cues of unharmed conspecifics, or a water control. I tested whether they were able to use these chemical cues to assign context to an ambiguous water disturbance and visual cues. The results indicate that guppies show adaptive interpretation of multimodal cues.

5.3 Materials and Methods

5.3.1 Fish origin and maintenance

Fish used in this study were wild caught in the Caura River, Trinidad, from a population experiencing high levels of predation, in June 2012 (UTM 20 P; E: 67952.77, N: 118037.64, elevation 112 m). They were shipped to Cardiff University (Cefas APB authorisation number CW054-D-187A), treated for infection using Binox® (Nitrofurazone; Jungle Laboratories Corporation®, Cibolo, Texas), and held for 3 weeks before testing. Fish were housed in 70 L aquaria of dechlorinated water at $24\pm 1^\circ\text{C}$, on a 12h Light:12h Dark lighting schedule (overhead fluorescent lighting), and fed daily on Aquarian® flakes supplemented with *Artemia* and bloodworm. Each tank had pea gravel substrate, an under gravel filter and standardised enrichment.

5.3.2 Cue production

All cues were produced in two batches. In each batch of alarm cue and the cue of unharmed conspecifics ('fish cue'), mature laboratory-bred females from the same population as the test fish were selected as donors. To make fish cue, seven donors *per* batch were held together for 20 hours in 2 L blank dechlorinated water. They were not fed during this isolation, and were subsequently returned to breeding tanks and were not used as either alarm cue donors or test fish. The holding water was divided into 10 ml aliquots and frozen until required. Alarm cue production followed the protocol of Brown *et al.* (2009). Seven donors were cold anaesthetised and immediately decapitated. The tail and viscera were also removed, leaving skeletal muscle and skin. All carcasses were added to 50 ml of chilled, dechlorinated water, homogenised and the solution filtered through glass wool. The concentration was adjusted to 0.1 cm^2 of skin ml^{-1} , following Brown *et al.* (2009). This 100% alarm cue solution was either divided into 10 ml aliquots and frozen at -20°C until required, or diluted with dechlorinated water to make a 10% alarm cue solution which was then similarly divided and frozen. The same volume of 'control' dechlorinated water was held overnight, divided and frozen until use.

5.3.3 Optomotor apparatus

The optomotor apparatus was adapted from Stephenson *et al.* (2011, 2012) and consisted of a cylindrical glass tank (diameter 18 cm, depth 10 cm) suspended from a steel frame (Fig. 5.1). The tank was surrounded by a drum (diameter 28 cm, depth 14 cm), which could be rotated in either direction by a motor at a constant speed of 10 rpm. The drum

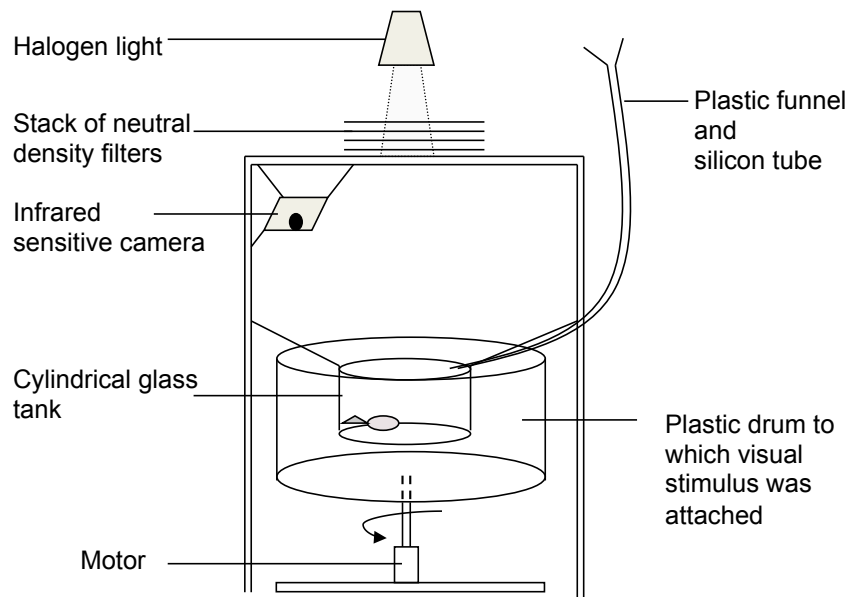


FIGURE 5.1: The apparatus used to elicit the optomotor response of guppies, and to use this response to test how visual behaviour was affected by chemical cues.

supported a visual cue consisting of alternating black and white stripes, each covering 20° of the arc of the drum circumference. Fish swim in the same direction and at the same speed as these stripes when they are able to see them. This optomotor response enables fish to maintain their position relative to stationary objects in moving water and is commonly used to test fish visual sensitivity, including that of the guppy (Anstis *et al.*, 1998). The fish were viewed and behaviour recorded using an infrared-sensitive video camera (Henelec 300c CCTV IR) supported from the top of the frame. The sides of the frame were covered in blackout fabric, and the top was covered with an MDF board. A 1 cm diameter circular hole was drilled into the board ('light hole'), and a halogen fibre optic light source (Schott KL 1500 LCD) was positioned above it to provide 1.5 lux of light at the surface of the water (approximately 2×10^{17} photons/s/m² using the calculations described by Stephenson *et al.*, 2011, 2012). In order to attenuate the light further, 7×7 cm squares of neutral-density (ND) filters (LEE filters; one layer of 299 and six of 209; nominal absorbances 1.2 and 0.3 respectively) were laid over the light hole. This light level was chosen during preliminary work as one at which the fish could see and respond to visual cues, but their response was limited compared to that at ambient light levels. During trials, chemical cues were introduced to the experimental tank in water using separate funnels and Nalgene® tubing. The tube was fed through a covered hole in the screen surrounding the frame, and the end hung 2 cm above the surface of the water in the experimental tank. The experimental room was held at $24 \pm 0.5^\circ\text{C}$.

5.3.4 Experimental protocol

Fish to be tested were held individually overnight in opaque white 1 L tanks and were not fed during their isolation. These tanks were wiped with 70% ethanol and rinsed thoroughly with dechlorinated water between uses. The experimental tank was filled with dechlorinated water to a depth of 4 cm, and a naïve test fish was added. An opaque board was placed over the light hole for 25 min to allow the fish to acclimatise and dark-adapt. Each trial began with the drum being rotated at 10 rpm for 30 s in each direction for two minutes. During the third minute, the chemical cue (100% alarm cue, 10% alarm cue, fish cue or control) was injected into the tank. Because the input tube hung 2 cm above the surface of the water, chemical cue input caused a disturbance at the surface of the water and therefore visual and mechanosensory as well as chemical cues. The rotation of the drum (30 s in each direction) was repeated during the four minutes immediately following chemical cue input. The visual cues were therefore rotated following this pattern during minutes 1 and 2 of each trial, and in minutes 4 to 7 (i.e. the four minutes following chemical cue input), but not during minute 3 (when chemical cue input took place). At the end of each trial the chemical cue input tube was rinsed with dechlorinated water. The fish was removed, weighed and measured and returned to a breeding tank. Both male and female guppies were tested using each of the four chemical cues. Ten blocks of these eight treatments were completed over the course of 14 days. Treatment order was randomised within block, and changed between block following a Latin square randomised design.

5.3.5 Ethical note

This work was conducted under the UK Home Office license (PPL 30/2876) with approval by the Cardiff University Animal Ethics Committee. As described above, during the course of this experiment, fish were subjected to social isolation, abnormal lighting conditions, and startling stimuli. Although these factors are likely to have temporarily elevated their stress levels, no fish showed any signs of having suffered lasting harm and resumed normal behaviour less than an hour after being returned to a breeding tank. Throughout the maintenance and use of these fish, I adopted the principle that ‘the best animal welfare is a prerequisite for the best science’, following the “Guidelines for the treatment of animals in behavioural research and teaching” recommended by The Association for the Study of Animal Behaviour (2012).

Model & response variable	Error family	Link function	Main effects	Two-way interactions	Random effect
1: Activity	Gaussian	Identity	Block (c) Length (n) Sex (c) Time (o)* Treatment (c)*	Time \times Length Time \times Treatment* Time \times Sex Sex \times Length	Fish identity
2: Proportion of time spent following the stripes			Activity (n)* Block (c) Length (n) Sex (c)* Time (o)* Treatment (c)*	Activity \times Time* Activity \times Treatment* Activity \times Sex Time \times Length Time \times Treatment Time \times Sex* Sex \times Length	

TABLE 5.1: Starting models used to test the hypothesis that chemical cues affect the way guppies respond to ambiguous disturbance and visual cues. These starting models were simplified using backwards stepwise deletion of non-significant fixed effects to minimise the Akaike’s Information Criteria (AIC); those that remained in the final model are denoted with an asterisk. ‘Block’ refers to the experimental block in which a particular trial was conducted, ‘Treatment’ refers to the chemical cue to which the fish was exposed (100% or 10% alarm cue, fish cue or control water), and ‘Time’ refers to the experimental time elapsed since the introduction of the chemical cue. Fixed effects were included as categorical (c), numeric (n), or ordinal (o) variables.

5.3.6 Data analysis

The proportion of each 30 s period that the fish spent following the stripes was calculated from the trial videos using JWatcher™ 1.0 (www.jwatcher.ucla.edu) by an observer unaware of the treatment. The observer additionally scored the number of times the fish swam through a quarter of the tank as a measure of activity. For the four minutes immediately after the input of the chemical cues, both fish activity (Model 1 in Table 5.1) and the proportion of each minute the fish spent following the stripes (Model 2 in Table 5.1) were used as the response variables in two linear mixed models in the *lme4* package in R 3.0.2 (LMM; Gaussian error family with identity link function; R Core Team, 2013; Bates *et al.*, 2014). In each model, fish identity was included as the random term to account for repeated measures through time. The sex of the fish, the chemical cue to which it was exposed (‘Treatment’), activity (Model 2 only), standard length, time since the chemical cue had been input and the experimental block in which the trial was conducted were all included as fixed effects, as well as two way interactions about which biologically relevant *a priori* hypotheses had been made (Table 5.1). Non-significant fixed effects were sequentially deleted from the starting models to minimise the Akaike’s Information Criteria (AIC), and only significant effects are reported. These analyses

were conducted on the raw data, but the data were converted to cumulative values for Figure 5.2 for clarity.

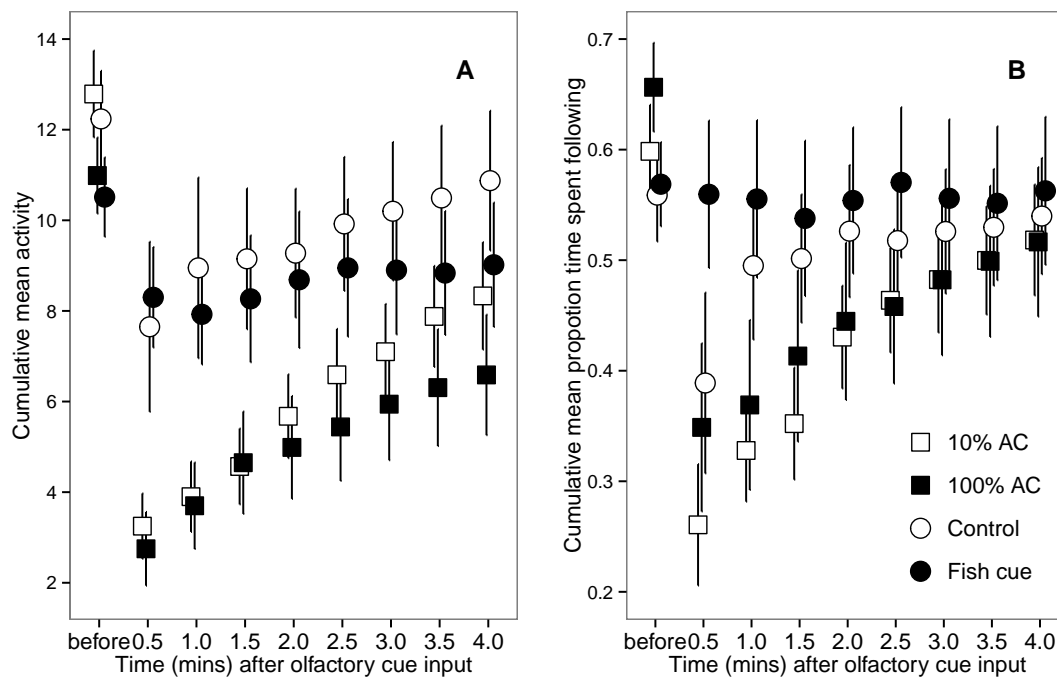


FIGURE 5.2: Guppy activity level depended on the nature of the chemical cue, and the time since chemical cue input (A), whereas the proportion of time fish spent following the visual cues depended on time since chemical cue input alone (B; alarm cue, ‘AC’, of different concentrations; dechlorinated water, ‘control’; or the cues of unharmed conspecifics, ‘fish cue’). The analyses described in the main text were conducted on the raw data, but these were converted to cumulative values for these plots for clarity. Error bars are the standard errors of the means.

5.4 Results

The chemical cue a fish was exposed to affected how its activity level changed through the four minutes following the disturbance caused by the chemical cue input (Fig. 5.2 A; Model 1 in Table 5.1 - treatment \times time interaction: $F_{21, 532} = 1.92$, $p = 0.009$). Guppies exposed to the chemical cues of unharmed conspecifics and control water showed significantly less of a decrease in activity level and recovered more quickly than the two alarm cue treatment groups (Fig. 5.2 A).

Exposure to 100% alarm cue thus reduced fish activity levels, but it increased the extent to which this activity was focused on responding to the visual cues: there was no significant difference in the proportion of time fish exposed to different chemical cues spent following the visual cues over the whole four minutes following chemical cue input

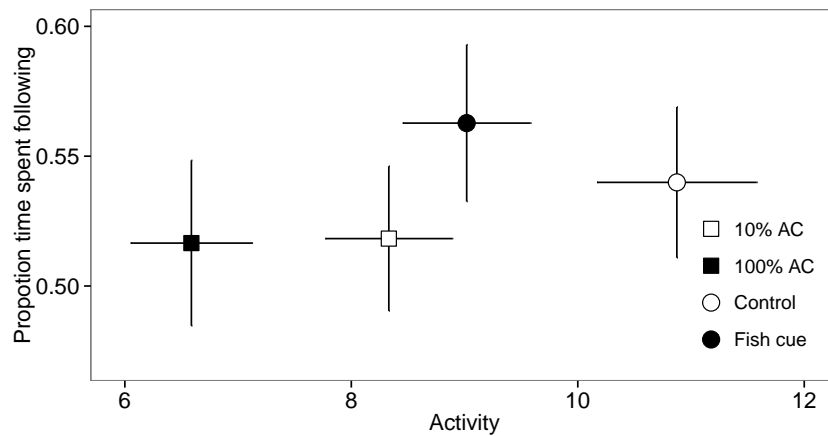


FIGURE 5.3: Guppies exposed to concentrated alarm cue (100% AC) showed a significant, threat-sensitive reduction in activity level, but no significant decrease in the proportion of time they spent following the visual cues, relative to those exposed to dilute alarm cue (10% AC), the cues of unharmed conspecifics (fish cue), or dechlorinated water control. Data points show the raw data means across the four minutes following chemical cue input, and the error bars are the 95% confidence intervals.

(Figs. 5.2 B & 5.3). This result supports the prediction that fish exposed to alarm cue are more responsive to visual cues than those exposed to either the cues of unharmed conspecifics or dechlorinated water (Fig. 5.4; Model 2 in Table 5.1 - activity \times treatment interaction: $F_{3, 505.9} = 3.98, p = 0.008$). Additionally, the proportion of time fish spent following the visual cues increased through time after the input of the chemical cue, but not among fish that remained highly active throughout (Fig. 5.2; Model 2 in Table 5.1 - activity \times time interaction: $F_{7, 538.03} = 5.30, p < 0.0001$). There was a significant sex difference in how guppies resumed following behaviour after the chemical cue was input; females showed less of a decrease in following behaviour and regained pre-disturbance levels faster than males (Fig. 5.5; Model 2 in Table 5.1 - sex \times time interaction: $F_{7, 530.1} = 2.62, p = 0.011$).

5.5 Discussion

Guppies used chemical cues to respond to a disturbance in a threat-sensitive manner. Those exposed to either concentration of conspecific alarm cue (10% or 100%) reduced their activity level significantly more than those exposed to the chemical cues of unharmed conspecifics or dechlorinated water (Fig. 5.2 A). Despite this difference in activity level after the input of the chemical cue, there was no overall difference between the groups exposed to the different chemical cues in their response to visual cues (Fig. 5.3). These results indicate that for a given activity level, guppies exposed to chemical

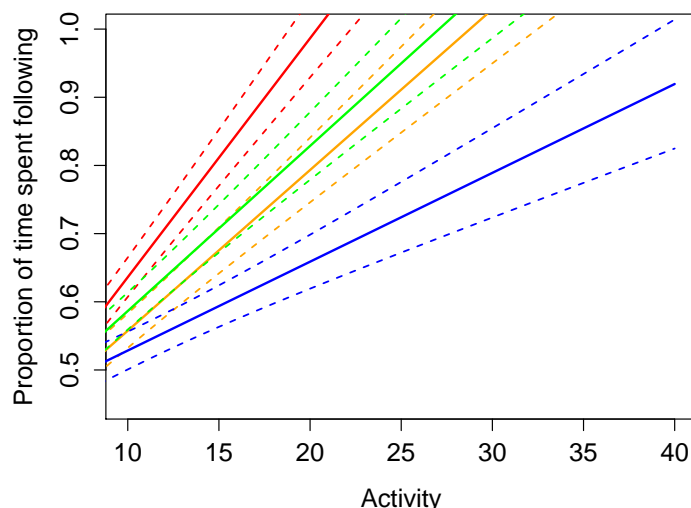


FIGURE 5.4: For a given activity level, guppies exposed to 100% alarm cue (red) spent significantly more time following the visual cues than those exposed to 10% alarm cue (orange), unharmed conspecifics (green), and dechlorinated water (blue). There was no difference between the proportion of time guppies exposed to cues of unharmed conspecifics and 10% alarm cue spent following the visual cues, but both groups spent more time following than those exposed to dechlorinated water. Solid lines are values predicted by the model (Model 2 in Table 5.1) described in the text over the range of fish activity observed in the raw data. Dashed lines are 95% confidence intervals.

alarm cue are more responsive to visual cues than those exposed to control chemical cues (Fig. 5.4). Guppies exposed to the chemical cues of unharmed conspecifics were minimally affected by the disturbance (Fig. 5.2), and females recovered faster than males (Fig. 5.5). By using chemical cues to assign context to otherwise ambiguous cues, therefore, guppies show adaptive interpretation of the cues received by multiple sensory modalities.

Attending to multiple cues across sensory systems is important in habitats where cues may be temporally or spatially unavailable. In situations such as fast flowing water, or areas of very turbulent flow, fish may not detect chemical cues; several species can transmit the same information visually. For example, naked characin *Gymnocharacinus bergii* inhabit fast flowing headwaters and responds equally strongly to alarm cue or to the sight of other individuals responding to alarm cue (Cordi *et al.*, 2005). Glowlight tetras, *Hemigrammus erythrozomus*, increase the frequency of ‘fin flicking’ when exposed to alarm cue, and observing this alone is enough to elicit the same behaviour in fish not exposed to alarm cue (Brown *et al.*, 1999). This propagation of alarm reactions can spread rapidly through shoals, purely on the basis of visual information (Magurran and Higham, 1988; Mathis *et al.*, 1996). That both chemical and visual cues can be used

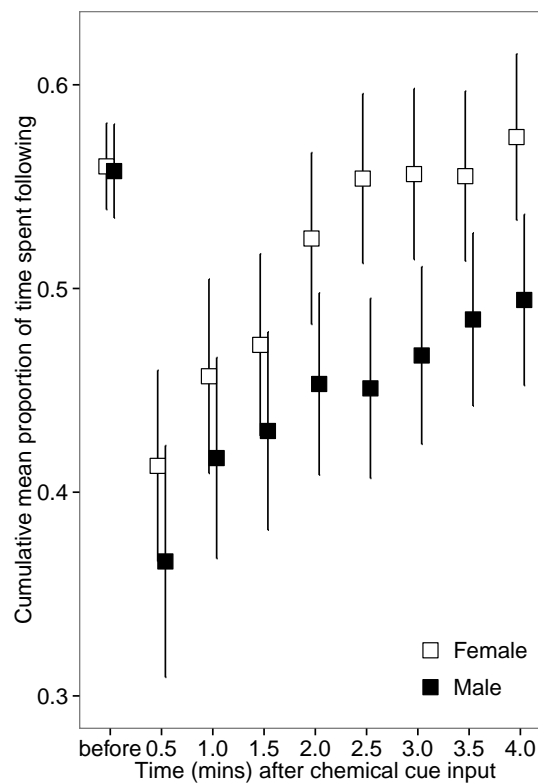


FIGURE 5.5: The mean proportion of time females spent following the visual cues after the input of the chemical cue was higher than that of males. Before chemical cue input, however, there was no difference between the sexes. The chemical cue input caused a disturbance in the vicinity of the fish. Error bars are the standard error of the mean.

to assess predation risk in this way may be key to fish survival as water bodies suffer more anthropogenic disturbance such as increased turbidity and pollution (Leahy *et al.*, 2011).

Guppies exposure to the chemical cues of unharmed conspecifics, in contrast to those exposed to chemical alarm cue, were not affected by the disturbance; both their activity level and response to the visual cues barely changed after the disturbance of the chemical cue input. The guppy is a social animal; assessing risk through attending to the cues emitted by individuals in close proximity is a common feature of sociality across taxa (reviewed by Griffin, 2004), including fish. The chemical cues of conspecifics can affect the extent to which fish respond to threatening cues. For example, rainbow trout, *Oncorhynchus mykiss*, in receipt of the chemical cues from undisturbed conspecifics show a reduced response to alarm cue compared to those exposed to cues from disturbed conspecifics (Ferrari *et al.*, 2008). Further, fathead minnows, *Pimephales promelas*, trust the response of conspecifics to ambiguous cues more than their own learned response (Crane and Ferrari, 2015). The result of the present study potentially reflects that this process can act across sensory systems: guppies in receipt of the chemical cues of

unharmful, undisturbed conspecifics use this information to infer the non-threatening nature of the water disturbance and subsequent visual cues and hence show no change in behaviour.

After the disturbance of the chemical cue input, male guppies took longer than females to return to the level of responsiveness to the visual cues they showed before the disturbance. This result seemingly contradicts previous findings; male guppies are bolder than female counterparts (Magurran and Seghers, 1994b; Harris *et al.*, 2010). Further, response to threatening cues is typically greater among females (Mirza *et al.*, 2001) and larger individuals (Pollock *et al.*, 2006), and as female guppies are larger than male guppies in natural populations (Chapter 2), these studies suggest that females should have taken longer to recover from the disturbance. The apparent disparity between my result and those of previous studies is likely to be due to the experimental context. Here, to ‘recover from the disturbance’ is to resume the pre-disturbance level of optomotor response, a behaviour that may complement female threat-responsiveness more than that of males. The cryptic colouration of female guppies means that remaining still relative to the background, or remaining with a shoal (both of which the optomotor response facilitates; Anstis *et al.*, 1998) is an efficient anti-predator strategy (Magurran, 1990; Magurran and Seghers, 1994b; Magurran *et al.*, 1994; Croft *et al.*, 2006). Male guppies, on the other hand, are brightly coloured; staying in the same area when threatened may therefore be maladaptive (Magurran and Seghers, 1994b; Magurran, 2005), particularly given that males do not show shoal fidelity to the same extent as females (Croft *et al.*, 2003a,b, 2012). In support of this explanation, there was no difference in the speed at which males and females resumed pre-disturbance activity levels; males were as active as females, but their activity was less focused on using the visual cues to hold their position.

Evidence from both electrophysiological and ethological studies indicate that the visual system of fish is affected by chemical cues (Maaswinkel and Li, 2003; Stephenson *et al.*, 2011), including alarm cue (Stephenson *et al.*, 2012). Whereas these studies demonstrated that visual sensitivity increases with chemical stimulation using the zebrafish, and invoked the terminal nerve as the physiological pathway, the present study found no evidence of such an effect in guppies. Currently all studies of the role of the terminal nerve in this interaction between sensory systems have been conducted on the cyprinids zebrafish *Danio rerio* (Maaswinkel and Li, 2003; Stephenson *et al.*, 2011, 2012) and goldfish *Carassius auratus* (Stell *et al.*, 1984; Fujita *et al.*, 1991); the findings of the present study could indicate that the results from these previous studies are not applicable to other families of fish.

This study builds on previous work by indicating that prey fish use the first cue they receive, in this case chemical, to assign a context to subsequent ambiguous cues received by other modalities in order to respond to them in an appropriate, threat-sensitive manner. It therefore provides further evidence for the importance of multimodal cues in driving adaptive animal behaviour (Rowe, 1999; McLennan, 2003).

5.6 Authorship Acknowledgement

The manuscript resulting from this chapter is authored by:

Jessica F. Stephenson.

5.7 Contribution to and position within the thesis

In this chapter I investigate the role of chemical cues in affecting fish response to cues in other sensory modalities, such as vision. The results indicate that chemical cues enable guppies to assign context to ambiguous cues detected by other sensory modalities, and therefore respond to them appropriately. In the next chapter I build on this work by testing how guppies use chemical and visual cues to detect infection in conspecifics, and use that information to employ avoidance behaviour.

Chapter 6

Visual and chemical cues inform guppies of the transmission risk posed by conspecifics

6.1 Abstract

Social animals take many attributes into account when deciding with whom to associate. Associating with diseased individuals likely increases transmission risk, but the risk may depend on the duration and severity of an individual's infection. When transmission risk is low, it may be beneficial to remain in a group; animals engaging in avoidance behaviour incur the cost of lost social benefits. This cost-benefit analysis predicts that animals should only avoid infected individuals when they pose a high transmission risk. We used the guppy *Poecilia reticulata*-*Gyrodactylus turnbulli* host-parasite system to test whether host avoidance behaviour is 'threat-sensitive', that is proportional to the level of transmission risk posed by infected conspecifics. Considering the variable nature of the sensory environment faced by these riverine fish, the need for cue reliability, and the selection pressure imposed by these parasites, we predicted that the cues guppies use to assess transmission risk elicit the same response in the receiver, termed 'redundant'. In dichotomous choice tests uninfected guppies avoided both the chemical and visual cues of conspecifics in the late, but not early stages of infection. A transmission experiment suggests that this is when *G. turnbulli* is most likely to transmit between guppies. Our results therefore confirm that guppies use redundant cues to avoid only those conspecifics that pose the greatest transmission risk. We discuss potential implications for the disease ecology of natural populations.

6.2 Introduction

Social animals take many attributes into account when deciding with whom to associate, including the health of conspecifics. Associating with individuals infected with directly transmitted diseases increases transmission risk (Poulin, 1999) and there is much evidence that animals across taxa avoid doing so (Goodall, 1986; Kiesecker *et al.*, 1999; Kavaliers *et al.*, 2003; Behringer *et al.*, 2006; Croft *et al.*, 2011; Schaller, 2011). For many animals, therefore, ‘social barriers’ to disease transmission may be as important as immunological or physical ones (Loehle, 1995; Daly and Johnson, 2011; Schaller, 2011; Zylberberg *et al.*, 2012; Johnson and Hoverman, 2014). This avoidance behaviour at the individual level can influence disease dynamics at the population level (Gudelj and White, 2004), but the mechanisms involved have been little investigated. Elucidating the factors affecting avoidance behaviour will facilitate more accurate predictions of how diseases spread through social networks and hence populations (Wilson *et al.*, 2014).

Animals engaging in avoidance behaviour incur costs associated with the loss of social benefits (e.g. foraging efficiency, mating opportunities, antipredator defence: Chivers *et al.*, 1995; Krause and Ruxton, 2002; Seppälä *et al.*, 2008a; Croft *et al.*, 2011; Schaller, 2011), and we can therefore predict that their avoidance of infectious conspecifics is threat-sensitive, or proportional to the level of risk, analogous to threat-sensitive antipredator behaviour (Lima and Dill, 1990). There is some empirical evidence that the cost of associating with infected individuals can be outweighed by other factors: stickleback, *Gasterosteus aculeatus* prefer infected shoals when they are larger than uninfected ones by a factor of three (Barber *et al.*, 1998). Additionally, this cost may become minimal if the transmission risk posed by, or ‘infectiousness’ of individuals is low. The infectiousness of potential social partners depends on the characteristics of their infection and is highly heterogeneous in natural populations (Chapter 8; Woolhouse *et al.*, 1997; Lloyd-Smith *et al.*, 2005; Paull *et al.*, 2012). For example, the number of parasites an individual is infected with, its ‘infection load’, is clearly linked to its infectiousness (Chapter 8). As well as variation between hosts, a single individual’s infectiousness is likely to change through time because infection load changes through the course of infection for many parasites (Fig. 6.1; Schmid-Hempel *et al.*, 1999; Poulin, 2007; Schmid-Hempel, 2011). It is therefore likely, though untested, that social animals display threat-sensitive avoidance of conspecifics based on their infectiousness, a task that requires constant assessment of a reliable cue.

Empirical studies of infection avoidance behaviour suggest that it is more commonly based on chemical, rather than visual cues (Kiesecker *et al.*, 1999; Behringer *et al.*, 2006; Arakawa *et al.*, 2012). Although many species avoid individuals with visually obvious symptoms of disease, these are not necessarily cues of infectiousness. For example, fishes

avoid conspecifics infected with indirectly transmitted parasites that change their visual appearance (Barber and Huntingford, 1995; Krause *et al.*, 1996; Barber *et al.*, 1998; Tobler and Schlupp, 2007). However, these fish are putatively avoiding the oddity effect in the absence of infectious disease (Landeau and Terborgh, 1986; Barber *et al.*, 1998). Visible attributes that can be affected by parasites may often serve as indirect cues of infection, such as colour (Hamilton and Zuk, 1982; Milinski and Bakker, 1990; Houde and Torio, 1992) or behaviour (Kennedy *et al.*, 1987). However, infected individuals may be able to tolerate parasites with no change in these cues (Folstad and Karter, 1992; Medzhitov *et al.*, 2012), actively disguise them (Aubert *et al.*, 1997; Lopes *et al.*, 2012), or there may be alternative explanations for these changes (e.g. dietary history and colouration; Grether, 2000). Conversely, changes in these attributes may make infected individuals more attractive social partners (e.g. Bouwman and Hawley, 2010). For many systems, therefore, chemical cues may provide more reliable, quantitative information about an individual's infection, including how infectious it is. For example, chemical cues change through the course of infection in *Plasmodium chaubadi* malaria-infected mice, *Mus musculus*, making the mouse a more attractive blood meal for the mosquito vector when the parasite reaches the infectious stage of its lifecycle (De Moraes *et al.*, 2014). Analogous temporal changes in the infectiousness of hosts infected with directly transmitted parasites may equally be linked to changes in the cues they emit, which could provide conspecifics with the opportunity to exhibit threat-sensitive avoidance of the most infectious individuals.

We report here on the first experimental investigation of threat-sensitive infection avoidance. We used the guppy *Poecilia reticulata*-*Gyrodactylus turnbulli* host-parasite system to test the hypothesis that individuals only show avoidance of the most infectious conspecifics. *G. turnbulli* is an ectoparasitic monogenean that reproduces on the host's skin and is transmitted directly through close contact between socially interacting hosts (Chapters 8 & 4; Richards *et al.*, 2010; Johnson *et al.*, 2011). *Gyrodactylus* spp. parasites negatively impact guppy fitness and are the most prevalent multicellular parasites in wild guppy populations (Chapters 4, 3 & 2). The ability to recognise and avoid infected individuals is therefore likely to be under strong selection and there is some evidence that it occurs: the presence of infected conspecifics reduces shoal cohesion in semi-natural conditions (Croft *et al.*, 2011). However, this reduction in cohesion will in turn reduce the efficacy of shoaling as an antipredator defence (Chivers *et al.*, 1995). We therefore predict that guppies balance their avoidance behaviour with an assessment of the infectiousness of individual conspecifics. We have demonstrated that infection load is an important determinant of infectiousness in this system, reaching a 'peak of infectiousness' when the number of *G. turnbulli* infecting a host surpasses *ca.* 40. Below this threshold, transmission can take up to four days, but above it transmission rarely takes longer

than one day (Chapter 8; Fig. 8.3). Because *G. turnbulli* has a generation time of 24-48 hours at 25°C (Cable, 2011), guppy infection load, and therefore infectiousness, initially increases rapidly over the course of infection in this system (Fig. 6.1). We therefore predicted that the longer a guppy has been infected, the more strongly it is avoided by conspecifics.

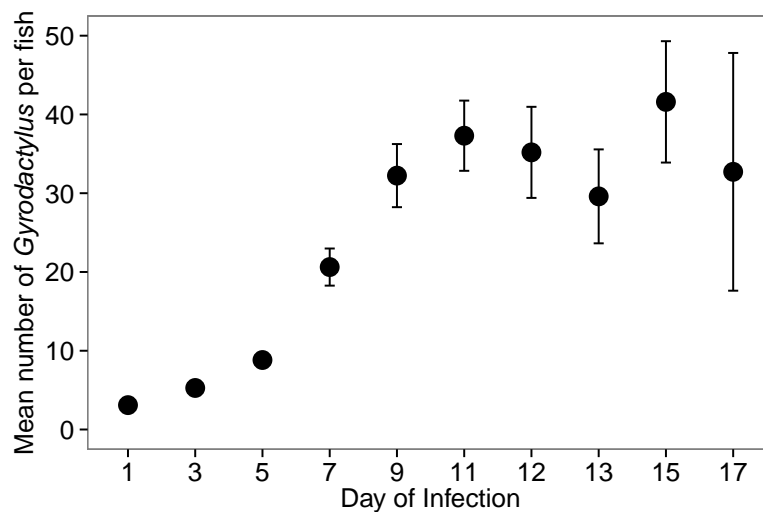


FIGURE 6.1: The number of *Gyrodactylus turnbulli* infecting individually housed guppies increased through time. Day 11 was the first point at which mean infection load was not significantly different from 40 parasites: the ‘peak of infectiousness’ of guppies (see Chapter 8). Error bars are the standard error of the mean.

As a conspecific’s infectiousness can change rapidly through time, discriminating between those that pose a high and low risk of transmission requires reliable information. Guppies are able to monitor other temporally variable physiological characteristics in conspecifics using chemical cues (reproductive status; Brask *et al.*, 2012), and they also have excellent vision (Anstis *et al.*, 1998). Given the costs of infection with *Gyrodactylus* spp. parasites (Chapters 3 & 2; Houde and Torio, 1992; López, 1999; van Oosterhout *et al.*, 2007), we suggest that this need for reliability has led to ‘redundancy’ in chemical and visual cues of infectiousness. Two cues can be considered redundant if they elicit the same response in receivers when presented in isolation; when presented simultaneously, redundant cues can either have an ‘enhanced’ or an ‘equivalent’ effect on receiver behaviour relative to the effect of either cue in isolation (Johnstone, 1996; Partan and Marler, 1999, 2005). We here test whether isolated visual and chemical cues of *G. turnbulli* infection in conspecifics elicit the same response from guppies. If supported, this cue redundancy would differentiate this system from others in which the cues of infection have been elucidated (e.g. Kiesecker *et al.*, 1999) but we consider cue redundancy likely, due to the sensory ecology of guppy habitat. Redundancy in these senses would compensate for the unidirectional flow and changeable nature of rivers: chemical cues are likely

only useful to receivers downstream of their origin, but they may be the only source of information in turbid conditions. Predation, another important selection pressure, has led to the evolution of an analogous cue redundancy in the riverine fishes naked characin, *Gymnocharacinus bergi* (see Cordi *et al.*, 2005), European minnows, *Phoxinus phoxinus* (see Magurran and Higham, 1988) and glowlight tetras, *Hemigrammus erythrozonus* (see Brown *et al.*, 1999): these fishes display antipredator behaviour in response to exposure to chemical cues of predation risk, or to the sight of other individuals responding to those chemical cues. We here present evidence that guppies use similarly redundant chemical and visual cues to avoid *G. turnbulli*-infected conspecifics when these pose the highest risk of parasite transmission.

6.3 Materials and Methods

6.3.1 Fish origin and maintenance

The guppies used in this study were wild caught fish and their laboratory bred descendants from the Caura River, Trinidad (Table 6.1; UTM 20 P; E: 67952.77, N: 118037.64, elevation 112m). In July 2012, approximately 600 fish were captured using a seine net and small bucket to avoid dislodging ectoparasites. The fish were shipped to Cardiff University (Cefas APB authorisation number CW054-D-187A). In Cardiff, 40 live fish and an additional 40 that were preserved in ethanol immediately following capture were screened for parasites; these data confirmed our finding from 2003 that this site is *Gyrodactylus*-free (Chapter 4). All fish were then prophylactically treated for infection using Binox® (Nitrofurazone; Jungle Laboratories Corporation®, Cibolo, Texas). Fish were housed at low densities in 70 L aquaria of dechlorinated water at $24\pm 1^\circ\text{C}$, on a 12h Light:12h Dark lighting schedule (overhead fluorescent lighting), and fed daily on Aquarian® flakes, supplemented with *Artemia* and bloodworm. Each tank had pea gravel substrate, an under gravel filter and standardised enrichment. Tanks were checked weekly for fry, and these were moved to separate rearing tanks. Males and females were separated once sex determination was possible (*ca.* 6-8 weeks), ensuring that all fish were virgins prior to the start of the experiment.

6.3.2 Experimental infections

We used F1 laboratory-bred virgin females to produce the chemical and visual cues of infection. Pairs (uninfected *vs.* infected) were size-matched ± 1 mm. The ‘infected’ stimulus fish were infected on Day 0 with the *Gt3* strain of *G. turnbulli*. Donor fish were killed with an overdose of anaesthetic (tricaine methanesulfonate; MS222;

PHARMAQ Ltd.) and brought close to the anaesthetised (0.02% MS222) recipient fish until 2-6 parasites had transferred, as observed under a dissecting microscope and fibre optic illumination. Recipients were then held individually in 1 L tanks under standard conditions, and the number of parasites infecting each was counted under anaesthetic every other day (Fig. 6.1). Control stimulus fish were anaesthetised before being held individually.

6.3.3 Cue production

At several points during infection, up to Day 19, these pairs were used as stimuli for the behavioural trials (Table 6.1). Because familiarity is an important factor in guppy association preferences (Griffiths and Magurran, 1997), we ensured that all test and stimulus fish were unfamiliar to one another. Chemical cues were produced according to two slightly different methodologies. In each method, F1 generation sexually mature virgin female offspring from the wild caught Caura fish were used as stimulus fish. During the production of each batch, five fish were held individually in 500 ml of dechlorinated water in food grade plastic containers for 24 hours. Fish were not fed during this isolation. These samples were then mixed together and frozen in 150 ml aliquots at -20°C . During the production of paired chemical cues, the same protocol was followed except that the samples from each stimulus fish were kept separate.

6.3.4 Experimental set-up and protocol

We used a 30×60 cm tank, filled to 5 cm water depth, with the layout kept constant between visual and chemical trials (Fig. 6.2). There were two glass cylinders at one end of the tank, separated by an opaque barrier. At the other end was a settling compartment (10×30 cm), separated from the test arena by a removable opaque barrier. Chemical cues were introduced via Nalgene® tubing on either side of the tank at 10 ml/min, maintained by flow meters (MMA-35, Dwyer Instruments UK). The tank was lit from above by 12 V DC white LEDs diffused by translucent white fabric. Test fish of both sexes were taken from the wild-caught parental and the laboratory bred F2 generation (Table 6.1) and were tested individually. Fish acclimatised in the settling compartment for 10 minutes. In visual trials, stimulus fish, one infected and one uninfected, were placed in the glass cylinders before this acclimatisation period. In chemical trials, the flow of chemical cues (infected *vs.* uninfected) was started two minutes before the end of acclimatisation. The barrier was lifted remotely *via* a pulley system at the end of the acclimatisation period and a 10 minute test period began when the fish crossed into the test arena. After each trial the tank and components were washed with 70% ethanol

TABLE 6.1: Visual and chemical cue production and use during behavioural trials to test for responses of guppies to *Gyrodactylus turnbulli* infection in conspecifics. Stimulus fish were first generation laboratory-bred female offspring of wild caught guppies from Trinidad and were sexually mature virgins. F2 test fish were second generation laboratory-bred sexually mature virgins of both sexes. Data are presented for the stage of infection rather than for each day for brevity. The ‘early’ stage of infection was up to Day 11, the first point at which the mean infection load of all stimulus fish was not significantly different from the apparent transmission threshold of 40 parasites (Chapter 8).

Year	Cue type	Stage of infection	Cue method	No. of stimulus pairs or batches	Days of infection on which the stimulus was used	Stimulus fish (females only)	Mean no. of parasites on the infected stimulus fish	Test fish (both sexes)	Mean no. of trials conducted with each pair or batch	Total no. of trials
2013	Visual	Early	Pairs	7	5, 6, 7, 8, 10	F1	12.5	Wild caught	5.1	36
		Late		7	15, 16, 20		63.5		4.3	30
	Chemical	Early	Batches	3	2, 8		9.4		13.3	40
		Late		1	17		83		14	14
2014	Visual	Early	Pairs	11	6, 8, 10		32.4	F2	1.2	13
		Late		23	13, 16, 19	23.3	1		24	
	Chemical	Early	Pairs	5	6, 9	16.5	1.6		8	
		Late		15	12, 14, 15, 17	57.7	1.3		20	

and rinsed with clean water. All behavioural trials were video recorded for later analysis using JWatcher™ 1.0 (www.jwatcher.ucla.edu).

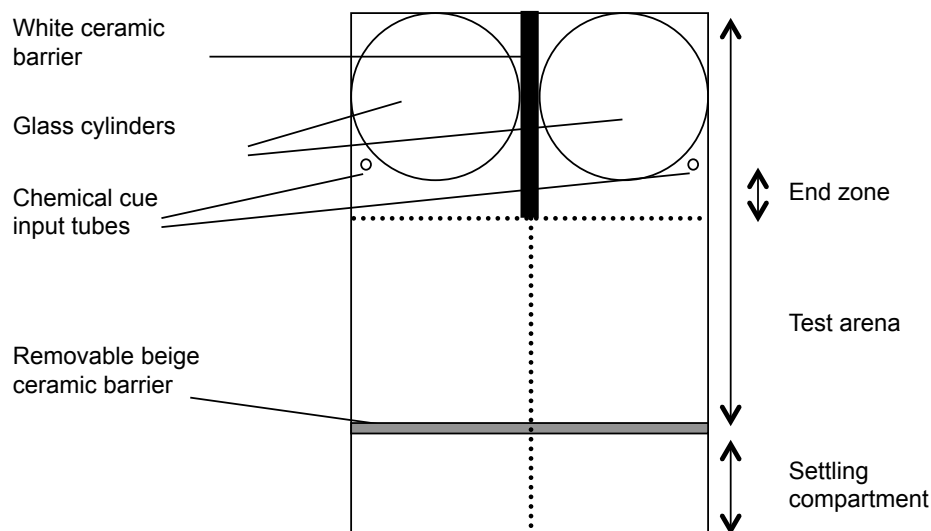


FIGURE 6.2: The choice chamber used to test for behavioural responses of guppies to chemical and visual cues of infection in conspecifics. The dotted lines were not present on the tank, but delineate the end zone and the sides of the tank as used during video analysis.

6.3.5 Test for a side-biased preference

A common issue with preference tests such as the one described in the present study is that test fish demonstrate a preference for one side of the experimental set up based on a factor out of the control of the researchers. In order to confirm that side-bias was not an issue during our experiment we conducted a preliminary trial in which no stimuli (visual or chemical) were presented to the test fish. This was the only difference between the preliminary and experimental trials. A total of ten preliminary trials was conducted, each with a naïve test fish (five male and five female). Each trial was recorded and analysed using JWatcher™ (version 1.0; www.jwatcher.ucla.edu), as in the experimental trials. In R (3.0.2, R Core Team, 2013), a t -test was used to test whether the proportion of time fish spent on the left hand side of the experimental set up was significantly different from 0.5. No side-bias was evident ($t(9) = -0.38$, $p = 0.71$), but to control for bias not detected in this preliminary work, we pseudo-randomised the side of the tank on which each cue was presented between trials in the main experiment, and included this as a fixed effect in our statistical model (Table 6.2).

6.3.6 Ethical note

This work was conducted under the UK Home Office license (PPL 30/2876) with approval by the Cardiff University Animal Ethics Committee. As described above, during the course of this experiment, both test and stimulus fish were subjected to social isolation, and some stimulus fish were additionally experimentally infected with parasites. Although social isolation is likely to have temporarily elevated their stress levels, uninfected stimulus and test fish showed no sign of having suffered lasting harm. To mitigate the effects of this isolation, fish were held in close proximity in transparent tanks; they were therefore in visual contact with conspecifics throughout. Among the infected stimulus fish, we minimised the number that were infected for longer than 10 days (Table 6.1), which is the point at which mortality increases (Dargent *et al.*, 2013). We monitored infected fish carefully and removed those that were exhibiting severe pathology (fused fin rays, difficulty swimming, loss of appetite Cable, 2011). These fish were either treated immediately or killed, if a full recovery was deemed unlikely by an experienced observer. Throughout the experiment, mortality was low ($< 10\%$), and infected fish recovered fully with treatment.

6.3.7 Data analysis

All analyses were conducted in the *lme4* package in R (3.0.2, R Core Team, 2013; Bates *et al.*, 2014). We used a linear mixed effects model (LMM; Gaussian error family and identity link function) to test for an effect of the day of infection on the (square-root transformed) number of *G. turnbulli* infecting the stimulus fish (Model 1 in Table 6.2). Fish identity was included as a random effect to control for repeated measures. We used *post-hoc t*-tests to confirm the day of infection at which this number was not significantly lower than the apparent threshold of 40 parasites: the ‘peak of infectiousness’ at which guppies become highly infectious (Chapter 8).

To test for an effect of the duration of the infection on the infected stimulus fish on test fish avoidance behaviour, we used the proportion of time the test fish spent associated with the infected fish as the response variable in an LMM (Gaussian error family and identity link function; Model 2 in Table 6.2). We used slightly different measures for the two senses to accommodate inherent differences between them: chemical cues could be detected across the whole side of the tank (Harden *et al.*, 2006), while visually mediated preference is measured in time spent in proximity to the stimulus fish (Houde, 1997). For chemical trials, therefore, we used the proportion of time test fish spent on the side of the tank that received the infected fish cue, whereas for visual trials we used the proportion of time test fish spent on the side of the ‘end zone’ of the tank next

Model & response variable	Error family	Link function	Main effects	Two-way interactions	Random effect
1: Square-root transformed number of <i>Gyrodactylus turnbulli</i> infecting the stimulus fish	Gaussian	Identity	Length (n) Day (o)*	Day \times Length	Fish identity
2: Proportion of time the test fish spent associated with the infected stimulus fish			Cue (c)* Day (o)* Load (n) Sex (c) Length (n) Tank side (c) Year (c)	Cue \times Day Cue \times Sex Cue \times Load Load \times Sex Load \times Length Sex \times Day	Stimulus pair identity

TABLE 6.2: Starting models used to test the hypotheses that the number of *Gyrodactylus turnbulli* infecting the stimulus fish, and test fish response to the cues of infection, changed over the course of infection. These starting models were simplified using backwards stepwise deletion of non-significant fixed effects to minimise the Akaike’s Information Criteria (AIC); those that remained in the final model are denoted with an asterisk. ‘Cue’ refers to the cue type that the fish were exposed to (chemical or visual), ‘Load’ refers to the infection load of the stimulus fish on the day it was tested, ‘Tank side’ refers to the side of the tank receiving the cue of the infected stimulus fish (to control for any potential side bias), and ‘Year’ refers to the year in which the trials were conducted. Fixed effects were included as categorical (c), numeric (n), or ordinal (o) variables.

to the infected fish (Fig. 6.2), out of the total time in the end zone. The identity of the stimulus pair (i.e. the pair of size-matched infected and uninfected stimulus fish) used in a trial was included as a random term to account for repeated measures (Tables 6.1 & 6.2). We included the following as fixed effects in the model: the cue type used in a trial (visual or chemical); the number of days the stimulus fish had been infected; the number of parasites infecting the stimulus fish; the length and sex of the test fish; the side of the tank in which the cue of infected conspecific was placed (Table 6.2). The year in which the tests were conducted, which encompassed changes in test fish generation (wild-caught parental *vs.* laboratory-bred F2; Table 6.1), and changes in stimulus production method (batch *vs.* pair), was also included in the model as a fixed effect. We also included the two-way interactions between these terms about which we had *a priori* hypotheses (Table 6.2). Non-significant fixed effects were eliminated in a stepwise manner using Akaike’s Information Criterion (AIC) to compare models; only significant terms are reported.

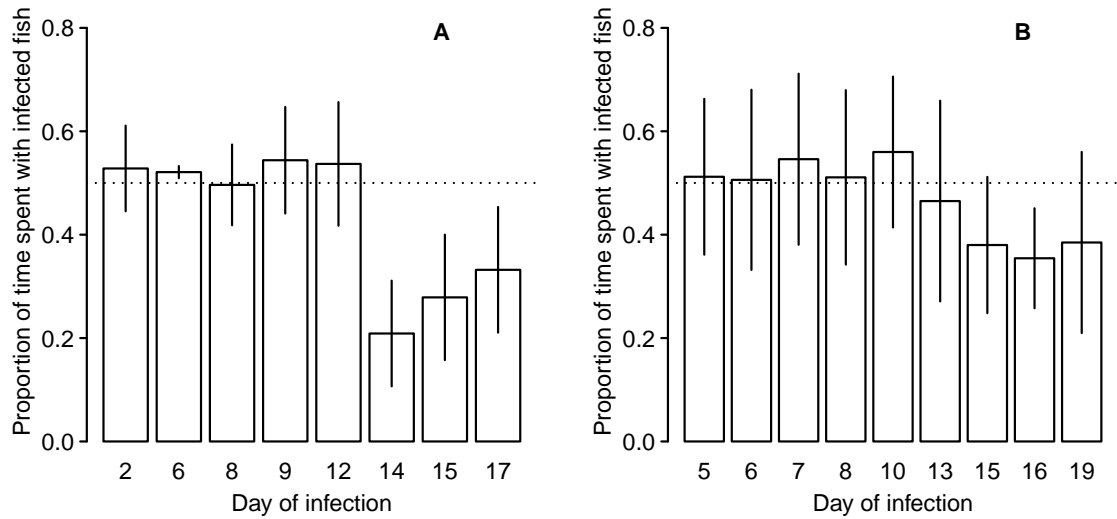


FIGURE 6.3: Guppies showed threat-sensitive avoidance of *Gyrodactylus turnbulli* infected conspecifics based on both chemical (A) and visual (B) cues. The error bars are the 95% confidence intervals. The dotted line illustrates 50%, hence guppies only showed a significant preference on days for which the error bars do not intersect this line.

6.4 Results

The number of *Gyrodactylus turnbulli* infecting individual stimulus fish increased over time (Fig. 6.1; Model 1 in Table 6.2; Day: $F_{12, 366.7} = 31.61$, $p < 0.0001$). The mean infection load of the stimulus fish was not significantly lower than 40 parasites from Day 11 onwards (Fig. 6.1; Day 11 t -test: $t(9) = -0.61$, $p = 0.56$). The proportion of time test fish spent associated with the infected stimulus fish depended on the length of time the stimulus fish had been infected (Fig. 6.3; Model 2 in Table 6.2; Day: $F_{1, 149.1} = 21.16$, $p < 0.0001$), and was only significantly lower than 50% after Day 11 (Fig. 6.3). There was remarkable redundancy between the responses of fish provided with chemical and visual cues. Guppies provided only with visual cues spent on average 6% more of their time associated with the infected fish compared with those provided with only chemical cues; this difference in response between the two senses was marginally non-significant (Fig. 6.3; Model 2 in Table 6.2; Cue: $F_{1, 15.8} = 2.45$, $p = 0.074$).

6.5 Discussion

Guppies avoided the cues of conspecifics in the late, but not early stages of *Gyrodactylus turnbulli* infection, and this behaviour was marginally stronger when based on chemical rather than on visual cues. We have shown that transmission occurs very rapidly during the ‘peak of infectiousness’, when the *G. turnbulli* infection load of an individual guppy reaches *ca.* 40 (Chapter 8). On the stimulus fish used in this study, the *G. turnbulli* infection load increased through time, reaching the threshold of 40 parasites after 11 days of infection (Fig. 6.1). This corresponds well to the point at which the test fish showed avoidance of the stimulus fish (Fig. 6.3). Our results therefore support our hypothesis that guppy avoidance of infected conspecifics is proportional to the transmission risk they pose, and is based on redundant visual and chemical cues.

Our results suggest that the cues of infection, both visual and chemical, elicit stronger responses in conspecifics the longer an individual has been infected. This increase in response is likely to be due to an increasing strength of these cues. Infected fish release a number of chemical cues as a result of infection, and these will change in concentration or composition as the infection progresses (De Moraes *et al.*, 2014). One component may be alarm cue, a chemical released from fish skin damaged during predation events (Brown *et al.*, 1999) and infection (Poulin *et al.*, 1999), which elicits avoidance behaviour in guppies (Brown *et al.*, 2009, 2010) and many other species (reviewed by Smith, 1992; Chapter 5). Clearly, the more skin damage a fish sustains through infection, the more alarm cue it will release. Other chemical cues are likely to include specific hormones such as cortisol (Stoltze and Buchmann, 2001), and chemical changes in mucous (Buchmann and Lindenstrøm, 2002) that increase in concentration during the course of gyrodactylid infection. Similarly, the visual cues of infection, such as the parasites themselves and the resultant host pathology and sickness behaviour, will become more obvious as the infection progresses. At later stages, and particularly at high *Gyrodactylus* spp. infection loads, guppies typically display clamped fins, paleness, and difficulty swimming (Cable, 2011).

However, given that one might predict that the changes in chemical and visual cues of infection would happen linearly as the infection load increased through time, it is somewhat surprising that we found evidence of a ‘step change’ in avoidance behaviour. This could reflect either a sudden change in the cues emitted, a sudden change in the response they elicit, or a combination of these two processes. The nonlinear relationship between infection load and transmission speed observed during our transmission experiment (Chapter 8: Fig. 8.3) lends weight to the hypothesis that the nature of the host-parasite interaction changes suddenly, increasing transmission risk and hence avoidance behaviour more rapidly than would be expected from a purely linear function

of infection load. Receivers of the cues of transmission risk may therefore be acting on a cost-benefit analysis: *ca.* 13 days of infection may be the first point at which associating with an infected individual is too costly. Certainly, our data suggest that this is after the point at which transmission of *G. turnbulli* occurs most rapidly (Fig. 6.1; Chapter 8).

Additionally, the cues emitted by an infected host may change suddenly as the infection reaches a point at which the cues cannot be suppressed any longer. Both the infectious host and its directly transmitted parasites are likely to have been under strong selection to disguise parasite appearance (Whittington, 1996; Johnson *et al.*, 2010) and host pathology and sickness behaviour (Aubert *et al.*, 1997; Lopes *et al.*, 2012) respectively. In doing so, parasites increase their fitness by increasing their chances of transmitting to new hosts (Poulin, 2007), and avoiding predation (Whittington, 1996). Infectious hosts able to disguise their infection continue to benefit from group living (Krause and Ruxton, 2002) and increase their relative fitness by passing parasites to shoal-mates; the more group members infected, the less detrimental infection becomes during intra-group competition. Response to this selection in the host and parasite may explain why we observed no avoidance of infected conspecifics in the early stages of infection, despite moderate infection loads (Table 6.1), and a moderate risk of transmission (Chapter 8). The change in the cues of infection may represent a ‘tipping point’ in the infection; it is possible that the parasites themselves are using cues from the host to determine when to begin transmission (Stephenson, 2012). Infected individuals may therefore be collaborating with their parasites in order to infect conspecifics (Rósza, 2000). Certainly, gyrodactylid-infected guppies attempt to ‘rub up’ against shoal-mates (Croft *et al.*, 2011; J. F. S. personal observation). This behaviour itself and the opportunity it provides shoal-mates to sample the host’s chemical and visual cues at close range, however, potentially explains their observed avoidance by conspecifics in semi-natural conditions (Croft *et al.*, 2011).

It is unclear how often guppies in natural populations reach the infection loads and resultant stage of morbidity of those in the present study, however; we rarely observed guppies with even moderate infection loads in extensive field surveys (Chapter 4; Appendix A). However, that we did not observe them may be due to the fact that infected guppies are more vulnerable to extrinsic sources of mortality such as spate conditions (males only; van Oosterhout *et al.*, 2007) and predation (Chapter 2); both these factors may remove heavily infected individuals from populations. Additionally, the threat of predation will be exacerbated by the social ostracism of heavily infected individuals that the present study suggests. Further, in natural settings, infected individuals may be able to moderate their own infection through transmission to shoalmates (Chapters 8 & 4; Richards *et al.*, 2010; Johnson *et al.*, 2011), an opportunity unavailable to the isolated

infected fish in this study. The cues from stimulus fish in the late stages of infection may therefore be extreme cues of parasite infection than would not normally be encountered in natural populations: test fish may be responding to the unfamiliar nature of the cues rather than to the threat of infection (Chapter 7). This neophobia would be analogous to that recently reported in guppies by Brown *et al.* (2013) as an evolutionary response to predation pressure. An additional consideration for the relevance of our findings to natural settings is that we did not test how the combination of visual and chemical cues of infection affected the response of the test fish to infected conspecifics. A combination of the two cues could have an effect equal to that of either cue alone, equivalence, or the response could be greater, enhancement (Partan and Marler, 1999, 2005). Enhancement could cause avoidance behaviour to occur earlier in infection: the artificiality of receiving only one cue may therefore explain why there appears to be a slight lag between the stimulus fish reaching the ‘peak of infectiousness’, and the test fish displaying avoidance behaviour.

The interaction between predators and parasites is likely to have important implications for the relevance of this finding to natural populations because wild guppies must balance the threat of infection with that of predation. Predation drives the evolution of a vast array of guppy traits (reviewed by Magurran, 2005) and its importance to the evolutionary ecology of this species may well dwarf that of parasites (Dargent *et al.*, 2013; Gotanda *et al.*, 2013; Stephenson, 2014): becoming infected does not reduce guppy fitness to the same extent as being eaten. Considering the constraints imposed by predation pressure leads to specific predictions about how our results may relate to wild populations. For example, if guppies are unable to employ avoidance behaviour because it will leave them vulnerable to predation, they should invest more in physiological immunity. This trade-off in behavioural and physiological immunity is supported by empirical data from other taxa (Schaller, 2011; Zylberberg *et al.*, 2012). Individual variation in physiological immunity may therefore contribute to the variation we observed in avoidance behaviour and suggests that this behaviour may be variable within natural populations. This system may therefore provide an excellent opportunity to test how physiological immunity correlates with animal personalities (Barber and Dingemans, 2010). A correlation between personality and parasite defence would have implications for the epidemiology of this disease in natural populations, given that personality affects guppy connectedness within social networks (Croft *et al.*, 2009). Intriguingly, the related prediction that better opportunities to employ avoidance behaviour in populations experiencing low levels of predation pressure leads to a reduction in physiological immunity is not borne out. Field surveys reveal higher infection burdens (Chapter 4; Martin and Johnsen, 2007; Gotanda *et al.*, 2013), and lower tolerance of infection (i.e. the ability to limit the fitness cost of a given infection: Roy and Kirchner,

2000; Råberg *et al.*, 2009; Chapter 3) among fish in high predation populations. How guppies from populations experiencing low levels of predation pressure use visual and chemical cues to inform their infection avoidance behaviour would help to elucidate the effect of predation pressure and physiological immunity on the sensory ecology of this host-parasite interaction. We therefore support recent calls for the unification of the study of host-parasite and predator-prey interactions (Hatcher *et al.*, 2006; Raffel *et al.*, 2008, 2010); considering the ‘enemy ecology’ of this system is likely to be a fruitful future research direction.

6.6 Authorship Acknowledgement

The manuscript resulting from this chapter is authored by:

Jessica F. Stephenson and Joanne Cable (Cardiff University).

6.7 Contribution to and position within the thesis

In this chapter I demonstrate that guppies use both visual and chemical cues to detect infection in conspecifics, but only avoid those in the later stages of infection. In the next chapter I test whether this avoidance behaviour is due to guppies avoiding an innate cue of infection, or whether they are simply avoiding conspecifics emitting abnormal chemical and visual cues. To do so, I test whether juvenile guppies can imprint on the chemical cues of infection and subsequently prefer to associate with these cues as adults.

Chapter 7

Better the devil you know: imprinting causes guppies to prefer infectious conspecifics

7.1 Abstract

Animals that recognise and associate with kin enjoy a wealth of benefits. The mechanisms behind this recognition vary between taxa, but many species use imprinting: the long-term memory of sensory cues encountered during development. Juveniles imprint on the cues of nearby individuals and, even during adulthood, may associate with those whose phenotypes match this ‘recognition template’. However, this process could lead to maladaptive social decisions if, for instance, individuals imprint on the cues of conspecifics infected with directly transmitted diseases. To investigate the role of imprinting in the sensory ecology of disease, we exposed guppies, *Poecilia reticulata*, *in utero* and as juveniles to the cues of either healthy conspecifics, or to those experiencing disease caused by the directly transmitted parasite *Gyrodactylus turnbulli*. In a dichotomous choice test, adult ‘disease-imprinted’ guppies preferred to associate with the chemical cues of *G. turnbulli*-infected conspecifics, whereas ‘healthy-imprinted’ guppies preferred to associate with the chemical cues of uninfected conspecifics. These responses were only observed when the stimulus fish were in the later stages of infection, and were therefore heavily infected and infectious. We discuss how this maladaptive imprinting may contribute to disease transmission in natural populations of a social host.

7.2 Introduction

Animals that recognise and associate with kin enjoy a wealth of benefits (reviewed by Krause and Ruxton, 2002). The mechanisms behind this recognition vary between taxa, but many species use imprinting: the long-term memory of sensory cues encountered during development (Harden *et al.*, 2006). Juveniles imprint on the sensory cues of nearby individuals and, even during adulthood, may associate with those whose phenotypes match this ‘recognition template’ (reviewed by Mateo, 2004).

Phenotype matching commonly leads to adaptive behaviour, but it is not infallible. For example wild buzzards, *Buteo buteo*, imprint on the colour morph of their mothers and select mates of the same morph, even among the less fit homozygous morphs, reducing the potential fitness of their offspring (Krüger *et al.*, 2001). Both sticklebacks, *Gasterosteus* spp. (Kozak *et al.*, 2011) and swordtails, *Xiphophorus* spp. (Verzijden and Rosenthal, 2011) imprint on the cues of heterospecifics when raised with them, and subsequently show maladaptive preferences for these heterospecifics during mate choice trials. Some populations of our study organism, the guppy *Poecilia reticulata*, use a similar mechanism to that of swordtails and sticklebacks and are attracted to heterospecifics after imprinting on their cues (Warburton and Lees, 1996). However, other populations can use sophisticated, potentially self-referent phenotype matching to associate with full- rather than half-siblings (Hain and Neff, 2007; Evans and Kelley, 2008).

Regardless of how well their phenotypes match the recognition template, animals should avoid associating with potentially harmful individuals, such as those infected with directly transmitted diseases (Chapter 6). Avoidance of infected conspecifics is based on chemical cues in a number of taxa (fish: Chapter 6; amphibians: Kiesecker *et al.*, 1999; mammals: Kavaliers *et al.*, 2005; crustaceans: Behringer *et al.*, 2006; insects: Conway *et al.*, in prep.). It is unclear whether this wide-spread behaviour is an innate avoidance of a specific infection cue, or a more general avoidance of phenotypes that differ markedly from an individual’s recognition template.

Here we used the guppy-*Gyrodactylus turnbulli* host-parasite system to test the hypothesis that the avoidance of infected conspecifics is based on phenotype matching and is therefore potentially vulnerable to maladaptive imprinting. Infection with the directly transmitted ectoparasite *G. turnbulli* reduces guppy fitness and survival (Chapters 2 & 3; Houde and Torio, 1992; van Oosterhout *et al.*, 2007). *G. turnbulli* transmission occurs through social contact (Chapters 4 & 8; Richards *et al.*, 2010; Johnson *et al.*, 2011), and previous work has shown that uninfected guppies use chemical and visual cues to avoid infected conspecifics when they are in the later stages of infection, and

hence most infectious (Chapters 6 & 8). We exposed guppies *in utero* and as juveniles to either adults experiencing *G. turnbulli*-induced disease, or to *G. turnbulli*-exposed but healthy adults. We then tested how the response of these fish as adults to the chemical cues of *G. turnbulli*-infected and uninfected conspecifics depended on their exposure as juveniles, and on the stage of infection of the infected fish.

7.3 Materials and Methods

7.3.1 Fish origin and maintenance

We used first (F1) and second (F2) generation laboratory-bred descendants of guppies from the lower Caura River, Trinidad (UTM: 20 P 67952.77 m E, 118037.64 m N; elevation 112m) to test for the importance of imprinting in determining guppy avoidance of the chemical cues of infected conspecifics. Wild fish ($n = ca. 600$) were transported to Cardiff University in June 2012 (Cefas APB authorisation number CW054-D-187A), where they were prophylactically treated for infection using Binox® (Nitrofurazone; Jungle Laboratories Corporation®, Cibolo, Texas). Fish were housed at low densities in 70 L aquaria of dechlorinated water at $24 \pm 1^\circ\text{C}$, on a 12h Light:12h Dark lighting schedule (overhead fluorescent lighting), and fed daily on Aquarian® flakes, supplemented with *Artemia* and bloodworm. Each tank had pea gravel substrate, an under gravel filter and standardised enrichment. Tanks were checked weekly for fry, and these were moved to separate rearing tanks. These F1 males and females were separated once sex determination was possible (*ca.* 6-8 weeks), ensuring that all fish were virgins prior to the start of the experimental infections.

7.3.2 Experimental infections

F1 fish ($n = 200$) were individually housed in 1 L tanks under standard conditions, and infected on Day 0 with 2 individuals of the *Gt3* strain of *Gyrodactylus turnbulli*, as part of a long-term breeding experiment. The *Gt3* culture is maintained on ornamental guppies ('culture fish'). During experimental infections, culture fish were killed using an overdose of tricaine methanesulfonate (MS222; PHARMAQ UK, Ltd.). Experimental fish were anaesthetised with 0.02% MS222 and then transferred to a shallow petri dish containing dechlorinated water. Under a dissecting microscope and fibre optic illumination, the tails of the culture fish and experimental fish were placed in close proximity until two individual *G. turnbulli* had transmitted. The number of parasites infecting each fish was counted under 0.02% MS222 every other day until Day 9. At this point, all fish were treated with levamisole to clear the infection, and were confirmed clear of infection

by visual inspection under 0.02% MS222 on three separate occasions with at least four days between (Schelkle *et al.*, 2009), before being moved to breeding tanks as described below.

7.3.3 Experimental rearing conditions

F2 laboratory-bred descendants of the wild-caught fish were exposed as fry to the cues of F1 fish that differed significantly in their response to *G. turnbulli* infection to test for a potential role of imprinting in the sensory ecology of disease. The 30% of the F1 guppies with the highest mean parasite count over the nine days of infection were placed in a breeding tank (mean parasite count \pm SEM = 21.3 \pm 0.7; n = 60: 30 males, 30 females). F2 fish gestated and born into this tank therefore imprinted on chemical cues of adults experiencing *G. turnbulli*-induced disease. The 30% of F1 guppies with the lowest mean parasite count (mean parasite count \pm SEM = 3.3 \pm 0.3; n = 60: 30 males, 30 females) were also placed in a breeding tank and their offspring imprinted on chemical cues of *G. turnbulli*-exposed but healthy adults. The difference between these two groups of fish in the mean number of parasites over the nine days of infection was large (difference between the means \pm SEM = 18.0 \pm 0.8) and statistically significant (*t*-test in R 3.0.2; R Core Team, 2013: $t(117) = 23.16$, $p < 0.0001$). F2 fry were left in these tanks for *ca.* 7 days (as in Warburton and Lees, 1996) before being moved to a tank of other F2 juveniles from the same breeding tank. Males and females were separated once sex determination was possible (*ca.* 6-8 weeks), ensuring that all fish were virgins prior to the start of the experiment.

7.3.4 Cue production

To produce chemical cues, virgin F1 females were size matched \pm 1 mm into pairs ('stimulus pairs'; n = 28). Because familiarity is an important factor in guppy association preferences (Griffiths and Magurran, 1997), we ensured that all test and stimulus fish were unfamiliar to one another. One fish in each stimulus pair was infected and the infection monitored following the protocol above. Control fish were sham infected to control for the effects of anaesthetic and handling time; they were not exposed to parasites, but otherwise the procedure was as used for those fish that were infected. We used these stimulus pairs to produce chemical cues of the 'early' and 'late' stages of infection of the stimulus fish (Table 7.1; early: infected for up to 9 days; late: infected for 12 days or more). Chemical cues were obtained by holding stimulus fish individually in 500 ml dechlorinated water in food-grade plastic containers for 24 hours. Fish were

Stage of infection	Days of infection on which the stimulus was used	Imprinting of test fish	No. of stimulus pairs	Mean no. of parasites on the infected stimulus fish	Mean no. of trials conducted with each pair	Total no. of trials
Early	6, 9	Healthy	5	16.5	1.6	8
		Diseased	7	21.1	1.3	9
Late	12, 14, 15, 17	Healthy	15	57.7	1.3	20
		Diseased	14	62.7	1.2	17

TABLE 7.1: Chemical cue production and use to test whether the avoidance of infected conspecifics is innate avoidance of infection, or the avoidance of cues identified as abnormal on the basis of imprinting and phenotype matching. See main text for methods, and the justification of using early (up to Day 9 of infection) and late (Day 12 of infection onwards) categories of the stage of infection during the analysis. Test fish were F2 laboratory-bred sexually mature virgins of both sexes. Stimulus fish were F1 laboratory-bred sexually mature virgin females.

not fed during this period and their holding water was subsequently decanted into two 250 ml aliquots and frozen at -20°C until use.

7.3.5 Categorisation of early and late stages of infection

We categorised the duration of the infection of the stimulus fish into early (up to Day 9) and late (Day 12 onwards; Table 7.1) stages of infection, rather than using the number of days, because guppies fail to show a response to the cues of infection in conspecifics up to *ca.* Day 11, but show a fairly consistent avoidance after this point (Chapter 6). This categorisation therefore does not mask ecologically important variation, and maximises the statistical power available to determine an effect. Additionally, Day 11 is the point at which the mean number of parasites *per* fish appears to plateau, and becomes more variable between fish (Fig. 6.1), suggesting that the immune response has a greater impact on the parasite infrapopulation after this point. This is also likely to be when fish start to be more affected by the infection; gyrodactylid-induced mortality increases after Day 10 of infection (Dargent *et al.*, 2013).

7.3.6 Experimental set-up and protocol

Guppies gestated and reared in either a tank with adults experiencing *G. turnbulli*-induced disease, or in a tank with *G. turnbulli*-exposed but healthy adults were tested for their response to the chemical cues of *G. turnbulli* infected and uninfected conspecifics using dichotomous choice tests. Behavioural trials were conducted in a glass tank (30×60 cm; Fig. 6.2) containing 9 L of dechlorinated water at $24 \pm 1^{\circ}\text{C}$. The tank was

separated into settling and test compartments by an opaque removable barrier. On either side of the test compartment, at the end furthest from the settling compartment, chemical cues were introduced via Nalgene® tubing at a rate of 10 ml/min using flow metres (MMA-35, Dwyer Instruments UK). The tank was lit from above by 12 V DC LEDs diffused by translucent white fabric. Test fish were placed in the settling compartment for 10 minutes to acclimatise. Chemical cues of infected and uninfected guppies were simultaneously run into the test arena two minutes before the end of acclimatisation. The opaque barrier was lifted at the end of acclimatisation and a 10 minute test period began when the fish crossed into the test arena. Each trial was recorded and behavioural data was subsequently quantified using JWatcher™ 1.0 (www.jwatcher.ucla.edu). A preliminary test confirmed that there was no side bias inherent in this set-up (Chapter 6). The chemical cue used in a trial (early or late), the sex of the test fish, its imprinting treatment (diseased or healthy), and which cue was run on the left hand side of the tank were pseudo-randomised following a Latin square design.

7.3.7 Ethical note

This work was conducted under the UK Home Office license (PPL 30/2876) with approval by the Cardiff University Animal Ethics Committee. As described above, during the course of this experiment, both test and stimulus fish were subjected to social isolation, and stimulus fish were additionally experimentally infected with parasites. Although social isolation is likely to have temporarily elevated their stress levels, neither stimulus nor test fish showed signs of having suffered lasting harm. To mitigate the effects of this isolation, fish were held in close proximity in transparent tanks; they were therefore in visual contact with conspecifics throughout. Among the infected stimulus fish, we minimised the number that were infected for longer than 10 days (Table 7.1), which is the point at which mortality increases (Dargent *et al.*, 2013). We monitored infected fish carefully and removed those that were exhibiting severe pathology (fused fin rays, difficulty swimming, loss of appetite Cable, 2011). These fish were either treated immediately or killed, if a full recovery was deemed unlikely by an experienced observer. Throughout the experiment, mortality was low (<5%), and infected fish recovered fully with treatment.

7.3.8 Data analysis

A Linear mixed model (LMM; Gaussian error family and identity link function; Table 7.2) was conducted in the *lme4* package in R (3.0.2; R Core Team, 2013; Bates *et al.*,

Response variable	Error family	Link function	Main effects	Two-way interactions	Random effect
Proportion of time spent associated with the infected stimulus fish	Gaussian	Identity	Load (n) Stage of infection (c)* Imprinting (c)* Sex (c) Length (n) Tank side (c)	Imprinting × Load Imprinting × Sex Imprinting × Stage of infection* Load × Sex Load × Length	Stimulus pair identity

TABLE 7.2: The starting model used to test the hypothesis that imprinting determines the response of guppies to the chemical cues of *Gyrodactylus turnbulli* infection in conspecifics. This model was simplified using backwards stepwise deletion of non-significant fixed effects to minimise the Akaike’s Information Criteria (AIC). Model terms that remained in the final model are denoted with an asterisk. ‘Load’ refers to the infection load of the stimulus fish on the day it was used, ‘Imprinting’ refers to the imprinting treatment the fish were exposed to, and ‘Tank side’ refers to the side of the tank receiving the cue of the infected stimulus fish (to control for any potential side bias). Fixed effects were included as categorical (c), numeric (n), or ordinal (o) variables.

2014). The proportion of time test fish spent on the side of the tank receiving the infected fish cue was used as the response variable. We included the following terms as fixed effects in the model: number of parasites on the stimulus fish; stage of infection of the stimulus fish (early or late); test fish imprinting treatment (diseased or healthy); test fish sex; test fish length; tank side that received the chemical cue of infected conspecific; two-way interactions about which we had *a priori* hypotheses (Table 7.2). The identity of the stimulus pair was included as a random term to control for repeated measures (Table 7.1). Non-significant fixed effects were eliminated in a stepwise manner using Akaike’s Information Criterion (AIC) to compare models; only significant effects are reported.

7.4 Results

Test fish showed no preference for the chemical cues of infected or uninfected stimulus fish when these were during the early stage of infection. During the late stage of the infection on the stimulus fish, however, healthy-imprinted guppies spent more time associated with the chemical cues of uninfected guppies, whereas *Gyrodactylus turnbulli*-induced disease-imprinted guppies spent more time associated with the chemical cues of *G. turnbulli*-infected guppies (Fig. 7.1; Table 7.2; imprinting × stage of infection: $F_{1, 36.7} = 15.18$, $p < 0.001$).

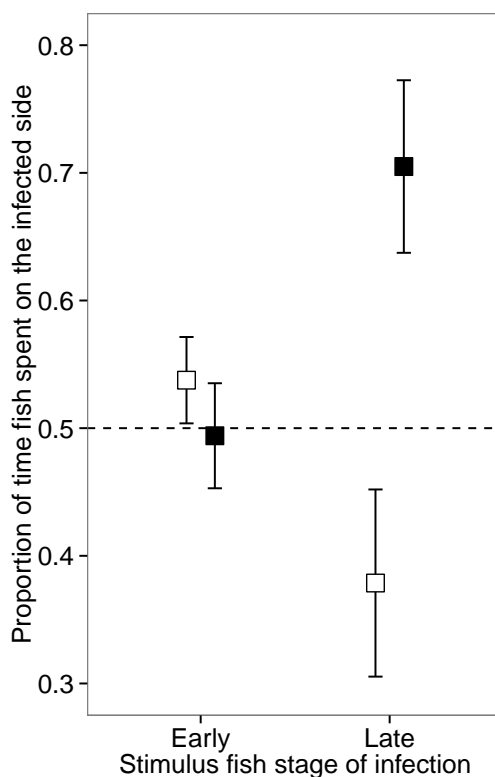


FIGURE 7.1: How guppies responded to the chemical cues of *Gyrodactylus turnbulli*-infected and uninfected conspecifics depended on their imprinting treatment. Those that had imprinted on the cues of *G. turnbulli*-exposed but healthy adults (white squares) avoided infected conspecifics during the late stage of infection. However, those that had imprinted on the cues of adults experiencing *G. turnbulli*-induced disease (black squares) spent more time associated with late-stage infected conspecifics. The error bars are the standard error of the mean, and the dashed line highlights 50% of the time, and therefore no preference.

7.5 Discussion

Fish exposed to the cues of healthy adults as juveniles avoided the chemical cues of *Gyrodactylus turnbulli*-infected conspecifics as adults, but only when these infected conspecifics were in the late stage of infection. By contrast, fish exposed to the cues of adults experiencing *G. turnbulli*-induced disease as juveniles showed a significant preference for the chemical cues of *G. turnbulli*-infected conspecifics as adults, again only when these were in the late stage of infection (Fig. 7.1). These results therefore support our hypothesis that the avoidance of infected conspecifics based on chemical cues is not innate, but is acquired through imprinting and subsequent phenotype matching.

The likelihood that this maladaptive imprinting will occur in natural guppy populations depends on the ecology of the population. Hain and Neff (2007) found evidence for self-referent phenotype matching among guppies from populations experiencing low, but not

high levels of predation. By contrast, guppies from populations experiencing high levels of predation may include information from prior experience to form their recognition templates ('familiarity'; Griffiths and Magurran, 1999; Croft *et al.*, 2004; Hain and Neff, 2007), and hence fail to show consistent kin-biased structuring in wild shoals (Russell *et al.*, 2004; Hain and Neff, 2007; Evans and Kelley, 2008; Piyapong *et al.*, 2011; Croft *et al.*, 2012). Our results complement this previous work: we used guppies from a population experiencing high levels of predation and the preference we observed cannot have been based on self-referent phenotype matching because disease-imprinted guppies were not exposed to the parasites themselves. Maladaptive imprinting may therefore more likely occur in populations that experience high levels of predation.

Exposure to the parasites themselves, prevented by our experimental design, may prevent wild juveniles from preferentially associating with the cues of infection. Juveniles with experience of *Gyrodactylus* spp. infection, such as those receiving the parasite from heavily infected mothers, are likely to learn to avoid the cues of infection; guppies show well developed learning abilities (Kotrschal *et al.*, 2013). It may seem unlikely that heavily infected females would survive to reproduce at all, but *Gyrodactylus* spp. infection affects wild females less negatively than wild males (Chapters 2 & 3) and may actually have a negligible effect on their survival (van Oosterhout *et al.*, 2007) and reproduction (Pérez-Jvostov *et al.*, 2012).

A further important consideration for the relevance of our findings to natural settings is cue concentration (Archard *et al.*, 2008). Cue concentration may explain the difference in test fish response to the cues of early and late stages of infection; the cue of infection is likely to increase in concentration through the infection of the stimulus fish and there may be a threshold concentration, below which test fish do not respond (similar to alarm cue; Chapter 5). This may limit the applicability of these findings to natural settings: the cues the fish imprinted on are likely to have been far more concentrated in our study than in the wild (Archard *et al.*, 2008). However, imprinting *in utero* may not be affected (Hain and Neff, 2007), and newborn fry would be in close proximity to their mother and therefore experience highly concentrated cues (Dusenberry, 1992), especially given the shallow, slow flowing habitats in which guppies generally give birth (Magurran, 2005). The time-frame of imprinting in guppies has not been investigated, but in zebrafish, imprinting on chemical cues takes place six days after fertilisation (Gerlach *et al.*, 2008), at which point guppies are still *in utero*. In summary, our results indicate that depending on the context, imprinting-mediated social behaviour may facilitate or prevent the transmission of this and other pathogens through natural populations.

7.6 Authorship Acknowledgement

The manuscript resulting from this chapter is authored by:

Jessica F. Stephenson and Michael Reynolds (Cardiff University).

7.7 Contribution to and position within the thesis

In this chapter I provide evidence that guppies use the cues they imprint on as juveniles, and subsequent phenotype matching as adults, to inform their association preferences, and that this process can lead to maladaptive preferences. The results of both this chapter and of Chapter 6 indicate that guppy avoidance of infected conspecifics only occurs when these are in the later stages of their infection. This behaviour is likely adaptive: in the next chapter I show that it is during this later stage of infection that *Gyrodactylus turnbulli* is most likely to transmit, and hence infected guppies are most infectious.

Chapter 8

Hand-me-down infections: do the quantity and quality of transmitted parasites depend on the previous host?

8.1 Abstract

It is well known that variation in resistance between infected hosts ('donors') contributes to heterogeneity in the quantity of parasites with which they are infected. Comparatively under-studied is the idea that variation in resistance may also affect parasite quality through immune damage or competition. During transmission, the susceptibility of parasite-naïve 'recipients' to becoming infected, and their subsequent resistance to that infection, may depend on this donor-mediated heterogeneity in the quantity and quality of transmitted parasites. Here, we exposed donor *Poecilia reticulata* with natural variation in *Gyrodactylus turnbulli* infection loads to parasite-naïve recipients. Variation in resistance between donors affected recipient susceptibility to becoming infected: the quantity of parasites transmitted and speed of transmission increased with donor infection load. However, transmitted parasite quality, inferred from the recipient's lifetime infection load, did not depend on the donor. Individual variation in resistance among donors therefore drives heterogeneity in susceptibility, but not resistance, among recipients.

8.2 Introduction

Investigating the impact of heterogeneity between infected hosts, or ‘donors’, on disease transmission is a central goal of modern epidemiology (Galvani and May, 2005; Lloyd-Smith *et al.*, 2005, 2006; Matthews *et al.*, 2006; Paull *et al.*, 2012). Epidemics such as HIV/AIDS, gonorrhoea and SARS, in which the majority of transmission events are due to a minority of donors, indicate marked heterogeneity in donor infectiousness (Woolhouse *et al.*, 1997; Galvani and May, 2005; Lloyd-Smith *et al.*, 2005, 2006; Stein, 2011; Paull *et al.*, 2012). One component of infectiousness is the number of infectious particles released by a donor, which is often highly correlated with the number of parasites it is infected with (‘infection load’; Dwyer *et al.*, 1997; Matthews *et al.*, 2006; Chase-Topping *et al.*, 2008; Lass *et al.*, 2013). Infection load is therefore commonly assumed to be a key indicator of infectiousness (Lloyd-Smith *et al.*, 2006; Matthews *et al.*, 2006), and is itself highly heterogeneous: parasite distribution within host populations is typically over-dispersed, such that the majority of hosts have low infection loads, and a small minority exhibit high infection loads (Shaw and Dobson, 1995; Perkins *et al.*, 2003). As an apparently logical extension of these observations, much of the infectious disease literature suggests that there is a positive, linear relationship between the infection load of a donor, or infectious particle density, and that of the parasite-naïve ‘recipient’ of its infection (e.g. Lloyd-Smith *et al.*, 2005; Chase-Topping *et al.*, 2008; Paull *et al.*, 2012).

While a small number of empirical studies provide partial support for a positive relationship between donor infection load, or infectious particle density, and recipient susceptibility (see Table 8.1 for definition; Anderson *et al.*, 1978; Keymer and Anderson, 1979; Karvonen *et al.*, 2003; Cobbold *et al.*, 2007), more data are required to rigorously test this assumption. There is also a need to decouple the effect of donor heterogeneity on a recipient’s susceptibility, a measure of the probability (e.g. Ben-Ami *et al.*, 2010; Cronin *et al.*, 2010; Civitello and Rohr, 2014) and quantity of parasites establishing (e.g. Anderson *et al.*, 1978; Keymer and Anderson, 1979; Raffel *et al.*, 2010), from that on its resistance, or ability to inhibit the growth of the parasite and hence limit infection load (Table 8.1; e.g. Roy and Kirchner, 2000; Råberg *et al.*, 2009). Even if there is a positive relationship between the infection load of the donor and that initially establishing on the recipient (a measure of recipient susceptibility), it is unclear how the recipient’s lifetime infection load (a measure of recipient resistance) will be determined by this initial infection load, or from whom it acquired the infection. However, most transmission experiments fail to monitor the recipient’s ongoing infection (but see Schmid-Hempel *et al.*, 1999) and therefore miss information vital to understanding the further spread of the disease in natural populations.

Recipient resistance may be affected by the donor from whom the infection was acquired because donor-specific characteristics of the infection could affect parasite quality (Dwyer *et al.*, 1997; Schmid-Hempel *et al.*, 1999; Karvonen *et al.*, 2003; Stein, 2011; Paull *et al.*, 2012). While previous work has indicated that factors such as host genetics, sex, reproductive condition and social status are important (Madhavi and Anderson, 1985; Poulin, 2007; Schmid-Hempel, 2011), the donor from whom the infection was acquired is an untested but potentially important source of variation in recipient resistance. For example, heavily infected donors could be infected with and therefore transmit more virulent parasite strains (Cobbold *et al.*, 2007; Chase-Topping *et al.*, 2008; Stein, 2011). Heavily infected donors may transmit poorer quality parasites, however, due to increased competition for resources (Poulin, 2007; Schmid-Hempel, 2011 and references therein). Conversely, donors with relatively low infection loads may transmit poorer quality parasites because they have been damaged by the host's immune response (Schmid-Hempel *et al.*, 1999). Variation in the time between infection of the donor and transmission to the recipient is also known to affect parasite establishment success (Schmid-Hempel *et al.*, 1999), and may therefore affect the recipient's ability to defend against the parasite. Both the strength of the host immune response and the host infection load change through the course of most parasitic infections (e.g. Scott and Anderson, 1984; Schmid-Hempel *et al.*, 1999; Bakke *et al.*, 2007; Poulin, 2007); if either host immune response or intraspecific competition affects parasite fitness, we predict that this will change through the course of infection.

In order to test how donor heterogeneity contributes to variation in the quantity and quality of transmitted parasites, we experimentally infected naïve donors, allowed them to develop natural variation in infection load, and then exposed them to naïve recipients. We tested whether there was an effect of the donor's infection load and the duration of its infection (early *versus* late) on the quantity of parasites transmitted and the speed at which transmission occurred, and hence recipient susceptibility (see Hypothesis 1 in Table 8.1). In order to test for donor-mediated variation in parasite quality, we calculated the integral of infection load over time for each recipient. This integral provides a single value encompassing both the duration and load of an individual's infection (Adelman *et al.*, 2013), and is therefore a useful measure of 'parasite quality', or the parasite's ability to exploit the host. We used this value to infer how recipient resistance depended on the parasites received from the donor (see Hypothesis 2 in Table 8.1).

We used the guppy *Poecilia reticulata*-*Gyrodactylus turnbulli* host-parasite system, which is particularly well-suited to addressing outstanding questions in disease ecology for several reasons. Infections of *Gyrodactylus* species, such as *G. turnbulli*, which feed and reproduce on the host skin can be easily quantified and monitored through time using non-destructive methods (Scott and Anderson, 1984; Bakke *et al.*, 2007). Because the

parasite can reproduce asexually, it is possible to minimise the potentially confounding effect of genetic variation between parasites by using a strain founded by a single individual. Here we used a strain initiated in this way and cultured on the same population of inbred guppies since 1997; any heterogeneity we observed between our experimental donors in the quality of transmitted parasites could not therefore be due to profound genetic differences between *G. turnbulli* individuals. Transmission events in this system are experimentally tractable because individual parasites move between hosts during social contact (Scott and Anderson, 1984; Johnson *et al.*, 2011). We also know that guppies differ in their resistance to *Gyrodactylus* spp. (Scott and Anderson, 1984; Madhavi and Anderson, 1985); infection loads are highly variable between hosts, with some hosts showing marked declines in infection load 9-12 days post-infection, possibly eliminating the parasite altogether, indicative of an effective immune response (Bakke *et al.*, 2007). Here, we illustrate the role of this variation among donors in driving heterogeneity in susceptibility and resistance among recipients, and hence parasite transmission across natural populations.

8.3 Materials and Methods

8.3.1 Fish origin and maintenance

The fish used during this study were laboratory-bred descendants of guppies, *Poecilia reticulata*, imported from the Lower Aripo River, Trinidad in June 2007. The fish were originally housed and bred at the University of Exeter, UK. In 2012, a subset of these fish was transported to Cardiff University, UK. In Cardiff, the fish were housed at low densities (15-20 fish per 70 L aquarium) at $25^{\circ}\text{C}\pm 1^{\circ}\text{C}$, on a 14h Light:10h Dark lighting schedule (overhead fluorescent lighting), and fed daily with live *Daphnia* and flake food (Aquarian®).

8.3.2 Experimental design

In order to test how variation between infected hosts ('donors') contributes to variation in the quantity and quality (inferred from the integral of each recipient's infection load over time) of transmitted parasites, we experimentally infected parasite-naïve donors, allowed them to develop natural variation in infection, and then exposed them to parasite-naïve conspecifics ('recipients'; Fig. 8.1). On Day 0 of the experiment, 60 female guppies (mean standard length [mm] \pm SE: 17.5 ± 0.4) were randomly selected as donors and infected with two *G. turnbulli* parasites each. On Day 1 each donor was screened to confirm its

TABLE 8.1: Definitions of susceptibility and resistance, hypothesised means by which donor heterogeneity could affect recipient susceptibility and resistance, and the data and models used to test those hypotheses. These data were recorded from all recipients, labelled ‘A’ in Fig. 8.1. Explanations of donor type (single or double) and donor day of infection (5 or 12) can be found in the main text. Models were simplified using backwards stepwise deletion of non-significant fixed effects to minimise Akaike’s Information Criteria (AIC); those that remained in the final model are denoted with an asterisk. Fixed effects were included as categorical (c) or numeric (n) variables.

Definition of key term	Hypothesis	Response variable(s)	Fixed effects	Random effect
Susceptibility: The probability and extent of recipient infection given contact with a donor.	1: Donor infection load drives heterogeneity in transmission speed and the quantity of parasites transmitted, and therefore recipient susceptibility.	The number of days until transmission occurred, and the count (log transformed) of parasites transmitted.	Main effects: donor type (c); donor integral (n); donor day of infection (c); fish length (n); donor infection load on the day the recipient was added (‘donor load’; n)*. Transmission speed model also included log donor load (n)* to account for nonlinearity. Two-way interactions: donor load \times recipient length; donor load \times donor type; donor load \times donor day of infection.	Donor identity, to control for the fact that some donors were given two opportunities to transmit.
Resistance: A host’s ability to inhibit the growth of the parasite and hence limit its infection load.	2: Donor infection load drives heterogeneity in the quality of parasites transmitted, and therefore recipient resistance.	The integral of each recipient’s infection load over time.	Main effects: donor type (c); donor integral (n); donor day of infection (c); fish length (n); infection load the recipient received from the donor (n). Two-way interactions: infection load the recipient received from the donor \times recipient length; infection load the recipient received from the donor \times donor integral.	

infection; any donors that had lost their infection were re-infected and the time reset to Day 0.

We tested whether there was an effect of the length of time the donor had been infected (early *versus* late) on the quantity and quality of parasites transmitted, and the speed of transmission. To this end, two time points were selected for exposure of naïve recipients to the donors: Day 5 and Day 12. On Day 5 in this system the parasite is established, but infection loads tend to be low and relatively uniform between hosts. On Day 12, however, infection loads are highly variable between hosts, with some hosts showing marked declines in infection load 9-12 days post-infection indicative of an effective immune response (see review by Bakke *et al.*, 2007). In order to control for the potentially confounding effect of a donor having transmitted before, we assigned donors to two groups: for ‘double donors’ ($n = 43$), a naïve recipient fish was added to the tank at both Day 5 and Day 12, whereas for ‘single donors’ ($n = 17$), one naïve recipient fish was added to the tank at Day 12 only (Fig. 8.1). At Day 5 ($n = 43$; double donors only) and Day 12 ($n = 60$; all donors), naïve female recipients were selected from stock, size matched within 2 mm (recipient mean standard length [mm] \pm SE: 17.5 ± 0.4) to each donor and added to the 1 L tanks. Each dyad was screened every 24 hours, and the number of *G. turnbulli* parasites on both donor and recipient recorded. If transmission had occurred, the recipient was isolated in a separate 1 L tank, its experimental time was set to Day 1, and it was screened every other day up to Day 30, or until the infection was lost. If no transmission was observed after four days, the recipient was removed from the experiment ($n = 8$). We used the integral of each recipient’s infection load over time to infer the quality of the parasites it received from its donor.

8.3.3 Experimental infections and parasite screening

The *G. turnbulli* strain (*Gt3*) used in this study was originally isolated from guppies from a pet shop in Nottingham, UK, in 1997. The *Gt3* laboratory culture is maintained on ornamental guppies (‘culture fish’). During experimental infections, culture fish were killed using an overdose of tricaine methanesulfonate (MS222; PHARMAQ UK, Ltd.). Experimental fish were anaesthetised with 0.02% MS222 and then transferred to a shallow petri dish containing dechlorinated water. Under a dissecting microscope and fibre optic illumination, the tails of the culture fish and experimental fish were placed in close proximity until two individual *G. turnbulli*, each pregnant with a mid-term embryo, had transmitted (Cable and Harris, 2002). Any additional parasites that transmitted were removed manually using watchmakers’ forceps. The same culture fish was used to infect 6 - 10 experimental fish before being replaced. Infected fish were revived

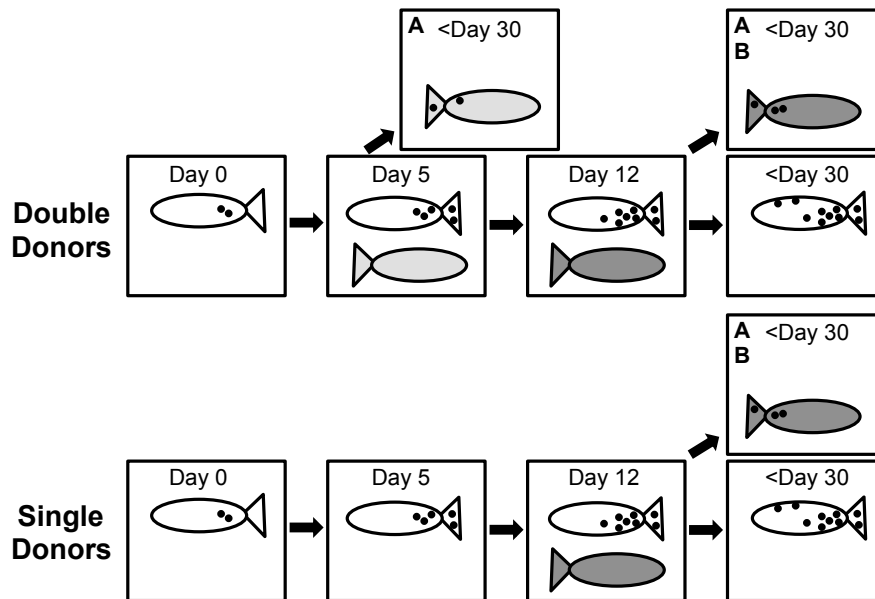


FIGURE 8.1: Diagram of the transmission experimental design. At Day 0, all donors (unshaded) were isolated and infected with two individual *Gyrodactylus turnbulli* (black dots). Their infection was monitored every other day for 30 days. At Days 5 (double donors only) and 12 (all donors), *G. turnbulli*-naïve recipients (light grey shading for Day 5, dark grey for Day 12) were added to the donor tanks. Both donor and recipient were screened for infection every 24 hours. Once a recipient had become infected, it was isolated and its infection monitored every other day for 30 days. **A:** Data from these recipients were used to test Hypotheses 1 and 2 (see Table 8.1). **B:** Data from these recipients were additionally used to test the *post hoc* hypothesis that transmission changes with donor experience.

and isolated in 1 L tanks. During this isolation, fish were maintained under standard conditions and fed flake food daily. Water in these individual tanks was changed every other day. All fish recovered from the infection procedure. Subsequent monitoring of the infection trajectory (‘screening’) involved briefly anaesthetising each fish (0.02% MS222) and counting the number of *G. turnbulli* present (Fig. 8.1).

8.3.4 Ethical note

This work was conducted under the UK Home Office license (PPL 30/2876) with approval by the Cardiff University Animal Ethics Committee. As described above, during the course of this experiment, donors and recipients were subjected to social isolation and parasite infection. Although social isolation is likely to have temporarily elevated their stress levels, previous work using these methods indicates that fish show no sign of having suffered lasting harm. To mitigate the effects of this isolation, fish were held in close proximity in transparent tanks; they were therefore in visual contact with conspecifics throughout. We monitored infected fish carefully and removed those that

were exhibiting severe pathology (fused fin rays, difficulty swimming, loss of appetite Cable, 2011). These fish were either treated immediately or killed, if a full recovery was deemed unlikely by an experienced observer.

8.3.5 Data Analysis

All statistical analyses were conducted in R (3.0.2; R Core Team, 2013). Table 8.1 gives an overview of the tests used. To test how variation between donors affected the quantity (count, log-transformed) of parasites transmitted, we used a general linear mixed model in the *lme4* package (GLMM; Gaussian error family and identity link function; Bates *et al.*, 2014). We included donor identity as a random factor to control for the fact that some donors had two opportunities to transmit their infection. As fixed factors, we included: donor type (single *versus* double); donor infection load on the day the recipient was added; donor infection integral (the integral of infection load over time following Adelman *et al.*, 2013); fish length; whether the donor was at Day 5 or 12 of infection; and two-way interactions. We used a second GLMM, again in *lme4* (Gaussian error family and identity link function; Bates *et al.*, 2014), with the number of days to transmission as the response variable, to test how donor heterogeneity affected the speed at which transmission occurred. In addition to those listed above, we included the log of donor infection load on the day the recipient was added as a fixed effect to account for nonlinearity. The models were simplified using stepwise deletion of non-significant terms to minimise Akaike's Information Criterion (AIC).

We tested whether the quality of the infection transmitted was affected by variation between donors, or the length of the donor's infection (early *versus* late), by calculating the infection integral of each recipient. We used the recipient integral as the response variable in a GLMM, again in the *lme4* package (Gaussian error family and identity link function; Bates *et al.*, 2014). Donor identity was again included as a random factor. Fixed factors included: the infection load the recipient received from the donor; donor integral; whether the donor was at Day 5 or 12 of infection; donor type (single *versus* double); fish length; and two way interactions. The same model simplification procedure was employed as above.

To test for a potential effect of donor experience of transmission on parasite transmission, we used only the data from the recipients added at Day 12. We used a general linear model (GLM; Gaussian error family and identity link function), with the proportion of its parasites a donor transmitted to the recipient as the response variable. Donor type (single *versus* double), donor infection load on the day the recipient was added,

fish length and two-way interactions were included as fixed factors. The same model simplification procedure was employed as above.

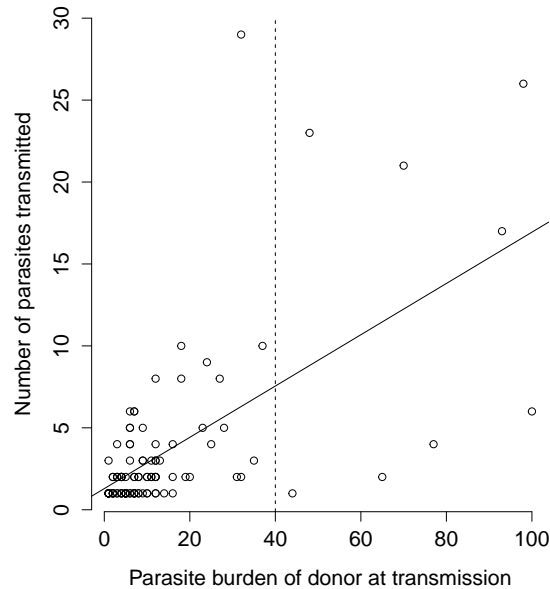


FIGURE 8.2: The number of parasites a donor transmitted depended on its infection load at transmission, with more heavily infected donors transmitting more parasites. The solid line is the regression of the parasite burden of the donor at transmission on the number of parasites transmitted, and the dashed line highlights an apparent ‘transmission threshold’ of 40 parasites. The number of parasites transmitted by donors above this threshold was significantly more variable than that transmitted by those below. These data are those collected from all recipients (labelled ‘A’ in Fig. 8.1), and were used to test Hypothesis 1 (see Table 8.1).

8.4 Results

Heterogeneity in the infection load of infected hosts (‘donors’) drives heterogeneity in parasite transmission to uninfected hosts (‘recipients’). In support of Hypothesis 1 (Table 8.1), The more heavily infected a donor on the day on which a recipient was added to its tank, the more parasites it transmitted ($F_{1, 86.3} = 55.29$, $p < 0.0001$; Fig. 8.2), and the faster transmission occurred (log donor infection load: $F_{1, 91.9} = 14.36$, $p = 0.0002$; Fig. 8.3). Our data suggest a ‘transmission threshold’ of *ca.* 40 parasites; transmission took longer than one day in 12.5% of trials above this threshold, compared to 55.7% of trials below (Fig. 8.3). The number of parasites transmitted by donors above this threshold was significantly more variable than that transmitted by those below (variance test: $F_{38, 63} = 2.44$, $p = 0.002$; Fig. 8.2), suggesting a role for factors other than infection load in determining the number of parasites transmitted by heavily

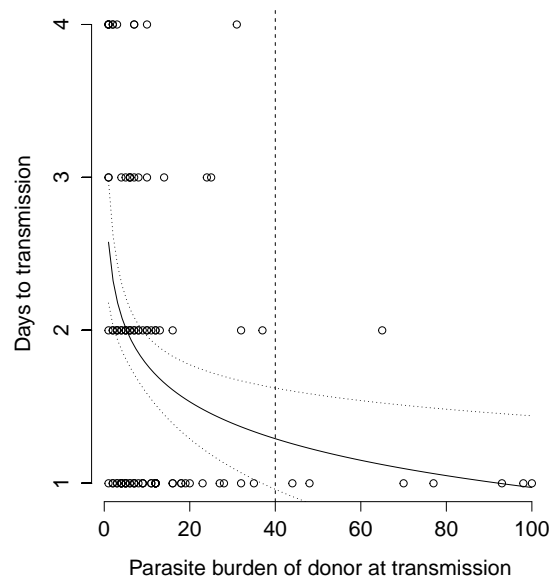


FIGURE 8.3: The speed of transmission depended on the infection load of the donor at transmission, with more heavily infected donors transmitting parasites more quickly. The solid line shows the values predicted by the final model (see text for details), and the dotted lines the 95% confidence intervals around the predictions. The dashed line highlights an apparent threshold of 40 parasites, above which transmission rarely took longer than one day. These data are those collected from all recipients (labelled ‘A’ in Fig. 8.1), and were used to test Hypothesis 1 (see Table 8.1).

infected hosts. Interestingly, despite a wide range of donor infection loads (range = 1 – 100) and transmitted infection loads (range = 1 – 29), we found no evidence that donor infection load explained any of the variation in parasite quality, inferred from the recipient infection integral (Hypothesis 2 in Table 8.1; Fig. 8.4). Parasite quality was not correlated with the quantity of parasites transmitted (Fig. 8.4), the speed of transmission, or the length of time the donor had been infected (Day 5 or 12; all $p > 0.05$). We additionally found that donors gave recipients a significantly higher proportion of their parasites on Day 12 if they had previous transmission experience ($F_{1,54} = 5.44$, $p = 0.023$; Fig. 8.5). In summary, while donor infection load and transmission experience impact the recipient’s susceptibility, over the course of a recipient’s infection this heterogeneity appears unimportant in determining its resistance and therefore contribution to onward parasite transmission.

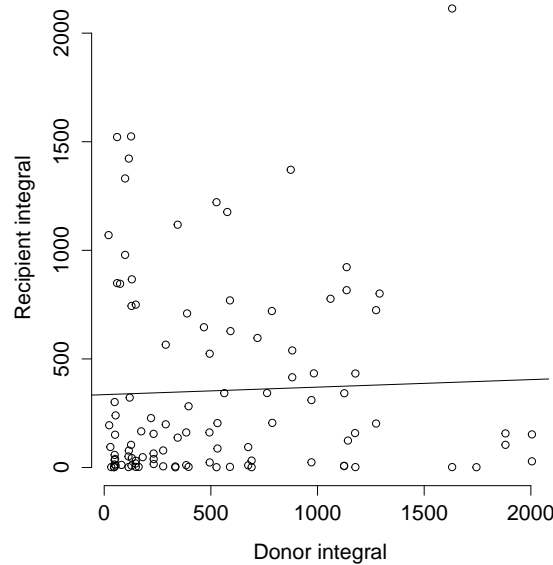


FIGURE 8.4: The integral of a donor's infection did not affect the integral of the recipient's infection, indicating that the quality of transmitted parasites was not dependent on the original host's infection. The integral is the area under the curve when the number of parasites a host is carrying is plotted against time. The solid line is the regression of donor integral on recipient integral. These data are those collected from all recipients (labelled 'A' in Fig. 8.1), and were used to test Hypothesis 2 (see Table 8.1).

8.5 Discussion

Our results suggest that heterogeneity between infected hosts ('donors') drives variation in the susceptibility of uninfected hosts ('recipients'), and is hence likely to drive heterogeneities in parasite transmission in natural populations. Heavily infected donors transmitted higher infection loads to their recipients (Hypothesis 1 in Table 8.1; Fig. 8.2), and transmitted infection more quickly (Hypothesis 1 in Table 8.1; Fig. 8.3). Despite a wide range of donor infection loads and transmitted infection loads, we found no evidence that donor-mediated heterogeneity in parasite quality explained any of the variation in recipient resistance (Hypothesis 2 in Table 8.1; Fig. 8.4). Our results therefore suggest that while recipient susceptibility to the establishment of infection may be affected by from whom the infection is acquired, a recipient's resistance is more dependent on its own characteristics. These findings call for susceptibility and resistance as we define them here to be considered as separate components of the host-parasite interaction, and disease transmission.

Our data provide empirical evidence from a directly transmitted parasite that the

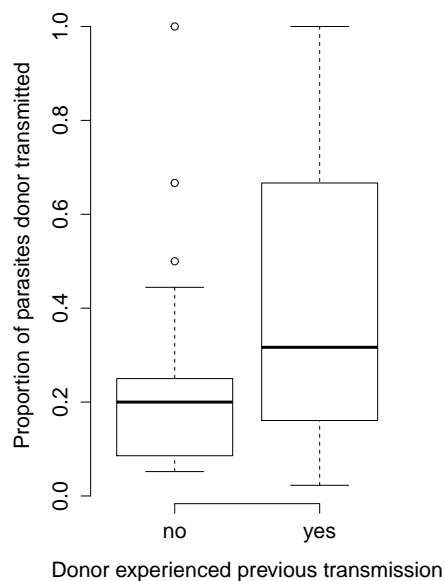


FIGURE 8.5: Donors with prior experience of transmitting an infection transmitted a significantly higher proportion of their parasites than those without experience after controlling for infection load. The data presented here are those collected from recipients added to donor tanks on Day 12 only, and the test is a comparison of double and single donors (labelled ‘B’ in Fig. 8.1), to test the *post hoc* hypothesis that transmission changes with donor experience.

infection load of a donor determines its infectiousness. Speed of transmission increased with donor infection load, but the relationship was not linear. This nonlinearity indicates that the increase in infectiousness with infection load is not simply a function of there being more parasites and thus a higher probability that some will transmit. Instead, it appears that the host-parasite interaction changes, encouraging parasites to transmit, once a certain infection load is reached. In our data, there appeared to be a threshold of *ca.* 40 parasites, above which transmission rarely took longer than one day. The existence of a threshold of infection load before rapid transmission occurs may be a common feature of *Gyrodactylus* spp. infections; Hendrichsen *et al.* (2015) found a similar pattern among Atlantic salmon infected with *G. salaris*.

The infection load transmitted by the donor also increased with donor infection load. Assumptions about this relationship pervade the infectious disease literature, but the little empirical evidence there is comes from single celled pathogens (Dwyer *et al.*, 1997; Schmid-Hempel *et al.*, 1999; Matthews *et al.*, 2006; Cobbold *et al.*, 2007), or from parasites with free-living transmission stages (Anderson *et al.*, 1978; Keymer and Anderson, 1979). Whilst the relationship we observed here is linear and positive (Fig. 8.2), at heavy infection loads (> 40) the variance is so large that the relationship

disintegrates. Donors with heavy infection loads are likely to fall into two categories: those that are tolerant of the parasites (that is they do not limit the infection but reduce or offset its fitness costs; Råberg *et al.*, 2009), and those that lack resistance to the parasite (that is they are unable to limit the infection or reduce its costs; Råberg *et al.*, 2009). Heavily infected, tolerant donors would be able to maintain behaviour promoting transmission. Donors lacking resistance, however, are likely to display sickness behaviours (Hart, 1988; Lloyd-Smith *et al.*, 2004), preventing them from interacting with the recipient to transmit infection, and providing a cue by which the recipient may avoid the donor (Chapter 6). Alternatively, tolerant individuals may be ideal hosts for the parasites, and they may therefore be less likely to leave, whereas those lacking resistance may mount an immune response or lack resources, encouraging parasites to transmit (Roy and Kirchner, 2000; Seppälä *et al.*, 2008b). That parasites leave the donor to avoid unfavourable conditions before they sustain damage potentially explains why we failed to find any evidence of donor-mediated variation in parasite quality (Scott and Anderson, 1984). We have no data on the tolerance of our experimental fish to their infection load, but how tolerance influences infectiousness is likely to be a fruitful, and as yet unexplored, research direction.

Additionally, we found evidence that donors that had transmitted before transmitted a significantly higher proportion of their parasites to naïve recipients than those that were transmitting for the first time, irrespective of infection load (Fig. 8.5). This indicates, for the guppy-gyrodactylid system at least, that a donor's ability to transmit parasites improves with the number of successful transmissions it makes. The number of transmission events a donor is involved in will depend on the rate at which it contacts other individuals; contact rate is highly heterogeneous in natural populations, with some individuals having much greater contact rates than others (May and Anderson, 1987; Woolhouse *et al.*, 1997; Lloyd-Smith *et al.*, 2004, 2005; Bansal *et al.*, 2007; Clay *et al.*, 2009). These highly connected individuals may give rise to 'super-spreading' events that can perpetuate epidemics (Galvani and May, 2005; Lloyd-Smith *et al.*, 2005; Small *et al.*, 2006; Stein, 2011). Theoretical work indicates that super-spreaders do not necessarily differ from the rest of the population in their infection characteristics (although this is common; Stein, 2011), but can arise simply due to their being highly connected (Small *et al.*, 2006). Despite the obvious importance of these super-spreaders, the present study is, to our knowledge, the first to quantify how the infection transmitted changes through sequential contacts; previous studies using a 'contact tracing' approach have considered the contact outcome to be binary (i.e. transmission or no transmission; Eames and Keeling, 2002; Lloyd-Smith *et al.*, 2005; Clay *et al.*, 2009; Paull *et al.*, 2012). Although we only compared donors that had transmitted infection once to those that had transmitted twice, our result suggests that sequential transmission events may increase

the proportion of its parasites that a donor will transmit. Whether this trend would continue over the course of further contacts and the mechanism involved require further investigation, but we suggest that changes in the donor's behaviour may be involved.

Variation in donor behaviour as a result of infection can alter its likelihood of transmitting (Lloyd-Smith *et al.*, 2004; Hampson *et al.*, 2009); in our system donors gain both therapeutic and ecological benefits from transmission so it is likely they will modify their behaviour to do so when possible. Indeed, infected guppies often swim in close proximity to others and attempt to initiate body contact, putatively in an attempt to rid themselves of the parasite (Croft *et al.*, 2011; JFS personal observation). Donors reduce their own infection load through transmission, at least temporarily, and may therefore gain respite from parasite-induced morbidity. The ecological benefit of transmission is clear: hosts will increase their relative fitness by transmitting to shoalmates because the more group members that are infected, the less detrimental infection becomes during intra-group competition. Infected members of a group are more vulnerable to predation (Seppälä, 2004), worse at competing for mates (Kennedy *et al.*, 1987; Worden *et al.*, 2000), and sustain increased physiological costs (Kennedy *et al.*, 1987; Kolluru *et al.*, 2009) relative to uninfected group members. These therapeutic and ecological rewards of transmission may encourage learning, which would explain our result that prior experience improved the donors' proficiency at transmission. The present study was designed explicitly to limit the recipient's ability to employ a full suite of avoidance behaviour but in more natural settings this may mitigate the infection to some extent, as has been demonstrated both theoretically (Gudelj and White, 2004; Bansal *et al.*, 2007) and empirically (Daly and Johnson, 2011; Johnson and Hoverman, 2014). It is also possible that changes in the host-parasite interaction and the demography of the infection between donors that transmitted once and twice resulted in the increased proportion of their infection load they transmitted with prior experience (Bakke *et al.*, 2007; Hendrichsen *et al.*, 2015). Further experimental work is required to elucidate the mechanism behind this intriguing result.

In conclusion, these results indicate that heterogeneity in infection load and transmission experience among donors leads to heterogeneity in disease transmission. Heavily infected donors transmit infection more quickly and transmit more parasites; they are therefore likely to contribute disproportionately more to onward epidemics than other donors. This may be particularly true in natural settings where host to host contact is fleeting and transmission is risky for *Gyrodactylus* spp. individuals: about 60% of parasites leaving the donor fail to infect a recipient (Scott and Anderson, 1984). Although a single gyrodactylid parasite is sufficient to establish an infection, the more individuals that attempt to transmit, the higher the probability of one finding a host, roughly

analogous to the ‘infective dose’ of single celled pathogens (Dwyer *et al.*, 1997; Ben-Ami *et al.*, 2010; Schmid-Hempel, 2011). Donor heterogeneity is therefore important in determining the susceptibility of recipients to infection establishment. Importantly, however, how resistant a recipient is appears ultimately unrelated to the characteristics of the donor from whom it acquired infection, but will determine how heavy its infection becomes and how long it lasts, and therefore the recipient’s role in an ongoing epidemic.

8.6 Authorship Acknowledgement

The manuscript resulting from this chapter is authored by:

Jessica F. Stephenson, Jordan Fox (Cardiff University), Kyle A. Young (Aberystwyth University), Joanne Cable (Cardiff University) and Sarah E. Perkins (Cardiff University).

8.7 Contribution to and position within the thesis

This chapter provides evidence that the more *Gyrodactylus turnbulli* a guppy is infected with, the more likely it is to transmit these parasites to conspecifics. Importantly, from whom an infection is acquired does not predict how an individual’s infection will develop, and therefore its role in a ongoing epidemic. In next chapter I discuss the key findings of this and all previous chapters, and indicate how future work could usefully address outstanding questions about how sensory and community ecology, through their impacts on social behaviour, affect disease dynamics in natural populations.

Chapter 9

General Discussion

This thesis is essentially composed of two related parts, each investigating a different force important in the interaction between the guppy, *Poecilia reticulata* and its gyrodactylid parasites: the ecological context of the host-parasite interaction, and the sensory ecology of the host. Here I indicate future research directions that could build on the salient findings of this thesis to further elucidate the role of each of these forces in disease ecology in natural systems.

9.1 Ecological context

As well as the interaction between a host and parasite, interactions with other species in natural communities are likely to affect disease dynamics. The first part of this thesis explores the effects of one such community interaction: predation. Selective predation of *Gyrodactylus* spp.-infected guppies represents a direct effect of predators on the parasites of their prey, and my results indicated that due to their smaller size and reduced ability to tolerate infection, males are more prone than females to *Gyrodactylus* spp. parasite-induced vulnerability to predation (Chapter 2). While these direct effects of predators likely contribute to the observed patterns in *Gyrodactylus* spp. parasitism in wild guppy populations, trait-mediated indirect effects of predators represent an additional mechanism. Predators have driven divergence in guppy life history strategies between upper and lower courses, and between males and females: females and upper course guppies are longer-lived than males or lower course guppies. Theory and empirical data from other systems predict that these longer-lived hosts should invest more in defence against parasites. In support of this prediction, I present evidence indicating that females and upper course guppies lose less body condition than males and lower course guppies

as a result of *Gyrodactylus* spp. infection in natural populations (Chapter 3). Predator-driven divergence in a second trait, shoaling, through increasing the rate of contact and thus *Gyrodactylus* spp. transmission between hosts, may drive higher prevalence among females relative to males in lower course populations, and among lower course relative to upper course populations (Chapter 4). Here I discuss future directions at different scales: first at the level of the host-parasite interaction, second the effect of predators on this interaction, and finally a broader scale investigation at the community level.

One of the key suggestions I make in this thesis is that host tolerance, the ability to mitigate the fitness costs of a given infection, rather than resistance, the ability to limit parasite growth (Roy and Kirchner, 2000; Råberg *et al.*, 2009), is the more important metric of guppy defence against *Gyrodactylus* spp. parasites. This system provides an experimentally tractable opportunity to rigorously test this suggestion, and to investigate the relationship between these two methods of host defence against pathogens; currently it is unclear whether they are positively or negatively correlated (Råberg *et al.*, 2009; Schmid-Hempel, 2011; Råberg, 2014). Previous work indicates that the major histocompatibility complex (MHC) genes are important in determining guppy resistance to *Gyrodactylus* spp. parasites (Fraser and Neff, 2010; Fraser *et al.*, 2010). Although more work is required to fully elucidate the genomic architecture of resistance, future studies should also consider that of tolerance. Beyond genetic factors, epigenetic factors such as parental effects are likely to be important in determining host defence against pathogens, be that tolerance or resistance, as has been found in other systems (Coltman *et al.*, 2001; Little *et al.*, 2003).

This thesis has focused on the ways in which predator-driven differences in host traits have affected the relationship of the host with its parasites, but this is just one side of the story. Future work should investigate how divergent host traits may impose selection on parasite traits, such as virulence (Coors and De Meester, 2010; Little *et al.*, 2010). The guppy-*Gyrodactylus* spp. system provides an excellent opportunity to test hypotheses about parasite adaptation to host ecology. A classic paradigm in disease ecology is the virulence-transmission trade-off; parasites that are highly virulent risk killing the host, and therefore themselves, unless they are able to transmit to a new host (Schmid-Hempel, 2011). This paradigm informs the prediction that the increase in transmission opportunities driven by predators, or by anthropogenic changes in host sensory environment as discussed below, should lead to increased virulence in *Gyrodactylus* spp. parasites. Variation in host tolerance and condition will also affect parasite traits (Seppälä *et al.*, 2008b; Coors and De Meester, 2010; Råberg, 2014), and as tolerance appears to be sex dependent in the guppy (Chapter 3), traits may differ between parasites infecting males and females (Fellous and Koella, 2009; Duneau and Ebert, 2012; Cousineau and Alizon, 2014). Controlled comparisons of parasites infecting

the sexes, and populations of guppies experiencing these divergent biotic and abiotic factors therefore has the potential to address outstanding questions in evolutionary parasitology.

By considering the interaction between parasites and predators, this thesis has made steps to move away from the traditional consideration of the host-parasite interaction in isolation, but this work is still far from a holistic consideration of the impact of community interactions on disease ecology. For example, interactions with other parasites may change how guppies respond to *Gyrodactylus* spp. (Johnson and Hoverman, 2012). As the prevalence of other multicellular parasites is very low in natural guppy populations (Appendix A), future work may consider the role of microbial pathogens and symbionts in mediating this interaction. Host microbiota, the microorganism community inhabiting the host, may determine the nature of its interaction with other classes of parasite (Koch and Schmid-Hempel, 2012). Although research has recently focused on the gut microbiota of teleosts including the guppy (Llewellyn *et al.*, 2014; Sullam *et al.*, 2015), the microbiota of the epithelial mucous will have a more direct impact on the interaction between teleost hosts and their ectoparasites, and has received far less attention (Llewellyn *et al.*, 2014). Incorporating this additional level of complexity into the predator-guppy-gyrodactylid system would enable researchers to address important questions. For example, an intriguing possibility is that microbiota may be transmitted during social contacts. Additionally, *Gyrodactylus* spp. parasites may vector microbiota between hosts: theoretical and empirical work suggests this is possible and may be important (Perkins and Fenton, 2006; Lacharme-Lora *et al.*, 2009; Kreisinger *et al.*, in press).

9.2 Sensory ecology

In the second part of this thesis, I investigated how individuals combine information about their surroundings and conspecifics from the chemical and visual sensory systems. I first demonstrated that chemical cues provide guppies with the context necessary to respond appropriately to ambiguous visual cues (Chapter 5). Given the proven importance of both visual and chemical cues to guppies, and that avoiding those infected with a directly transmitted disease may prevent an individual from contracting an infection, it is unsurprising that both senses are important in detecting *Gyrodactylus turnbulli* infection in conspecifics (Chapter 6). However, the chemical cues guppies use to avoid infected conspecifics appear not to elicit innate recognition; juvenile guppies can imprint on the cues of adults experiencing disease caused by *G. turnbulli*, and choose to associate with these cues in adulthood (Chapter 7). The mechanism behind the

avoidance of infection behaviour may therefore simply be that guppies avoid individuals that emit cues that are abnormal. Whatever the mechanism, avoidance of infected conspecifics only occurs during the later stages of their infection (Chapters 6 & 7). This finding indicates that heterogeneity in infectiousness during the course of infection may mean that the benefits of sociality are only outweighed by the cost of associating with diseased conspecifics when infectiousness is high. Certainly, the transmission experiment presented in Chapter 8 showed that the onset of avoidance coincides with the stage of infection at which conspecifics are most infectious. Based on these findings, it is clear that the sensory ecology of the host could strongly affect disease transmission in natural populations.

Identifying the chemistry of the cues of *G. turnbulli* infection that guppies respond to would help elucidate whether they are specific to this infection, and whether a change in concentration or composition is responsible for the apparent threshold in avoidance behaviour we observed (De Moraes *et al.*, 2014). The change in the cues of infection may represent a ‘tipping point’ in the infection; it is possible that the parasites themselves are using cues from the host to determine when to begin transmission (Stephenson, 2012). Knowledge of cue chemistry would facilitate direct tests of this, and the other hypotheses laid out in Chapter 6 and 7 that either the host and parasite are collaboratively suppressing the cues of disease, or that the receiver of the cues is balancing the benefits of sociality with the risk of transmission.

Pollution and turbidity caused by anthropogenic disturbance mean that pristine freshwater bodies are becoming increasingly rare; the resulting changes in sensory environment have implications for the evolutionary ecology of the communities occupying these habitats (van der Sluijs *et al.*, 2011). Turbidity reduces the utility of vision, and pollution can reduce or eliminate a fish’s ability to detect and respond to olfactory or visual cues (Dutta *et al.*, 1992; Hartman and Abrahams, 2000; van der Sluijs *et al.*, 2011; Elvidge *et al.*, 2012; McIntyre *et al.*, 2012; Lonnstedt *et al.*, 2013). The impact of these changes on fish sensory ecology is therefore likely to be marked and may well lead to differentiation between populations under different conditions (Boughman, 2002; Tezuka *et al.*, 2014), or degradation of forces that could lead to differentiation (Candolin *et al.*, 2007; Wong *et al.*, 2007; Vonlanthen *et al.*, 2012). Compensation and overlap between the olfactory and visual sensory systems enables some fish to overcome the difficulties posed by anthropogenic alterations in their sensory environment, but this research has largely focused on mate choice, foraging and antipredator behaviours (Chapman *et al.*, 2010; van der Sluijs *et al.*, 2011; Elvidge *et al.*, 2012; McIntyre *et al.*, 2012; Lonnstedt *et al.*, 2013). Tests using the guppy-*Gyrodactylus* spp. system could elucidate how changes in sensory environment may affect disease transmission in natural populations. Turbidity reduces key measures of social behaviour in guppies from northern Trinidad (Borner

et al., 2015), with potentially important implications for disease transmission (Wilson *et al.*, 2014). Streams here generally run clear, however; a comparison of how changes in turbidity and pollution affect social behaviour and disease transmission among guppies from these typically clear northern streams to those from southern, consistently more turbid streams could elucidate how host and parasite may respond to chronic and acute changes in sensory conditions. These experiments would essentially manipulate the amount of information available to uninfected hosts about infection in their immediate social group and as such have relevance to disease transmission across host-parasite systems. How these processes affect disease dynamics across social networks and hence populations could have fundamental implications for epidemiology.

Appendix A

Supplementary figures and tables of data presented in Chapter 4

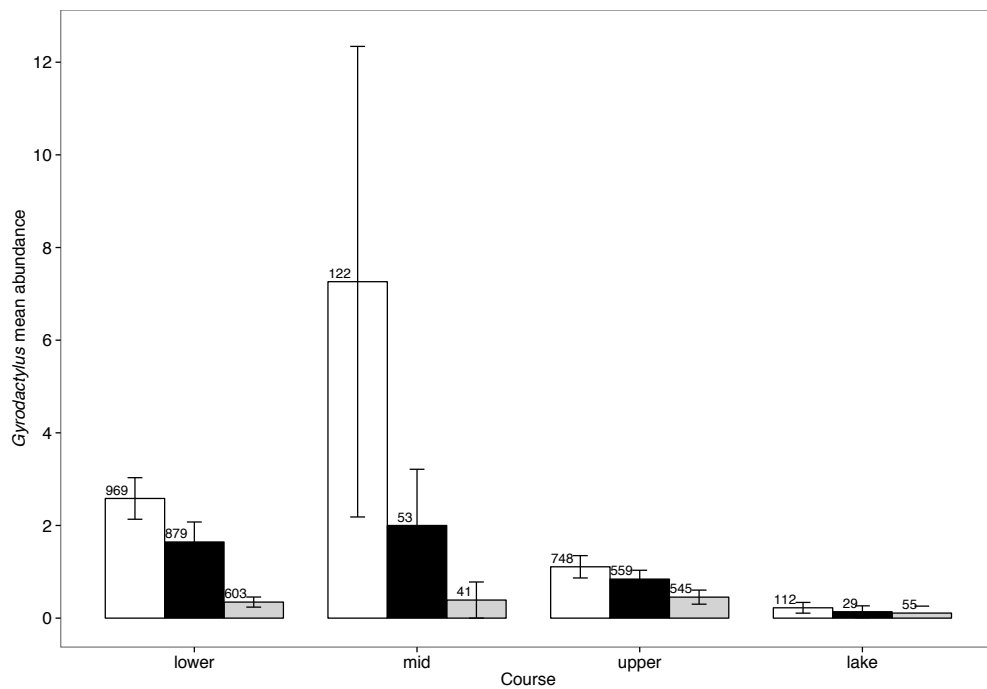


FIGURE A.1: *Gyrodactylus* spp. mean abundance in fish from the lower, mid and upper courses and the Pitch Lake ('lake'). White bars are data from females, black from males, and gray from juveniles. Error bars are the 95% confidence intervals. The numbers give the total number of fish that contributed to each bar.

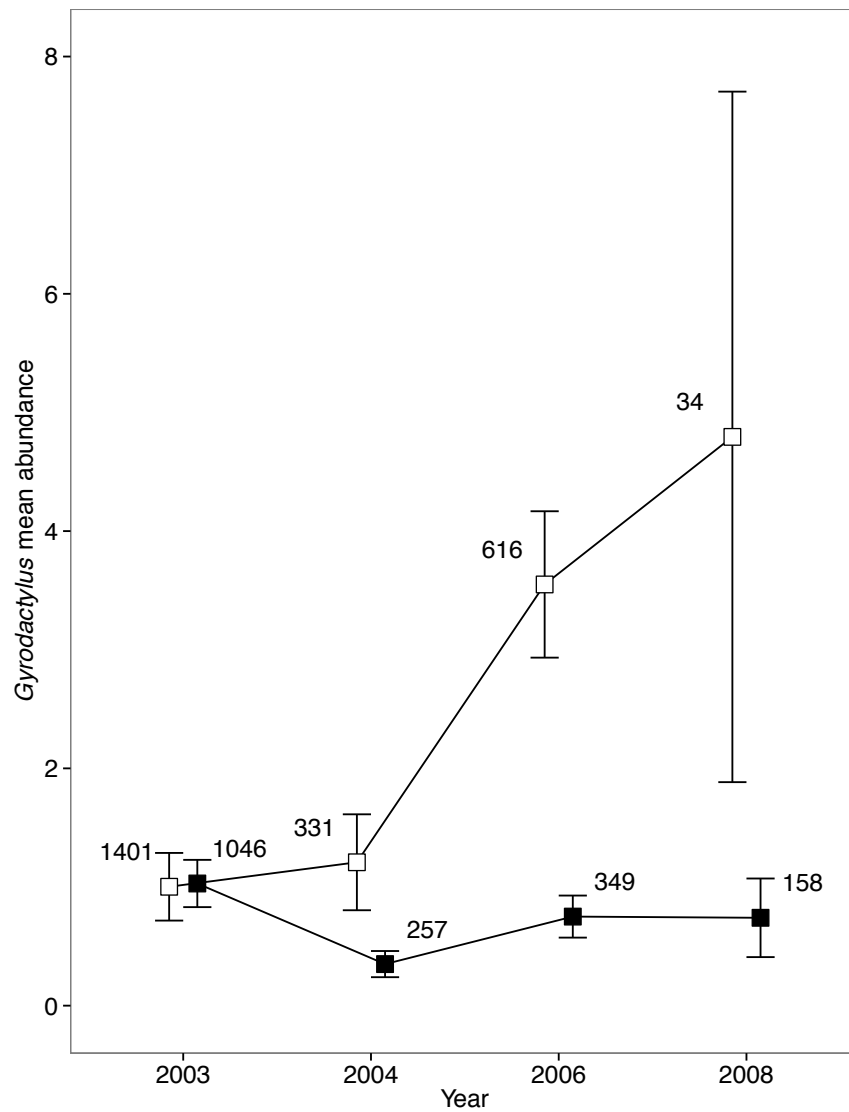


FIGURE A.2: Change in *Gyrodactylus* spp. abundance over the years sampled in this study. White squares represent data from fish from lower course sites and black squares are fish from the upper. The data are the means from all fish sampled and the error bars are the 95% confidence intervals around those means. The numbers on the plot give the overall number of fish contributing to each data point.

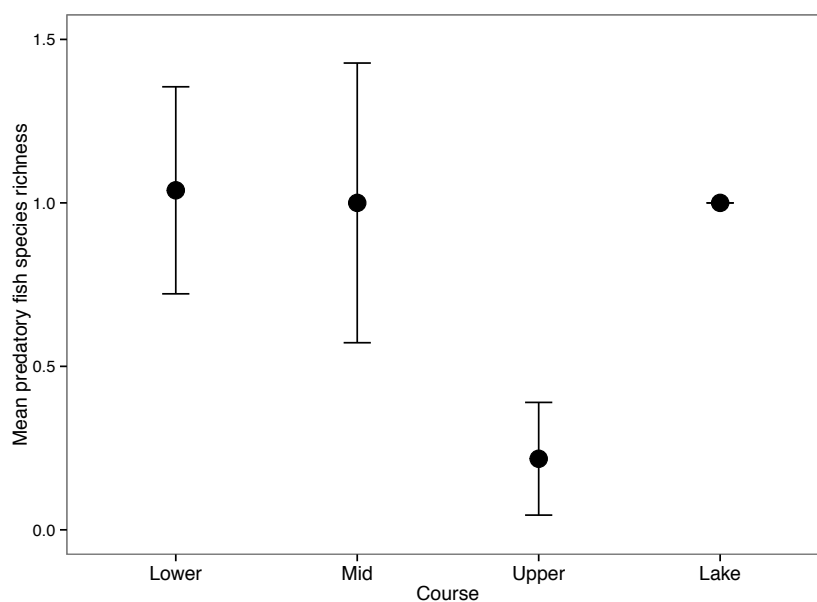


FIGURE A.3: Mean predatory fish species richness (with 95% confidence intervals) recorded at each site in each of the four ‘course’ categories. The four predator species included in the richness score (*Polycentrus schomburgkii*, *Crenichla alta*, *Hoplias malabaricus* and *Gobiomorus dormitor*) were those we recorded that had a trophic level of greater than 3.5 (Fishbase; www.fish-base.org), which indicates they are generally ichthyophagous (see Chapter 4).

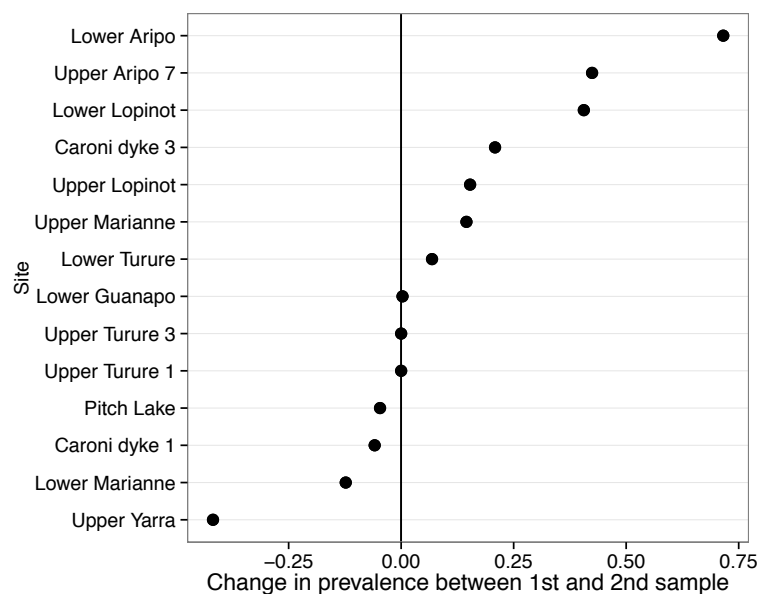


FIGURE A.4: Change in *Gyrodactylus* spp. prevalence between the first and second time a site was sampled. The sites shown are the only ones from the sample that were visited more than once, and more details are given about them in Table A.1

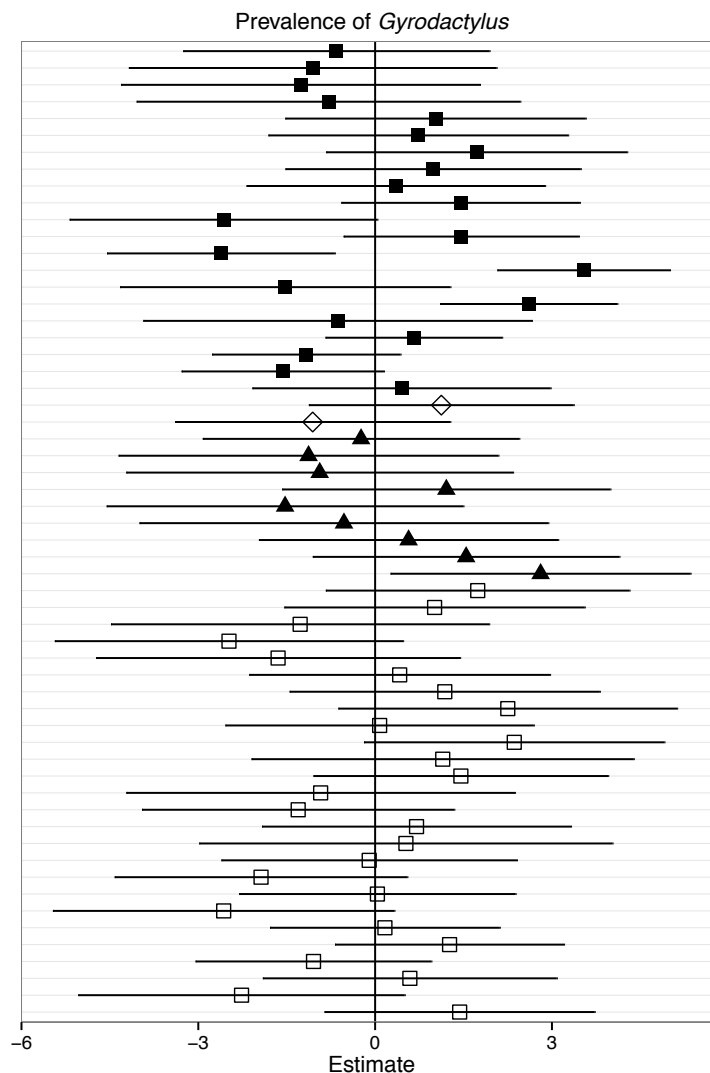


FIGURE A.5: Variation between sample sites in likelihood of infection with *Gyrodactylus* spp. Each data point represents a single sample site: black squares are sites in the upper courses of the rivers; white diamonds are sites in the Pitch Lake; black triangles are mid-river sites; white squares are lower course sites. The vertical line represents the overall mean, thus sites for which the 95% confidence interval error bars do not overlap this line are significantly different from the mean. The lack of a consistent difference between courses may seem counterintuitive given the fact that we found an important difference between the courses by modeling course as a fixed effect. We can reconcile this result because the random effect tests for overall sources of variation, and the ‘course’ term in the random effect is actually ‘site nested within course’ and has 42 levels. Individual sites ($n=62$) are more variable than sites nested within course, but overall the fixed course effect (with 4 levels) shows us that there is a strong, consistent pattern between courses. The random effect shows that besides a true course effect a lot of variation remains, indicating spatial heterogeneity between sites.

TABLE A.1: Summary of the dataset.

Drainage	River	Course	Site	Years Sampled	UTM (20P)		Sample size	<i>Gyrodactylus</i> spp. prevalence	<i>Gyrodactylus</i> spp. mean intensity (on infected fish)	Other symbionts present (prevalence)	
					GPS E	GPS N					
Caroni	Arima	Lower	1	2006	689982	1173762	50	0	-	Fungus (0.020)	
			2	2003	689016	1176837	53	0.84	6.69		
		Mid		2003, 2006	687034	1179246	69	0.58	24.15	<i>Trichodina</i> (0.145), <i>Ichthyophthirius</i> (0.029), Fungus (0.014)	
		Upper		2003	687672	1178320	64	0.14	2.44		
Aripo		Lower		2003, 2006, 2008	694410	1177783	291	0.40	5.44	<i>Trichodina</i> (0.007), Fungus (0.003), Digenean (0.034)	
			Upper	1	2006, 2008	694030	1182128	73	0.08	1.00	Fungus (0.028)
				2	2008	692771	1182303	30	0.38	3.90	
				3	2006, 2008	693230	1182328	145	0.67	1.10	<i>Trichodina</i> (0.076), Fungus (0.069)
			4	2006	693221	1181917	56	0.07	2.64		
			5	2003	693277	1181843	4	0.00	-		
			6	2003	?		56	0.50	1.54		
			7	2003	?		24	0.43	2.50		
			8	2003	693277	1181843	102	0.62	3.29	Fungus (0.008)	
			9	2006	693823	1180613	48	0.02	2.00	<i>Ichthyophthirius</i> (0.104)	
Caura		Lower		2003	678436	1177236	227	0.00	-		
			Upper	1	2003, 2004	699539	1182054	153	0.24	1.81	Fungus (0.007)
				2	2003	679627	1182692	110	0.00	-	
				3	2003	679757	1182475	77	0.19	1.47	
Dyke		Lower	1	2003, 2004, 2006	674748	1177894	92	0.10	26.56	<i>Trichodina</i> (0.022), Fungus (0.022)	
			2	2003, 2006	675080	1177892	184	0.61	4.71	<i>Trichodina</i> (0.016), Digenean (0.016)	
			3	2003, 2004	674869	1177433	177	0.21	1.81	<i>Trichodina</i> (0.751)	
Guanapo		Lower		2003, 2004, 2006	691385	1174569	232	0.23	2.68	<i>Trichodina</i> (0.103)	
			Mid		2003	691815	1176691	20	0.20	4.00	
			Upper		2003	690247	1182015	91	0.11	1.80	
Lopinot		Lower		2003, 2004, 2006	683553	1175663	453	0.60	5.00	Digenean (0.002)	
			Mid		2006	684127	1156566	30	0.40	1.50	Fungus (0.024)
			Upper		2003, 2004, 2006	683520	1182443	187	0.38	2.80	
Maracas		Upper		2003	671265	1183604	145	0.44	9.05		
Coffee	Coffee	Lower		2009	651072	1132467	41	0.00	-	Fungus (0.024)	
Cunupia	Dyke	Lower	1	2006	678485	1168663	22	0.23	1.00	<i>Trichodina</i> (0.500)	
			2	2006	673445	1165676	50	0.02	1.00	<i>Trichodina</i> (0.920), <i>Ichthyophthirius</i> (0.020)	

Table A.1 – continued from previous page

Drainage	River	Course	Site	Years Sampled	UTM (20P)		Sample size	<i>Gyrodactylus</i> spp. prevalence	<i>Gyrodactylus</i> spp. mean intensity	Other symbionts present (prevalence)
					GPS E	GPS N				
Guapo	Guapo	Lower	1	2006	646956	1124820	1	1.00	5.00	<i>Trichodina</i> (1.000)
			2	2006	646609	1125009	10	0.80	1.63	<i>Trichodina</i> (0.800), Digenean (0.300)
La Seiva	La Seiva	Lower		2006			61	0.08	1.40	<i>Ichthyophthirius</i> (0.016), Fungus (0.033)
Lizard	Lizard	Lower		2004	715789	1122959	12	0.00	-	
Marianne	Marianne	Lower		2003, 2004	685890	1193642	70	0.71	3.10	
			1	2006			4	0.00	-	
		2	2006			31	0.00	-	<i>Ichthyophthirius</i> (0.065), Fungus (0.032)	
		Upper		2003, 2004	685891	1192747	171	0.13	1.59	
Matura	Matura	Lower		2006	713012	1181533	49	0.39	1.47	Fungus (0.041)
Mayaro	Pilote	Lower		2004	711697	1121927	16	0.00	-	
	Tributary	Lower	1	2006	719160	1138112	20	1.00	10.50	<i>Trichodina</i> (0.950), Fungus (0.100), Digenean (0.05)
			2	2006	719153	1137295	20	0.95	16.89	<i>Trichodina</i> (0.800),
Nariva	Manzanilla	Lower		2006			3	1.00	1.33	
North East	Toco	Lower		2006	727327	1198431	3	0.00	-	
Oropuche	Oropuche	Lower		2006			45	0.60	1.85	
		Mid		2006	704386	1178808	4	0.50	2.50	
		Upper		2006			52	0.67	2.46	
	Turure	Lower		2003, 2004	700333	1178556	158	0.22	1.47	
Upper		1	2003			56	0.00	-		
		2	2006	700442	1183253	16	0.00	-		
			3	2003, 2004	700494	1180614	91	0.00	-	
Pitch Lake	Pitch Lake	Lake	1	2004, 2006	650309	1131471	118	0.03	1.00	<i>Trichodina</i> (0.009),
			2	2006	650309	1131471	78	0.27	1.52	<i>Ichthyophthirius</i> (0.423), Digenean (0.013)
	Visigney	Lower		2009	650110	1129992	28	0.11	1.00	Fungus (0.036)
Silverstream	Silverstream	Lower		2006	657517	1129995	25	0.00	-	
		Mid		2006	656792	1128331	11	0.00	-	<i>Trichodina</i> (0.091), Fungus (0.273), Digenean (0.273)
Tarouba	Tarouba	Mid		2006	659331	1130492	12	0.00	-	Digenean (0.333)
Vance	Vance	Mid		2006	649782	1127854	35	0.09	1.00	
		Upper		2009	649751	1127459	42	0.02	1.00	Fungus (0.024)
Yarra	Yarra	Lower		2003	680414	1194065	58	0.52	2.07	
		Upper		2004, 2006	683427	1189518	59	0.14	2.63	<i>Camallanus</i> (0.017)
TOTAL							4715			

Model	Degrees of Freedom	Log Likelihood Ratio	AIC	Δ AIC	AIC Weight
2/3/4/5/6/7/8	23	-4094.74	8235.49	0.00	0.69
1/2/3/4/5/6/7/8	24	-4094.56	8237.13	1.64	0.31

TABLE A.2: The top-ranked candidate models explaining variation in *Gyrodactylus* spp. abundance. AIC = Akaike information criteria value; the terms are coded as follows: 1: Presence of Trichodina; 2: Course; 3: Host class, 4: Year, 5: Weight, 6: Course:Host class, 7: Course:Weight; 8: Sex:Weight.

Predictor	Standardised coefficient	Unconditional SE	95% CI		Relative importance of overall predictor
			2.5%	97.5%	
(Intercept)	-0.561	0.873	-2.272	1.151	
Course-lower	1.239	0.899	-0.522	3.000	Course = 1
Course-mid	1.515	1.026	-0.495	3.525	
Course-upper	1.054	0.921	-0.751	2.859	
Host class-j	1.037	0.633	-0.203	2.277	Sex = 1
Host class-m	-0.023	0.290	-0.590	0.545	
Year-2004	0.059	0.064	-0.065	0.184	Year = 1
*Year-2006	0.801	0.067	0.669	0.933	
*Year-2008	1.095	0.169	0.764	1.426	
Weight	0.672	0.431	-0.173	1.517	Weight = 1
lower:j	-1.014	0.629	-2.247	0.219	Course:Sex = 1
*mid:j	-3.101	0.695	-4.463	-1.739	
upper:j	-1.141	0.634	-2.383	0.101	
lower:m	-0.111	0.295	-0.690	0.468	
mid:m	-0.349	0.396	-1.124	0.427	
upper:m	-0.062	0.303	-0.656	0.532	
lower:weight	-0.249	0.436	-1.103	0.605	Course:Weight = 1
mid:weight	0.578	0.524	-0.448	1.604	
upper:weight	-0.599	0.441	-1.463	0.264	
*j:weight	1.118	0.302	0.526	1.710	Sex:Weight = 1
m:weight	-0.066	0.154	-0.368	0.236	
Trichodina	0.068	0.113	-0.153	0.289	Trichodina = 0.31

TABLE A.3: Model averaged standardised coefficients, unconditional standard error, and 95% confidence intervals of model predictors for *Gyrodactylus* spp. abundance. Predictors for which the confidence intervals do not bound zero are denoted with an *.

Bibliography

- Adelman, J. S., Kirkpatrick, L., Grodio, J. L., and Hawley, D. M. (2013). House finch populations differ in early inflammatory signaling and pathogen tolerance at the peak of *Mycoplasma gallisepticum* infection. *The American Naturalist* **181**: 674–689.
- Alzaga, V., Vicente, J., Villanua, D., Acevedo, P., Casas, F., and Gortazar, C. (2008). Body condition and parasite intensity correlates with escape capacity in Iberian hares (*Lepus granatensis*). *Behavioral Ecology and Sociobiology* **62**: 769–775.
- Anderson, R. M., Whitfield, P. J., and Dobson, A. P. (1978). Experimental studies of infection dynamics: infection of the definitive host by the cercariae of *Transversotrema patialense*. *Parasitology* **77**: 189–200.
- Anderson, R. M. and May, R. M. (1981). The population dynamics of microparasites and their invertebrate hosts. *Philosophical Transactions: Biological Sciences* **291**: 451–524.
- Anstis, S., Hutahajan, P., and Cavanagh, P. (1998). Optomotor test for wavelength sensitivity in guppyfish (*Poecilia reticulata*). *Vision Research* **38**: 45–53.
- Arakawa, H., Cruz, S., and Deak, T. (2012). Attractiveness of illness-associated odorant cues in female rats is modulated by ovarian hormones, but not associated with pro-inflammatory cytokine levels. *Brain Behaviour and Immunity* **26**: 40–49.
- Archard, G. A., Cuthill, I., Partridge, J. C., and van Oosterhout, C. (2008). Female guppies (*Poecilia reticulata*) show no preference for conspecific chemosensory cues in the field or an artificial flow chamber. *Behaviour* **145**: 1329–1346.
- Archer, S. N., Endler, J. A., Lythgoe, J. N., and Partridge, J. C. (1987). Visual pigment polymorphism in the guppy *Poecilia reticulata*. *Vision Research* **27**: 1243–1252.
- Archer, S. N. and Lythgoe, J. N. (1990). The visual pigment basis for cone polymorphism in the guppy, *Poecilia reticulata*. *Vision Research* **30**: 225–233.
- Arendt, J. D., Reznick, D. N., and López-Sepulcre, A. (2014). Replicated origin of female-biased adult sex ratio in introduced populations of the Trinidadian guppy (*Poecilia reticulata*). *Evolution* **68**: 2343–2356.

- Aubert, A., Goodall, G., Dantzer, R., and Gheusi, G. (1997). Differential effects of lipopolysaccharide on pup retrieving and nest building in lactating mice. *Brain Behaviour and Immunity* **11**: 107–118.
- Bakke, T. A., Cable, J., and Harris, P. D. (2007). The biology of gyrodactylid monogeneans: the “Russian-doll killers”. *Advances in Parasitology* **64**: 161–460.
- Bansal, S., Grenfell, B. T., and Meyers, L. A. (2007). When individual behaviour matters: homogeneous and network models in epidemiology. *Journal of the Royal Society Interface* **4**: 879–891.
- Barber, I. and Huntingford, F. A. (1995). The effect of *Schistocephalus solidus* (Cestoda: Pseudophyllidea) on the foraging and shoaling behaviour of three-spined sticklebacks, *Gasterosteus aculeatus*. *Behaviour* **132**: 15–16.
- Barber, I., Downey, L. C., and Braithwaite, V. (1998). Parasitism, oddity and the mechanism of shoal choice. *Journal of Fish Biology* **53**: 1365–1368.
- Barber, I., Hoare, D. J., and Krause, J. (2000). Effects of parasites on fish behaviour: a review and evolutionary perspective. *Reviews in Fish Biology and Fisheries* **10**: 131–165.
- Barber, I., Walker, P., and Svensson, P. A. (2004). Behavioural responses to simulated avian predation in female three-spined sticklebacks: the effect of experimental *Schistocephalus solidus* infections. *Behaviour* **141**: 1425–1440.
- Barber, I. and Dingemanse, N. J. (2010). Parasitism and the evolutionary ecology of animal personality. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **365**: 4077–4088.
- Barson, N. J., Cable, J., and van Oosterhout, C. (2009). Population genetic analysis of microsatellite variation of guppies (*Poecilia reticulata*) in Trinidad and Tobago: evidence for a dynamic source-sink metapopulation structure, founder events and population bottlenecks. *Journal of Evolutionary Biology* **22**: 485–497.
- Bartoń, K. (2014). *MuMIn: Multi-model inference*. R package version 1.10.0. URL: <http://CRAN.R-project.org/package=MuMIn>.
- Bates, D., Maechler, M., Bolker, B., and Walker, S. (2014). *lme4: Linear mixed-effects models using Eigen and S4*. R package version 1.1-6. URL: <http://CRAN.R-project.org/package=lme4>.
- Behringer, D. C., Butler, M. J., and D., S. J. (2006). Avoidance of disease by social lobsters. *Nature* **441**: 421.

- Ben-Ami, F., Ebert, D., and Regoes, R. R. (2010). Pathogen Dose Infectivity Curves as a Method to Analyze the Distribution of Host Susceptibility: A Quantitative Assessment of Maternal Effects after Food Stress and Pathogen Exposure. *The American Naturalist* **175**: 106–115.
- Berejikian, B. A., Smith, R. J. F., Tezak, E. P., Schroder, S. L., and Knudsen, C. M. (1999). Chemical alarm signals and complex hatchery rearing habitats affect antipredator behaviour and survival of Chinook salmon (*Oncorhynchus tshawytscha*) juveniles. *Canadian Journal of Fisheries and Aquatic Sciences* **56**: 830–838.
- Berejikian, B. A., Tezak, E. P., and LaRae, A. (2003). Innate and enhanced predator recognition in hatchery-reared Chinook salmon. *Environmental Biology of Fishes* **67**: 241–251.
- Bergstrom, C. A. (2002). Fast-start swimming performance and reduction in lateral plate optnumber in threespine stickleback. *Canadian Journal of Zoology* **80**: 207–213.
- Bettini, S., Lazzari, M., Ciani, F., and Franceschini, V. (2009). Immunohistochemical and histochemical characteristics of the olfactory system of the guppy, *Poecilia reticulata* (Teleostei, Poeciliidae). *The Anatomical Record Part A: Discoveries in Molecular, Cellular and Evolutionary Biology* **292**: 1569–1576.
- Binning, S. A., Roche, D. G., and Layton, C. (2013). Ectoparasites increase swimming costs in a coral reef fish. *Biology Letters* **9**: 20120927.
- Blais, J., Rico, C., van Oosterhout, C., Cable, J., Turner, G. F., and Bernatchez, L. (2007). MHC adaptive divergence between closely related and sympatric African cichlids. *PLoS ONE* **2**: e734.
- Blake, R. W., Kwok, P. Y. L., and Chan, K. H. S. (2006). Effects of two parasites, *Schistocephalus solidus* (Cestoda) and *Bunodera* spp. (Trematoda), on the escape fast-start performance of three-spined sticklebacks. *Journal of Fish Biology* **69**: 1345–1355.
- Blasco-Costa, I., Koehler, A. V., Martin, A., and Poulin, R. (2013). Upstream-downstream gradient in infection levels by fish parasites: a common river pattern? *Parasitology* **140**: 266–274.
- Bolker, B., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., and White, J.-S. S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution* **24**: 127–135.
- Boonstra, R., Hik, D., Singleton, G. R., and Tinnikov, A. (1998). The impact of predator-induced stress on the snowshoe hare cycle. *Ecology Monographs* **79**: 371–394.

- Borner, K. K., Krause, S., Mehner, T., Uusi-Heikkilä, S., Ramnarine, I. W., and Krause, J. (2015). Turbidity affects social dynamics in Trinidadian guppies. *Behavioral Ecology and Sociobiology* **69**: 645–651.
- Boughman, J. W. (2002). How sensory drive can promote speciation. *Trends in Ecology and Evolution* **17**: 571–577.
- Bouwman, K. M. and Hawley, D. M. (2010). Sickness behaviour acting as an evolutionary trap? Male house finches preferentially feed near diseased conspecifics. *Biology Letters* **6**: 462–465.
- Bowmaker, J. K. (2008). Evolution of vertebrate visual pigments. *Vision Research* **48**: 2022–2041.
- Brask, J. B., Croft, D. P., Thompson, K., Dablesteen, T., and Darden, S. K. (2012). Social preferences based on sexual attractiveness: a female strategy to reduce male sexual attention. *Proceedings of the Royal Society of London B: Biological Sciences* **279**: 1748–1753.
- Brooks, R. and Endler, J. (2001). Female guppies agree to differ: phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. *Evolution* **55**: 1644–1651.
- Brown, G. E. and Smith, R. J. F. (1998). Acquired predator recognition in juvenile rainbow trout (*Oncorhynchus mykiss*): conditioning hatchery-reared fish to recognize chemical cues of a predator. *Canadian Journal of Fisheries and Aquatic Sciences* **55**: 611–617.
- Brown, G. E., Godin, J.-G. J., and Pedersen, J. (1999). Fin-flicking behaviour: a visual antipredator alarm signal in a characin fish, *Hemigrammus erythrozonus*. *Animal Behaviour* **58**: 469–475.
- Brown, G. E. (2003). Learning about danger: chemical alarm cues and local risk assessment in prey fishes. *Fish and Fisheries* **4**: 227–234.
- Brown, G. E. and Magnavacca, G. (2003). Predator inspection behaviour in a characin fish: an interaction between chemical and visual information? *Ethology* **109**: 739–750.
- Brown, G. E., Poirier, J.-F., and Adrian Jr., J. C. (2004). Assessment of local predation risk: the role of subthreshold concentrations of chemical alarm cues. *Behavioural Ecology* **15**: 810–815.
- Brown, G. E., Macnaughton, C. J., Elvidge, C. K., Ramnarine, I. W., and Godin, J.-G. J. (2009). Provenance and threat-sensitive predator avoidance patterns in wild-caught Trinidadian guppies. *Behavioral Ecology and Sociobiology* **63**: 699–706.

- Brown, G. E., Elvidge, C. K., Macnaughton, C. J., Ramnarine, I. W., and Godin, J. G. J. (2010). Cross-population responses to conspecific chemical alarm cues in wild Trinidadian guppies, *Poecilia reticulata*: evidence for local conservation of cue production. *Canadian Journal of Zoology* **88**: 139–147.
- Brown, G. E., Ferrari, M. C. O., Elvidge, C. K., Ramnarine, I. W., and Chivers, D. P. (2013). Phenotypically plastic neophobia: a response to predation risk. *Proceedings of the Royal Society of London B: Biological Sciences* **280**: 20122712.
- Buchmann, K. and Lindenstrøm, T. (2002). Interactions between monogenean parasites and their fish hosts. *International Journal for Parasitology* **32**: 309–319.
- Burnham, K. P. and Anderson, D. R. (2002). *Model selection and multi-model inference: a practical information-theoretic approach*. 2nd Edition. New York: Springer.
- Bush, A. O., Lafferty, K. D., Lotz, J. M., and Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology* **85**: 575–583.
- Cable, J. and Harris, P. D. (2002). Gyrodactylid developmental biology: historical review, current status and future trends. *International Journal of Parasitology* **32**: 255–280.
- Cable, J., Scott, E. C. G., Tinsley, R. C., and Harris, P. D. (2002). Behavior favoring transmission in the viviparous monogenean *Gyrodactylus turnbulli*. *Journal of Parasitology* **88**: 183–184.
- Cable, J. and van Oosterhout, C. (2007a). The impact of parasites on the life history evolution of guppies (*Poecilia reticulata*): The effects of host size on parasite virulence. *International Journal for Parasitology* **37**: 1449–1458.
- Cable, J. and van Oosterhout, C. (2007b). The role of innate and acquired resistance in two natural populations of guppies (*Poecilia reticulata*) infected with the ectoparasite *Gyrodactylus turnbulli*. *Biological Journal of the Linnean Society* **90**: 647–655.
- Cable, J. (2011). “Poeciliid parasites”. *Ecology and Evolution of Poeciliid Fishes*. Ed. by Evans, J. P., Pilastro, A., and Schlupp, I. Chicago: The University of Chicago Press.
- Cable, J., Archard, G. A., Mohammed, R. S., McMullan, M., Stephenson, J. F., Hansen, H., and van Oosterhout, C. (2013). Can parasites use predators to spread between primary hosts? *Parasitology* **140**: 1138–1143.
- Candolin, U., Salesto, T., and Evers, M. (2007). Changed environmental conditions weaken sexual selection in sticklebacks. *Journal of Evolutionary Biology* **20**: 233–239.

- Carleton, K. and Kocher, T. (2001). Cone opsin genes of African cichlid fishes: Tuning spectral sensitivity by differential gene expression. *Molecular Biology and Evolution* **18**: 1540–1550.
- Chapman, B. B., Morrell, L. J., Tosh, C. R., and Krause, J. (2010). Behavioural consequences of sensory plasticity in guppies. *Proceedings of the Royal Society of London B: Biological Sciences* **277**: 1395–1401.
- Chase-Topping, M., Gally, D., Low, C., Matthews, L., and Woolhouse, M. E. (2008). Super-shedding and the link between human infection and livestock carriage of *Escherichia coli* O157. *Nature Reviews Microbiology* **6**: 904–912.
- Chivers, D. P., Brown, G. E., and Smith, R. J. F. (1995). Familiarity and shoal cohesion in fathead minnows (*Pimephales promelas*): implications for antipredator behaviour. *Canadian Journal of Zoology* **73**: 955–960.
- Chivers, D. and Smith, R. J. (1998). Chemical alarm signaling in aquatic predator-prey systems: a review and prospectus. *Ecoscience* **5**: 338–352.
- Civitello, D. J. and Rohr, J. R. (2014). Disentangling the effects of exposure and susceptibility on transmission of the zoonotic parasite *Schistosoma mansoni*. *Journal of Animal Ecology* **83**: 1379–1386.
- Clay, C. A., Lehmer, E. M., Previtali, A., St. Jeor, S., and Dearing, M. D. (2009). Contact heterogeneity in deer mice: implications for Sin Nombre virus transmission. *Proceedings of the Royal Society of London B: Biological Sciences* **276**: 1305–1312.
- Clutton-Brock, T. and Isvaran, K. (2007). Sex differences in ageing in natural populations of vertebrates. *Proceedings of the Royal Society of London B: Biological Sciences* **274**: 3097–3014.
- Cobbold, R. N., Hancock, D. D., Rice, D. H., Berg, J., Stillborn, R., Hovde, C. J., and Besser, T. J. (2007). Rectoanal junction colonization of feedlot cattle by *Escherichia coli* O157:H7 and its association with supershedders and excretion dynamics. *Applied Environmental Microbiology* **73**: 1563–1568.
- Coltman, D. W., Pilkington, J., Kruuk, L. E. B., Wilson, K., and Pemberton, J. M. (2001). Positive genetic correlation between parasite resistance and body size in a free-living ungulate population. *Evolution* **55**: 2116–2125.
- Conway, C., Kean, E. K., Müller, C., and Perkins, S. E. (in prep.). The scent of infection: mechanisms driving behavioural avoidance of infected individuals.
- Coors, A. and De Meester, L. (2010). Fitness and virulence of a bacterial endoparasite in an environmentally stressed crustacean host. *Parasitology* **138**: 122–131.

- Cordi, V., Ortubay, S., and Lozada, M. (2005). Visual cues during the alarm reaction of *Gymnocharacinus bergi* (Pisces, Characidae). *Journal of Applied Ichthyology* **21**: 487–491.
- Côté, I. M. and Poulin, R. (1995). Parasitism and group size in social animals: a meta-analysis. *Behavioral Ecology* **6**: 159.
- Cousineau, S. V. and Alizon, S. (2014). Parasite evolution in response to sex-based host heterogeneity in resistance and tolerance. *Journal of Evolutionary Biology* **27**: 2753–2766.
- Crane, A. L. and Ferrari, M. C. O. (2015). Minnows trust conspecifics more than themselves when faced with conflicting information about predation risk. *Animal Behaviour* **100**: 184–190.
- Croft, D. P., Albanese, B., Arrowsmith, B. J., Botham, M., Webster, M., and Krause, J. (2003a). Sex-biased movement in the guppy (*Poecilia reticulata*). *Oecologia* **137**: 62–68.
- Croft, D. P., Arrowsmith, B. J., Bielby, J., and Skinner, K. (2003b). Mechanisms underlying shoal composition in the Trinidadian guppy, *Poecilia reticulata*. *Oikos* **100**: 429–438.
- Croft, D. P., Arrowsmith, B. J., Webster, M., and Krause, J. (2004). Intrasexual preferences for familiar individuals in male guppies (*Poecilia reticulata*). *Journal of Fish Biology* **64**: 279–283.
- Croft, D. P., Morrell, L. J., Wade, A. S., Piyapong, C., Ioannou, C. C., Dyer, J. R. G., Chapman, B. B., Wong, Y., and Krause, J. (2006). Predation risk as a driving force for sexual segregation: a cross-population comparison. *American Naturalist* **167**: 867–878.
- Croft, D. P., Krause, J., Darden, S. K., Ramnarine, I. W., Faria, J. J., and James, R. (2009). Behavioural trait assortment in a social network: patterns and implications. *Behavioral Ecology and Sociobiology* **63**: 1495–1503.
- Croft, D. P., Edenbrow, M., Darden, S. K., Ramnarine, I. W., van Oosterhout, C., and Cable, J. (2011). Effect of gyrodactylid ectoparasites on host behaviour and social network structure in guppies, *Poecilia reticulata*. *Behavioral Ecology and Sociobiology* **65**: 2219–2227.
- Croft, D. P., Hamilton, P. B., Darden, S. K., Jacoby, M. P., James, R., Bettaney, E. M., and Tyler, C. R. (2012). The role of relatedness in structuring the social network of a wild guppy population. *Oecologia* **170**: 955–963.

- Cronin, J. P., Welsh, M. E., Dekkers, M. G., Abercrombie, S. T., and Mitchell, C. E. (2010). Host physiological phenotype explains pathogen reservoir potential. *Ecology Letters* **13**: 1221–1232.
- Crow, R. T. and Liley, N. R. (1979). A sexual pheromone in the guppy, *Poecilia reticulata* (Peters). *Canadian Journal of Zoology* **57**: 184–188.
- Daly, E. W. and Johnson, P. T. J. (2011). Beyond immunity: quantifying the effects of host anti-parasite behavior on parasite transmission. *Oecologia* **165**: 1043–1050.
- Dargent, F., Scott, M. E., Hendry, A. P., and Fussman, G. F. (2013). Experimental elimination of parasites in nature leads to the evolution of increased resistance in hosts. *Proceedings of the Royal Society of London B: Biological Sciences* **280**: 20132371.
- De Moraes, C. M., Stanczyk, N. M., Betz, H. S., Pulido, H., Sim, D. G., Read, A. F., and Mescher, M. C. (2014). Malaria-induced changes in host odors enhance mosquito attraction. *Proceedings of the National Academy of Sciences* **111**: 11079–11084.
- DeCaestecker, E., De Meester, L., and Ebert, D. (2002). In deep trouble: habitat selection constrained by multiple enemies in zooplankton. *Proceedings of the National Academy of Sciences* **99**: 5481–5485.
- Derby, C. D. and Sorensen, P. W. (2008). Neural processing, perception, and behavioral responses to natural chemical stimuli by fish and crustaceans. *Journal of Chemical Ecology* **34**: 898–914.
- Domenici, P. and Blake, R. (1997). The kinematics and performance of fish fast-start swimming. *The Journal of Experimental Biology* **200**: 1165–1178.
- Doving, K. and Selset, R. (1980). Behavior patterns in cod released by electrical stimulation of olfactory tract bundles. *Science* **207**: 559–560.
- Duffy, M. A., Housley, J. M., Penczykowski, R. M., Cáceres, C. E., and Hall, S. R. (2011). Unhealthy herds: indirect effects of predators enhance two drivers of disease spread. *Functional Ecology* **25**: 945–953.
- Duneau, D. and Ebert, D. (2012). Host sexual dimorphism and parasite adaptation. *PLoS Biology* **10**: e1001217.
- Dusenberry, D. B. (1992). *Sensory Ecology*. New York: Freeman.
- Dutta, H., Marcelino, J., and Richmonds, C. (1992). Brain acetylcholinesterase activity and optomotor behavior in bluegills, *Lepomis macrochirus* exposed to different concentrations of diazinon. *Archives of Physiology and Biochemistry* **100**: 331–334.

- Dwyer, G., Elkinton, J. S., and Buonaccorsi, J. P. (1997). Host Heterogeneity in susceptibility and disease dynamics: tests of a mathematical model. *The American Naturalist* **150**: 685–707.
- Eames, K. T. D. and Keeling, M. J. (2002). Modeling dynamic and network heterogeneities in the spread of sexually transmitted diseases. *Proceedings of the National Academy of Sciences* **99**: 13330–13335.
- Eizaguirre, C. and Lenz, T. L. (2010). Major histocompatibility complex polymorphism: Dynamics and consequences of parasite-mediated local adaptation in fishes. *Journal of Fish Biology* **77**: 2023–2047.
- Elvidge, C. K., Macnaughton, C. J., and Brown, G. E. (2012). Sensory complementation and antipredator behavioural compensation in acid-impacted juvenile Atlantic salmon. *Oecologia* **172**: 69–78.
- Endler, J. A. (1978). A predator's view of animal color patterns. *Evolutionary Biology* **11**: 319–364.
- Endler, J. A. (1980). Natural selection on color patterns in *Poecilia reticulata*. *Evolution* **34**: 76–91.
- Endler, J. A., Basolo, A. L., Glowacki, S., and Zerr, J. (2001). Variation in response to artificial selection for light sensitivity in guppies (*Poecilia reticulata*). *The American Naturalist* **158**: 36–48.
- Eraud, C., Jacquet, A., and Faivre, B. (2009). Survival cost of an early immune soliciting in nature. *Evolution* **63**: 1036–1043.
- Evans, J. P. and Kelley, J. L. (2008). Implications of multiple mating for offspring relatedness and shoaling behaviour in juvenile guppies. *Biology Letters* **4**: 623–626.
- Fellous, S. and Koella, J. C. (2009). Different transmission strategies of a parasite in male and female hosts. *Journal of Evolutionary Biology* **22**: 582–588.
- Ferrari, M. C. O., Trowell, J., Brown, G. E., and Chivers, D. (2005). The role of learning in the development of threat-sensitive predator avoidance by fathead minnows. *Animal Behaviour* **70**: 777–784.
- Ferrari, M. C. O., Vavrek, M. A., and Elvidge, C. K. (2008). Sensory complementation and the acquisition of predator recognition by salmonid fishes. *Behavioral Ecology and Sociobiology* **63**: 113–121.
- Fischer, E. K., Harris, R. M., Hofmann, H. A., and Hoke, K. L. (2014). Predator exposure alters stress physiology in guppies across timescales. *Hormones and Behavior* **65**: 165–172.

- Foam, P., Harvey, M., Mirza, R. S., and Brown, G. E. (2005). Heads up: juvenile convict cichlids switch to threat-sensitive foraging tactics based on chemosensory information. *Animal Behaviour* **70**: 601–607.
- Folstad, I. and Karter, A. J. (1992). Parasites, bright males, and the immunocompetence handicap. *American Naturalist* **139**: 603–622.
- Fraser, B. A. and Neff, B. D. (2010). Parasite mediated homogenizing selection at the MHC in guppies. *Genetica* **138**: 273–278.
- Fraser, B. A., Ramnarine, I. W., and Neff, B. D. (2010). Temporal variation at the MHC class IIB in wild populations of the guppy, (*Poecilia reticulata*). *Evolution* **64**: 2086–2096.
- Fujita, I., Sorensen, P. W., Stacey, N. E., and Hara, T. J. (1991). The olfactory system, not the terminal nerve, functions as the primary chemosensory pathway mediating responses to sex-pheromones in male goldfish. *Brain Behaviour and Evolution* **38**: 313–321.
- Gadgil, M. and Bossert, P. W. (1970). Life historical consequences of natural selection. *American Naturalist* **104**: 1–24.
- Galvani, A. P. and May, R. M. (2005). Epidemiology: dimensions of superspreading. *Nature* **438**: 293–295.
- Gelman, A. and Su, Y.-S. (2014). *arm: Data Analysis Using Regression and Multi-level/Hierarchical Models*. R package version 1.7-03. URL: <http://CRAN.R-project.org/package=arm>.
- Gerlach, G., Hodgins-Davis, A., Avolio, C., and Schunter, C. (2008). Kin recognition in zebrafish: a 24-hour window for olfactory imprinting. *Proceedings of the Royal Society of London B: Biological Sciences* **275**: 2165–2170.
- Ghalambor, C. K., Reznick, D. N., and Walker, J. A. (2004). Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). *American Naturalist* **164**: 38–50.
- Gheorghiu, C., Marcogliese, D. J., and Scott, M. E. (2012). Waterborne zinc alters temporal dynamics of guppy *Poecilia reticulata* epidermal response to *Gyrodactylus turnbulli* (Monogenea). *Diseases of Aquatic Organisms* **98**: 143–153.
- Giaquinto, P. C. and Hoffman, A. (2010). Role of olfaction and vision cues in feeding behaviour and alarm reaction in the catfish pintado, *Pseudoplatystoma corruscans*. *Journal of Ethology* **28**: 21–27.

- Goodall, J. (1986). Social rejection, exclusion, and shunning among the Gombe chimpanzees. *Ethology and Sociobiology* **7**: 227–236.
- Gordon, S. P., Reznick, D. N., Kinnison, M. T., Bryant, M. J., Weese, D. J., Rasanen, K., Millar, N. P., and Hendry, A. P. (2009). Adaptive changes in life history and survival following a new guppy introduction. *The American Naturalist* **174**: 34–45.
- Gotanda, K. M., Delaire, L. C., Raeymaekers, J. A. M., Pérez-Jvostov, F., Dargent, F., Bentzen, P., Scott, M. E., Fussman, G. F., and Hendry, A. P. (2013). Adding parasites to the guppy-predation story: insights from field surveys. *Oecologia* **172**: 155–166.
- Grear, D. A., Perkins, S. E., and Hudson, P. J. (2009). Does elevated testosterone result in increased exposure and transmission of parasites? *Ecology Letters* **12**: 528–537.
- Grether, G. (2000). Carotenoid limitation and mate preference evolution: a test of the indicator hypothesis in guppies (*Poecilia reticulata*). *Evolution* **54**: 1712–1724.
- Griffin, A. S. (2004). Social learning about predators: a review and prospectus. *Learning and Behaviour* **32**: 131–140.
- Griffiths, S. and Magurran, A. E. (1997). Familiarity in schooling fish: how long does it take to acquire? *Animal Behaviour* **53**: 945–949.
- Griffiths, S. W. and Magurran, A. E. (1998). Sex and schooling behaviour in the Trinidadian guppy. *Animal Behaviour* **56**: 689–693.
- Griffiths, S. and Magurran, A. E. (1999). Schooling decisions in guppies (*Poecilia reticulata*) are based on familiarity rather than kin recognition by phenotype matching. *Behavioral Ecology and Sociobiology* **45**: 437–443.
- Grueber, C. E., Nakagawa, S., Laws, R. J., and Jamieson, I. G. (2011). Multimodel inference in ecology and evolution: challenges and solutions. *Journal of Evolutionary Biology* **24**: 699–711.
- Gudelj, I. and White, K. A. J. (2004). Spatial heterogeneity, social structure and disease dynamics of animal populations. *Theoretical Population Biology* **66**: 139–149.
- Guevara-Fiore, P., Stapley, J., Krause, J., Ramnarine, I. W., and Watt, P. J. (2010). Male mate-searching strategies and female cues: how do male guppies find receptive females? *Animal Behaviour* **79**: 1191–1197.
- Hain, T. J. A. and Neff, B. D. (2007). Multiple paternity and kin recognition mechanisms in a guppy population. *Molecular Ecology* **16**: 3938–3946.
- Haislip, N. A., Hoverman, J. T., Miller, D. L., and Gray, M. J. (2012). Natural stressors and disease risk: does the threat of predation increase amphibian susceptibility to ranavirus? *Canadian Journal of Zoology* **90**: 893–902.

- Hall, D. and Suboski, M. D. (1995). Visual and olfactory stimuli in learned release of alarm reactions by zebra danio fish (*Brachydanio rerio*). *Neurobiology of Learning and Memory* **63**: 229–240.
- Hamdani, E. and Doving, K. (2007). The functional organization of the fish olfactory system. *Progress in Neurobiology* **82**: 80–86.
- Hamilton, W. D. and Zuk, M. (1982). Heritable true fitness and bright birds: a role for parasites? *Science* **218**: 384–387.
- Hampson, K., Dushoff, J., Cleaveland, S., Haydon, D. T., Kaare, M., Packer, C., and Dobson, A. (2009). Transmission Dynamics and Prospects for the Elimination of Canine Rabies. *PLoS Biology* **7**: e1000053.
- Hansen, A., Rolen, S. H., Anderson, K., Morita, Y., Caprio, J., and Finger, T. E. (2003). Correlation between olfactory receptor cell type and function in the channel catfish. *Journal of Neuroscience* **23**: 9328–9339.
- Hansen, A. and Zielinski, B. S. (2005). Diversity in the olfactory epithelium of bony fishes: development, lamellar arrangement, sensory neuron cell types and transduction components. *Journal of Neurocytology* **34**: 183–208.
- Hara, T. J. (1994). Olfaction and gustation in fish: an overview. *Acta Physiol Scand* **152**: 207–217.
- Hara, T. J. (2006). Feeding behaviour in some teleosts is triggered by single amino acids primarily through olfaction. *Journal of Fish Biology* **68**: 810–825.
- Harden, M. V., Newton, L. A., Lloyd, R. C., and Whitlock, K. E. (2006). Olfactory imprinting is correlated with changes in gene expression in the olfactory epithelia of the zebrafish. *Journal of Neurobiology* **66**: 1452–1466.
- Harris, P. D., Soleng, A., and Bakke, T. A. (2000). Increased susceptibility of salmonids to the monogenean *Gyrodactylus salaris* following administration of hydrocortisone acetate. *Parasitology* **120**: 57–64.
- Harris, S., Ramnarine, I. W., Smith, H. G., and Pettersson, L. B. (2010). Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata*. *Oikos* **119**: 1711–1718.
- Hart, B. J. (1988). Biological basis of the behavior of sick animals. *Neuroscience & Biobehavioral Reviews* **12**: 123–137.
- Hartman, E. J. and Abrahams, M. V. (2000). Sensory compensation and the detection of predators: the interaction between chemical and visual information. *Proceedings of the Royal Society of London B: Biological Sciences* **267**: 571–575.

- Haskins, C. P., Haskins, E. F., McLaughlin, J. J. A., and Hewitt, R. E. (1961). "Polymorphism and population structure in *Lebistes reticulata*, an ecological study". *Vertebrate Speciation*. Ed. by Blair, W. F. Austin, TX: University of Texas Press.
- Hatcher, M. J., Dick, J. T. A., and Dunn, A. M. (2006). How parasites affect interactions between competitors and predators. *Ecology Letters* **9**: 1253–1271.
- Hawkins, L., Magurran, A. E., and Armstrong, J. D. (2007). Innate abilities to distinguish between predator species and cue concentration in Atlantic salmon. *Animal Behaviour* **73**: 1051–1057.
- Helfman, G. (1989). Threat-sensitive avoidance in damselfish-trumpetfish interaction. *Behavioral Ecology and Sociobiology* **24**: 47–58.
- Hendrichsen, D. K., Kristoffersen, R., Gjelland, K. Ø., Knudsen, R., Kusterle, S., Rikardsen, A. H., Henriksen, E. H., Smalås, A., and Olstad, K. (2015). Transmission dynamics of the monogenean *Gyrodactylus salaris* under seminatural conditions. *Journal of Fish Diseases* **38**: 541–550.
- Hockley, F. A., Wilson, C. A. M. E., Brew, A., and Cable, J. (2013). Fish responses to flow velocity and turbulence in relation to size, sex and parasite load. *Journal of the Royal Society Interface* **11**: 20130814.
- Hoffmann, M., Tripathi, N., Henz, S. R., Lindholm, A. K., Weigel, D., Breden, F., and Dreyer, C. (2007). Opsin gene duplication and diversification in the guppy, a model for sexual selection. *Proceedings of the Royal Society of London B: Biological Sciences* **274**: 33–42.
- Holt, R. D. and Roy, M. (2007). Predation can increase the prevalence of infectious disease. *The American Naturalist* **169**: 690–699.
- Houde, A. and Torio, A. J. (1992). Effect of parasitic infection on male color pattern and female choice in guppies. *Behavioral Ecology* **3**: 346–351.
- Houde, A. (1997). *Sex, color and mate choice in guppies*. Monographs in Behavior and Ecology. Princeton: Princeton University Press.
- Hudson, P. J., Dobson, A. P., and Newborn, D. (1992). Do parasites make prey vulnerable to predation? Red grouse and parasites. *Journal of Animal Ecology* **61**: 681–692.
- Huizinga, M., Ghalambor, C. K., and Reznick, D. N. (2009). The genetic and environmental basis of adaptive differences in shoaling behaviour among populations of Trinidadian guppies, *Poecilia reticulata*. *Journal of Evolutionary Biology* **22**: 1860–1866.

- Janssens, L. and Stocks, R. (2014). Reinforcing effects of non-pathogenic bacteria and predation risk: from physiology to life history. *Oecologia* **176**: 323–332.
- Johnson, M. B., Lafferty, K. D., van Oosterhout, C., and Cable, J. (2011). Parasite transmission in social interacting hosts: monogenean epidemics in guppies. *PLoS ONE* **6**: e22634.
- Johnson, P. T. J., Stanton, D. E., Preu, E. R., Forshay, K. J., and Carpenter, S. R. (2006). Dining on disease: how interactions between infection and environment affect predation risk. *Ecology* **87**: 1973–1980.
- Johnson, P. T. J., Dobson, A. P., Lafferty, K. D., Marcogliese, D. J., Memmott, J., Orlofske, S. A., Poulin, R., and Thieltges, D. W. (2010). When parasites become prey: ecological and epidemiological significance of eating parasites. *Trends in Ecology and Evolution* **25**: 362–371.
- Johnson, P. T. J. and Hoverman, J. T. (2012). Parasite diversity and coinfection determine pathogen infection success and host fitness. *Proceedings of the National Academy of Sciences* **109**: 9006–9011.
- Johnson, P. T. J., Rohr, J. R., Hoverman, J. T., Kellermanns, E., Bowerman, J., and Lunde, K. B. (2012). Living fast and dying of infection: host life history drives interspecific variation in infection and disease risk. *Ecology Letters* **15**: 235–242.
- Johnson, P. T. J. and Hoverman, J. T. (2014). Heterogeneous hosts: how variation in host size, behaviour and immunity affects parasite aggregation. *Journal of Animal Ecology* **83**: 1103–1112.
- Johnstone, R. A. (1996). Multiple displays in animal communication: ‘backup signals’ and ‘multiple messages’. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **351**: 329–338.
- Jokela, J., Schmid-Hempel, P., and Rigby, M. C. (2000). Dr. Pangloss restrained by the Red Queen - steps towards a unified defence theory. *Oikos* **89**: 267–274.
- Joly, D. O. and Messier, F. (2004). The distribution of *Echinococcus granulosus* in moose: evidence for parasite-induced vulnerability to predation by wolves? *Oecologia* **140**: 586–590.
- Karvonen, A., Paukku, S., Valtonen, E. T., and Hudson, P. J. (2003). Transmission, infectivity and survival of *Diplostomum spathaceum* cercariae. *Parasitology* **127**: 217–224.

- Karvonen, A. and Seehausen, O. (2012). The role of parasitism in adaptive radiations—when might parasites promote and when might they constrain ecological speciation? *International Journal of Ecology* **2012**: 280169.
- Kavaliers, M., Fudge, M. A., Colwell, D. D., and Choleris, E. (2003). Aversive and avoidance responses of female mice to the odors of males infected with an ectoparasite and the effects of prior familiarity. *Behavioral Ecology and Sociobiology* **54**: 423–430.
- Kavaliers, M., Choleris, E., and Pfaff, D. W. (2005). Genes, odours and the recognition of parasitized individuals by rodents. *Trends in Ecology and Evolution* **21**: 423–429.
- Kennedy, C. E. J., Endler, J. A., Poynton, S. L., and McMinn, H. (1987). Parasite load predicts mate choice in guppies. *Behavioral Ecology and Sociobiology* **21**: 291–295.
- Kerby, J. L., Hart, A. J., and Storfer, A. (2011). Combined effects of virus, pesticide and predator cue on the larval tiger salamander (*Ambystoma tigrinum*). *EcoHealth* **8**: 46–54.
- Keymer, A. E. and Anderson, R. M. (1979). The dynamics of infection of *Tribolium confusum* by *Hymenolepis diminuta*: the influence of infective-stage density and spatial distribution. *Parasitology* **79**: 195–207.
- Kiesecker, J. M., Skelly, D. K., Beard, K. H., and Preisser, E. (1999). Behavioral reduction of infection risk. *Proceedings of the National Academy of Sciences* **96**: 9165–9168.
- Kim, J.-W., Wood, J. L. A., Grant, J. W. A., and Brown, G. E. (2011). Acute and chronic increases in predation risk affect the territorial behaviour of juvenile Atlantic salmon in the wild. *Animal Behaviour* **81**: 93–99.
- Koch, H. and Schmid-Hempel, P. (2012). Gut microbiota instead of host genotype drive the specificity in the interaction of a natural host-parasite system. *Ecology Letters* **15**: 1095–1103.
- Kolluru, G. R., Grether, G. F., South, S. H., Dunlop, E., Cardinali, A., Liu, L., and Carapiet, A. (2006). The effects of carotenoid and food availability on resistance to a naturally occurring parasite (*Gyrodactylus turnbulli*) in guppies (*Poecilia reticulata*). *Biological Journal of the Linnean Society* **89**: 301–309.
- Kolluru, G., Grether, G., Dunlop, E., and South, S. (2009). Food availability and parasite infection influence mating tactics in guppies (*Poecilia reticulata*). *Behavioral Ecology* **20**: 131–137.
- Kotrschal, A., Rogell, B., Bundsen, A., Svensson, B., Zajitschek, S. R. K., Brännström, I., Immler, S., Maklakov, A. A., and Kolm, N. (2013). Artificial selection on relative brain

- size in the guppy reveals costs and benefits of evolving a larger brain. *Current Biology* **23**: 168–171.
- Kozak, G. M., Head, M. L., and Boughman, J. W. (2011). Sexual imprinting on ecologically divergent traits leads to sexual isolation in sticklebacks. *Proceedings of the Royal Society of London B: Biological Sciences* **278**: 2604–2610.
- Krause, J., Godin, J. G. J., and Brown, D. (1996). Size-assortativeness in multi-species fish shoals. *Journal of Fish Biology* **49**: 221–225.
- Krause, J., Loader, S. P., McDermott, J., and Ruxton, G. D. (1998). Refuge use by fish as a function of body length-related metabolic expenditure and predation risks. *Proceedings of the Royal Society of London B: Biological Sciences* **265**: 2373–2379.
- Krause, J. and Ruxton, G. D. (2002). *Living in Groups*. Oxford Series in Ecology and Evolution. Oxford University Press, Oxford.
- Kreisinger, J., Bastien, G., Hauffe, H., Marchesi, J., and Perkins, S. E. (in press). Interactions between multiple helminths and the gut microbiota in wild rodents. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*.
- Krüger, O., Lindström, J., and Amos, W. (2001). Maladaptive mate choice maintained by heterozygote advantage. *Evolution* **55**: 1207–1214.
- Lacharme-Lora, L., Perkins, S. E., Humphrey, T. J., Hudson, P. J., and Salisbury, V. (2009). Use of bioluminescent bacterial biosensors to investigate the role of free-living helminths as reservoirs and vectors of *Salmonella*. *Environmental Microbiology Reports* **1**: 198–207.
- Lafferty, K. D. and Kuris, A. M. (2002). Trophic strategies, animal diversity and body size. *Trends in Ecology and Evolution* **17**: 507–513.
- Lafferty, K. D., Allesina, S., Arim, M., Briggs, C. J., De Leo, G., Dobson, A. P., Dunne, J. A., Johnson, P. T. J., Kuris, A. M., Marcogliese, D. J., Martinez, N. D., Memmott, J., Marquet, P. A., McLaughlin, J. P., Mordecai, E. A., Pascual, M., Poulin, R., and Thieltges, D. W. (2008). Parasites in food webs: the ultimate missing links. *Ecology Letters* **11**: 553–546.
- Landeau, L. and Terborgh, J. (1986). Oddity and the confusion effect in predation. *Animal Behaviour* **34**: 1372–1380.
- Larson, J. K. and McCormick, M. I. (2005). The role of chemical alarm signals in facilitating learned recognition of novel chemical cues in a coral reef fish. *Animal Behaviour* **69**: 51–57.

- Lass, S., Hudson, P. J., Thakar, J., Saric, J., Harvali, E., R., A., and Perkins, S. E. (2013). Generating super-shedders: co-infection increases bacterial load and egg production of a gastrointestinal helminth. *Journal of the Royal Society Interface* **10**: 20120588.
- Laver, C. and Taylor, J. (2011). RT-qPCR reveals opsin gene upregulation associated with age and sex in guppies (*Poecilia reticulata*) - a species with color-based sexual selection and 11 visual-opsin genes. *BMC Evolutionary Biology* **11**: 81.
- Law, R. (1979). Optimal life histories under age-specific predation. *American Naturalist* **114**: 399–417.
- Leahy, S. M., McCormick, M. I., Mitchell, M. D., and Ferrari, M. C. O. (2011). To fear or to feed: the effects of turbidity on perception of risk by a marine fish. *Biology Letters* **7**: 811–813.
- Leduc, A. O. H. C., Kim, J.-W., Macnaughton, C. J., and Brown, G. E. (2010). Sensory complement model helps to predict alarm response patterns in juvenile Atlantic salmon (*Salmo salar*) under natural conditions. *Canadian Journal of Zoology* **88**: 398–403.
- Lee, K. A., Wikelski, M., Robinson, W. D., Robinson, T. R., and Klasing, K. C. (2008). Constitutive immune defences correlate with life-history variables in tropical birds. *Journal of Animal Ecology* **77**: 356–363.
- Legendre, P. (2014). *lmodel2: Model II Regression*. R package version 1.7-2. URL: <http://CRAN.R-project.org/package=lmodel2>.
- Lima, S. L. and Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* **68**: 619–640.
- Little, T. J., O'Connor, B., Colegrave, N., Watt, K., and Read, A. F. (2003). Maternal transfer of strain-specific immunity in an invertebrate. *Current Biology* **13**: 489–492.
- Little, T. J., Shuker, D. M., Colegrave, N., Day, T., and Graham, A. L. (2010). The coevolution of virulence: tolerance in perspective. *PLoS Pathogens* **6**: e1001006.
- Llaurens, V., McMullan, M., and van Oosterhout, C. (2012). Cryptic MHC polymorphism revealed but not explained by selection on the class IIB peptide-binding region. *Molecular Biology and Evolution* **29**: 1631–1644.
- Llewellyn, M. S., Boutin, S., Hoseinifar, S. H., and Derome, N. (2014). Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries. *Frontiers in Microbiology* **5**: 207.

- Lloyd-Smith, J. O., Getz, W. M., and Westerhoff, H. V. (2004). Frequency-dependent incidence in models of sexually transmitted diseases: portrayal of pair-based transmission and effects of illness on contact behaviour. *Proceedings of the Royal Society of London B: Biological Sciences* **271**: 625–634.
- Lloyd-Smith, J. O., Schreiber, S. J., Kopp, P. E., and Getz, W. M. (2005). Superspreading and the effect of individual variation on disease emergence. *Nature* **438**: 355–359.
- Lloyd-Smith, J. O., Schreiber, S. J., and Getz, W. M. (2006). Moving beyond averages: Individual-level variation in disease transmission. *Contemporary Mathematics* **410**: 235–258.
- Lochmiller, R. L. and Deerenberg, C. (2000). Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88**: 87–98.
- Loehle, C. (1995). Social barriers to pathogen transmission in wild animal populations. *Ecology* **76**: 326–335.
- Lönngstedt, O. M. and McCormick, M. I. (2011). Chemical alarm cues inform prey of predation threat: The importance of ontogeny and concentration in a coral reef fish. *Animal Behaviour* **82**: 213–218.
- Lonnstedt, O. M., Munday, P. L., McCormick, M. I., Ferrari, M. C. O., and Chivers, D. P. (2013). Ocean acidification and responses to predators: can sensory redundancy reduce the apparent impacts of elevated CO₂ on fish? *Ecology and Evolution* **10**: 3565–3575.
- Lopes, P. C., Adelman, J., Wingfield, J. C., and Bentley, G. E. (2012). Social context modulates sickness behaviour. *Behavioral Ecology and Sociobiology* **66**: 1421–1428.
- López, S. (1998). Acquired resistance affects male sexual display and female choice in guppies. *Proceedings of the Royal Society of London B: Biological Sciences* **265**: 717–723.
- López, S. (1999). Parasitized female guppies do not prefer showy males. *Animal Behaviour* **57**: 1129–1134.
- Lopez-Sepulcre, A., Gordon, S. P., Paterson, I. G., Bentzen, P., and Reznick, D. N. (2013). Beyond lifetime reproductive success: the posthumous reproductive dynamics of male Trinidadian guppies. *Proceedings of the Royal Society of London B: Biological Sciences* **280**: 20131116.
- Lythgoe, J. N. (1979). *The Ecology of Vision*. Oxford: Clarendon Press.
- Lythgoe, J. N. (1980). *Vision in fishes: ecological adaptations*. Environmental Physiology of Fishes. New York: Plenum Publishing Corporation.

- Maan, M., Hofker, K., van Alphen, J., and Seehausen, O. (2006). Sensory drive in cichlid speciation. *American Naturalist* **167**: 947–954.
- Maaswinkel, H. and Li, L. (2003). Olfactory input increases visual sensitivity in zebrafish: a possible function for the terminal nerve and dopaminergic interplexiform cells. *Journal of Experimental Biology* **206**: 2201–2209.
- Madhavi, R. and Anderson, R. M. (1985). Variability in the susceptibility of the fish host, *Poecilia reticulata*, to infection with *Gyrodactylus bullatarudis* (Monogenea). *Parasitology* **91**: 531–544.
- Magurran, A. E. and Higham, A. (1988). Information transfer across fish shoals under predator threat. *Ethology* **78**: 153–158.
- Magurran, A. E. (1990). The adaptive significance of schooling as an anti-predator defence in fish. *Annales Zoologici Fennici* **27**: 51–66.
- Magurran, A. E. and Seghers, B. H. (1990). Population differences in the schooling behaviour of newborn guppies, *Poecilia reticulata*. *Ethology* **84**: 334–342.
- Magurran, A. E. and Nowak, M. A. (1991). Another battle of the sexes: the consequences of sexual asymmetry in mating costs and predation risk in the guppy, *Poecilia reticulata*. *Proceedings of the Royal Society of London B: Biological Sciences* **246**: 31–38.
- Magurran, A. E. and Seghers, B. H. (1994a). Predator inspection behaviour covaries with schooling tendency amongst wild guppy, *Poecilia reticulata*, populations in Trinidad. *Behaviour* **128**: 121–134.
- Magurran, A. E. and Seghers, B. H. (1994b). Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata*, populations in Trinidad. *Proceedings of the Royal Society of London B: Biological Sciences* **255**: 31–36.
- Magurran, A. E., Seghers, B. H., Shaw, P. W., and Carvalho, G. R. (1994). Schooling preferences for familiar fish in the guppy, *Poecilia reticulata*. *Journal of Fish Biology* **29**: 83–95.
- Magurran, A. E., Seghers, B. H., Shaw, P. W., and Carvalho, G. R. (1995). The behavioural diversity and evolution of guppy, *Poecilia reticulata*, populations in Trinidad. *Advances in the Study of Behavior* **24**: 155–202.
- Magurran, A. E., Irving, P., and Henderson, P. (1996). Is there a fish alarm pheromone? A wild study and critique. *Proceedings of the Royal Society of London B: Biological Sciences* **263**: 1551–1556.
- Magurran, A. E. and Macías Garcia, C. (2000). Sex differences in behaviour as an indirect consequence of mating system. *Journal of Fish Biology* **57**: 839–857.

- Magurran, A. E. (2005). *Evolutionary ecology: the Trinidadian guppy*. Oxford Series in Ecology and Evolution. New York: Oxford University Press.
- Marcogliese, D. J. and Pietrock, M. (2011). Combined effects of parasites and contaminants on animal health: parasites do matter. *Trends in Parasitology* **27**: 123–130.
- Martin, C. H. and Johnsen, S. (2007). A field test of the Hamilton-Zuk hypothesis in the Trinidadian guppy (*Poecilia reticulata*). *Behavioural Ecology and Sociobiology* **61**: 1897–1909.
- Martin, L. B., Weil, Z. M., and Nelson, R. J. (2007). Immune defense and reproductive pace of life in *Peromyscus* mice. *Ecology* **88**: 2516–2528.
- Mateo, J. M. (2004). Recognition systems and biological organization: the perception component of social recognition. *Annales Zoologici Fennici* **41**: 729–745.
- Mathis, A., Chivers, D., and Smith, R. J. F. (1996). Cultural transmission of predator recognition in fishes: intraspecific and interspecific learning. *Animal Behaviour* **51**: 185–201.
- Matthews, L., Low, J. C., Gally, D. L., Pearce, M. C., Mellor, D. J., Heesterbeek, J. A. P., Chase-Topping, M., Naylor, S. W., Shaw, D. J., Reid, J., S. W., Gunn, G. J., and Woolhouse, M. E. (2006). Heterogeneous shedding of *Escherichia coli* O157 in cattle and its implications for control. *Proceedings of the National Academy of Sciences* **103**: 547–552.
- May, R. M. and Anderson, R. M. (1987). Transmission dynamics of HIV infection. *Nature* **326**: 137–142.
- Maynard Smith, J. and Harper, D. G. C. (2003). *Animal Signals*. Oxford Series in Ecology and Evolution. New York: Oxford University Press.
- McCoy, M. W. and Bolker, B. M. (2008). Trait-mediated interactions: influence of prey size, density and experience. *Journal of Animal Ecology* **77**: 478–486.
- McIntyre, J. K., Baldwin, D. H., Beauchamp, D. A., and Scholz, N. L. (2012). Low-level copper exposures increase visibility and vulnerability of juvenile coho salmon to cutthroat trout predators. *Ecological Applications* **22**: 1460–1471.
- McKellar, A. E., Turcotte, M. M., and Hendry, A. P. (2009). Environmental factors influencing adult sex ratio in Trinidadian guppies. *Oecologia* **159**: 735–745.
- McKellar, A. E. and Hendry, A. P. (2011). Environmental factors influencing adult sex ratio in *Poecilia reticulata*: laboratory experiments. *Journal of Fish Biology* **79**: 937–953.

- McLennan, D. (2003). The importance of olfactory signals in the gasterosteid mating system: sticklebacks go multimodal. *Biological Journal of the Linnean Society* **80**: 555–572.
- Medzhitov, R., Schneider, D. S., and Soares, M. P. (2012). Disease tolerance as a defense strategy. *Science* **335**: 936–941.
- Michod, R. E. (1979). Evolution of life histories in response to age-specific mortality factors. *American Naturalist* **113**: 531–550.
- Milinski, M. and Bakker, T. C. M. (1990). Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature* **344**: 330–333.
- Miller, M. R., White, A., and Boots, M. (2007). Host life span and the evolution of resistance characteristics. *Evolution* **61**: 2–14.
- Mirza, R. S. and Chivers, D. P. (2000). Predator-recognition training enhances survival of brook trout: evidence from laboratory and field-enclosure studies. *Canadian Journal of Zoology* **78**: 2198–2208.
- Mirza, R. S., Scott, J. J., and Chivers, D. P. (2001). Differential responses of male and female red swordtails to chemical alarm cues. *Journal of Fish Biology* **59**: 716–728.
- Mirza, R. S. and Chivers, D. P. (2003). Response of juvenile rainbow trout to varying concentrations of chemical alarm cue: response thresholds and survival during encounters with predators. *Canadian Journal of Zoology* **81**: 88–95.
- Mohammed, R. S., van Oosterhout, C., Schelkle, B., Cable, J., and McMullan, M. (2012). Upstream guppies (*Poecilia reticulata*, Peters, 1859) go against the flow. *Biota Neotropica* **12**: 68–72.
- Nakagawa, S. and Freckleton, R. P. (2011). Model averaging, missing data and multiple imputation: a case study for behavioural ecology. *Behavioral Ecology and Sociobiology* **65**: 103–116.
- Nakagawa, S. and Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution* **4**: 133–142.
- Nosil, P. (2012). *Ecological Speciation*. Oxford Series in Ecology and Evolution. Oxford University Press, Oxford.
- Östlund-Nilsson, S., Curtis, L., Nilsson, G. E., and Grutter, A. S. (2005). Parasitic isopod *Anilocra apogonae*, a drag for the cardinal fish *Cheilodipterus quinquelineatus*. *Marine Ecology Progress Series* **287**: 209–216.

- Packer, C., Holt, R. D., Hudson, P. J., Lafferty, K. D., and Dobson, A. P. (2003). Keeping the herds healthy and alert: implications of predator control for infectious disease. *Ecology Letters* **6**: 797–802.
- Pagán, I., Alonso-Blanco, C., and García-Arenal, F. (2008). Host responses in life-history traits and tolerance to virus infection in *Arabidopsis thaliana*. *PLoS Pathogens* **4**: e1000124.
- Pap, P. L., Vágási, C. I., Vincze, O., Osváth, G., Veres-Szászka, J., and Czirják, G. A. (2014). Physiological pace of life: the link between constitutive immunity, developmental period, and metabolic rate in European birds. *Oecologia* **177**: 147–158.
- Parris, M. J. and Beaudoin, J. G. (2004). Chytridiomycosis impacts predator-prey interactions in larval amphibian communities. *Oecologia* **140**: 626–632.
- Partan, S. R. and Marler, P. (1999). Communication goes multimodal. *Science* **283**: 1272–1273.
- Partan, S. R. and Marler, P. (2005). Issues in the classification of multimodal communication signals. *The American Naturalist* **166**: 231–245.
- Paull, S. H., Song, S., McClure, K. M., Sackett, L. C., Kilpatrick, A. M., and Johnson, P. T. J. (2012). From superspreaders to disease hotspots: linking transmission across hosts and space. *Frontiers in Ecology and the Environment* **10**: 75–82.
- Peig, J. and Green, A. J. (2009). New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos* **118**: 1883–1891.
- Pérez-Jvostov, F., Hendry, A. P., Fussman, G. F., and Scott, M. E. (2012). Are host-parasite interactions influenced by adaptation to predators? A test with guppies and *Gyrodactylus* in experimental stream channels. *Oecologia* **170**: 77–88.
- Perkins, S. E., Cattadori, I. M., Tagliapietra, V., Rizzoli, A. P., and Hudson, P. J. (2003). Empirical evidence for key hosts in persistence of a tick-borne disease. *International Journal for Parasitology* **33**: 909–917.
- Perkins, S. E. and Fenton, A. (2006). Helminths as vectors of pathogens in vertebrate hosts: a theoretical approach. *International Journal for Parasitology* **36**: 887–894.
- Pettersson, L. B., Ramnarine, I., Becher, S. A., Mahabir, R., and Magurran, A. E. (2004). Sex ratio dynamics and fluctuating selection pressures in natural populations of the Trinidadian guppy, *Poecilia reticulata*. *Behavioral Ecology and Sociobiology* **55**: 461–468.

- Piyapong, C., Butlin, R. K., Faria, J. J., Scruton, K. J., Wang, J., and Krause, J. (2011). Kin assortment in juvenile shoals in wild guppy populations. *Heredity* **106**: 749–756.
- Pollock, M. S., Pollock, R. J., and Chivers, D. P. (2006). Effects of body size, body condition, and breeding state on response to alarm cues by fathead minnows. *Canadian Journal of Zoology* **84**: 1351–1357.
- Poulin, R. (1999). Parasitism and shoal size in juvenile sticklebacks: conflicting selection pressures from different ectoparasites? *Ethology* **105**: 959–968.
- Poulin, R., Marcogliese, D. J., and McLaughlin, J. D. (1999). Skin-penetrating parasites and the release of alarm substances in juvenile rainbow trout. *J Fish Biology* **55**: 47–53.
- Poulin, R. (2007). *Evolutionary Ecology of Parasites*. 2nd Edition. Princeton University Press.
- Poulin, R. (2010). Parasite manipulation of host behavior: an update and frequently asked questions. *Advances in the Study of Behavior* **41**: 151–186.
- Preston, D. L., Boland, C. E., Hoverman, J. T., and Johnson, P. T. J. (2014). Natural enemy ecology: comparing the effects of predation risk, infection risk and disease on host behaviour. *Functional Ecology* **28**: 1472–1481.
- Previtali, M. A., Ostfeld, R. S., Keesing, F., Jolles, A. E., Hanselmann, R., and Martin, L. B. (2012). Relationship between pace of life and immune responses in wild rodents. *Oikos* **121**: 1483–1492.
- R Core Team (2013). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria. URL: <http://www.R-project.org/>.
- Råberg, L., Graham, A. L., and Read, A. F. (2009). Decomposing health: tolerance and resistance to parasites in animals. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **364**: 37–49.
- Råberg, L. (2014). How to live with the enemy: understanding tolerance to parasites. *PLoS Biology* **12**: e1001989.
- Raffel, T. R., Martin, L. B., and Rohr, J. R. (2008). Parasites as predators: unifying natural enemy ecology. *Trends in Ecology and Evolution* **23**: 610–618.
- Raffel, T. R., Hoverman, J. T., Halstead, N. T., Michel, P. J., and Rohr, J. R. (2010). Parasitism in a community context: trait-mediated interactions with competition and predation. *Ecology* **91**: 1900–1907.

- Rantala, M. J., Honkavaara, J., Dunn, D. W., and Suhonen, J. (2011). Predation selects for increased immune function in male damselflies, *Calopteryx splendens*. *Proceedings of the Royal Society of London B: Biological Sciences* **278**: 1231–1238.
- Rennison, D. J., Owens, G. L., Allison, W. T., and Taylor, J. S. (2011). Intra-retinal variation of opsin gene expression in the guppy (*Poecilia reticulata*). *The Journal of Experimental Biology* **214**: 3248–3254.
- Reznick, D. N. and Endler, J. (1982). The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* **36**: 160–177.
- Reznick, D. N. and Bryga, H. (1987). Life-history evolution in guppies. 1. Phenotypic and genotypic changes in an introduction experiment. *Evolution* **41**: 1370–1385.
- Reznick, D. N., Bryga, H., and Endler, J. A. (1990). Experimentally induced life-history evolution in a natural population. *Nature* **346**: 357–359.
- Reznick, D. N., Butler IV, M. J., Rodd, F. H., and Ross, P. (1996). Life-history evolution in guppies (*Poecilia reticulata*) 6. Differential mortality as a mechanism for natural selection. *Evolution* **50**: 1651–1660.
- Reznick, D. N., Shaw, F. H., Rodd, F. H., and Shaw, R. G. (1997). Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* **275**: 1934–1937.
- Reznick, D. N., Butler IV, M. J., and Rodd, F. H. (2001). Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *The American Naturalist* **157**: 126–140.
- Richards, E. L., van Oosterhout, C., and Cable, J. (2010). Sex-specific differences in shoaling affect parasite transmission in guppies. *PLoS ONE* **5**: e13285.
- Ricklefs, R. E. and Wikelski, M. (2002). The physiology/life-history nexus. *Trends in Ecology and Evolution* **17**: 482–468.
- Rigby, M. C. and Jokela, J. (2000). Predator avoidance and immune defence: costs and trade-offs in snails. *Proceedings of the Royal Society of London B: Biological Sciences* **267**: 171–176.
- Rózsa, L. (2000). Spite, xenophobia, and collaboration between hosts and parasites. *Oikos* **91**: 396–400.
- Rowe, C. (1999). Receiver psychology and the evolution of multicomponent signals. *Animal Behaviour* **58**: 921–931.
- Rowe, C. and Guildford, T. (1999). Hidden colour aversions in domestic chicks triggered by pyrazine odours of insect warning displays. *Nature* **383**: 520–522.

- Roy, B. A. and Kirchner, J. W. (2000). Evolutionary dynamics of pathogen resistance and tolerance. *Evolution* **54**: 51–63.
- Rundle, H. D. and Nosil, P. (2005). Ecological speciation. *Ecology Letters* **8**:3, 336–352.
- Russell, S. T., Kelley, J. L., Graves, J. A., and Magurran, A. E. (2004). Kin structure and shoal composition dynamics in the guppy, *Poecilia reticulata*. *Oikos* **106**: 520–526.
- Sala-Bozano, M., van Oosterhout, C., and Mariani, S. (2012). Impact of a mouth parasite in a marine fish differs between geographical areas. *Biological Journal of the Linnean Society* **105**: 842–852.
- Sandkam, B., Young, C. M., and Breden, F. (2015). Beauty in the eyes of the beholders: colour vision is tuned to mate preference in the Trinidadian guppy (*Poecilia reticulata*). *Molecular Ecology* **24**: 596–609.
- Schaller, M. (2011). The behavioural immune system and the psychology of human sociality. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **366**: 3418–3426.
- Schelkle, B., Shinn, A. P., Peeler, E., and Cable, J. (2009). Treatment of gyrodactylid infections in fish. *Diseases of Aquatic Organisms* **86**: 65–75.
- Schelkle, B., Mohammed, R. S., Coogan, M. P., McMullan, M., Gillingham, E. L., van Oosterhout, C., and Cable, J. (2012). Parasites pitched against nature: Pitch Lake water protects guppies (*Poecilia reticulata*) from microbial and gyrodactylid infections. *Parasitology* **139**: 1772–1780.
- Schmid-Hempel, P., Pühr, K., Krüger, N., Reber, C., and Schmid-Hempel, R. (1999). Dynamic and genetic consequences of variation in horizontal transmission for a micro-parasitic infection. *Evolution* **53**: 426–434.
- Schmid-Hempel, P. (2011). *Evolutionary Parasitology*. Oxford University Press.
- Scott, M. E. and Anderson, R. M. (1984). The population dynamics of *Gyrodactylus bullatarudis* (Monogenea) within laboratory populations of the fish host *Poecilia reticulata*. *Parasitology* **89**: 159–194.
- Scott, M. E. and Nokes, D. J. (1984). Temperature-dependent reproduction and survival of *Gyrodactylus bullatarudis* (Monogenea) on guppies (*Poecilia reticulata*). *Parasitology* **89**: 221–228.
- Seghers, B. H. (1974). Schooling behaviour in the guppy (*Poecilia reticulata*): an evolutionary response to predation. *Evolution* **28**: 486–489.
- Sekuler, R., Sekuler, A. B., and Lau, R. (1997). Sound alters visual motion perception. *Nature* **285**: 308.

- Seppälä, O. (2004). Parasite-induced change in host behaviour and susceptibility to predation in an eye fluke-fish interaction. *Animal Behaviour* **68**: 257–263.
- Seppälä, O., Karvonen, A., and Valtonen, E. T. (2008a). Shoaling behaviour of fish under parasitism and predation risk. *Animal Behaviour* **75**: 145–150.
- Seppälä, O., Liljeroos, K., Karvonen, A., and Jokela, J. (2008b). Host condition as a constraint for parasite reproduction. *Oikos* **117**: 749–753.
- Shaw, D. J. and Dobson, A. P. (1995). Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology* **111**: 111–133.
- Shohet, A. J. and Watt, P. J. (2004). Female association preferences based on olfactory cues in the guppy, *Poecilia reticulata*. *Behavioral Ecology and Sociobiology* **55**: 363–369.
- Small, M., Tse, C. K., and Walker, D. M. (2006). Super-spreaders and the rate of transmission of the SARS virus. *Physica D: Nonlinear Phenomena* **215**: 146–158.
- Smith, R. J. F. (1992). Alarm signals in fishes. *Reviews in Fish Biology and Fisheries* **2**: 33–63.
- Sparkman, A. M. and Palacios, M. G. (2009). A test of life-history theories of immune defence in two ecotypes of the garter snake, *Thamnophis elegans*. *Journal of Animal Ecology* **78**: 1242–1248.
- Stein, R. A. and Magnuson, J. J. (1976). Behavioral response of crayfish to a fish predator. *Ecology* **57**: 751–761.
- Stein, R. A. (2011). Super-spreaders in infectious diseases. *International Journal of Infectious Diseases* **15**: e510–e513.
- Stell, W. K., Walker, S. E., Chohan, K. S., and Ball, A. K. (1984). The goldfish nervus terminalis: A lutenizing hormone-releasing hormone and molluscan cardioexcitatory peptide immunoreactive olfactoretinal pathway. *Proceedings of the National Academy of Sciences* **81**: 940–944.
- Stephenson, J. F., Whitlock, K. E., and Partridge, J. C. (2011). Zebrafish preference for light or dark is dependent on ambient light levels and olfactory stimulation. *Zebrafish* **8**: 17–22.
- Stephenson, J. F. (2012). The chemical cues of male sea lice *Lepeophtheirus salmonis* encourage others to move between host Atlantic salmon *Salmo salar*. *Journal of Fish Biology* **81**: 1118–1123.
- Stephenson, J. F., Partridge, J. C., and Whitlock, K. E. (2012). Food and conspecific chemical cues modify visual behaviour of zebrafish, *Danio rerio*. *Zebrafish* **9**: 68–73.

- Stephenson, J. F. (2014). The experimental evolution of parasite resistance in wild guppies: artificial selection, resource availability and predation pressure. *Proceedings of the Royal Society of London B: Biological Sciences* **281**: 20140541.
- Stephenson, J. F., van Oosterhout, C., Mohammed, R. S., and Cable, J. (2015). Parasites of Trinidadian guppies: evidence for sex- and age-specific trait-mediated indirect effects of predators. *Ecology* **96**: 489–498.
- Stoehr, A. M. and Kokko, H. (2006). Sexual dimorphism in immunocompetence: what does life-history theory predict? *Behavioral Ecology* **17**: 751–756.
- Stoltze, K. and Buchmann, K. (2001). Effect of *Gyrodactylus derjavini* infections on cortisol production in rainbow trout fry. *Journal of Helminthology* **75**: 291–294.
- Sullam, K. E., Rubin, B. E. R., Dalton, C. M., Kilham, S., Flecker, A. S., and Russell, J. A. (2015). Divergence across diet, time and populations rules out parallel evolution in the gut microbiomes of Trinidadian guppies. *The ISME Journal* **2015**: 1–15.
- Tadiri, C. P., Dargent, F., and Scott, M. E. (2012). Relative host body condition and food availability influence epidemic dynamics: a *Poecilia reticulata*-*Gyrodactylus turnbulli* host-parasite model. *Parasitology* **140**: 343–351.
- Terai, Y., Mayer, W., Klein, J., Tichy, H., and Okada, N. (2002). The effect of selection on a long wavelength-sensitive (LWS) opsin gene of Lake Victoria cichlid fishes. *Proceedings of the National Academy of Sciences* **99**: 15501–15506.
- Terai, Y., Seehausen, O., Sasaki, T., Takahashi, K., Mizoiri, S., Sugawara, T., Sato, T., Watanabe, M., Konijnendijk, N., Mrosso, H. D. J., Tachida, H., Imai, H., Shichida, Y., and Okada, N. (2006). Divergent selection on opsins drives incipient speciation in Lake Victoria cichlids. *PLoS Biology* **4**: e433.
- Tezuka, A., Yamamoto, H., Yokoyama, J., van Oosterhout, C., and Kawata, M. (2011). The MC1R gene in the guppy (*Poecilia reticulata*): genotypic and phenotypic polymorphisms. *BMC Research Notes* **4**: 31–39.
- Tezuka, A., Kasagi, S., van Oosterhout, C., McMullan, M., Iwasaki, W. M., Kasai, D., Yamamichi, M., Innan, H., Kawamura, S., and Kawata, M. (2014). Divergent selection for opsin gene variation in guppy (*Poecilia reticulata*) populations of Trinidad and Tobago. *Heredity* **113**: 381–389.
- The Association for the Study of Animal Behaviour (2012). Guidelines for the treatment of animals in behavioural research and teaching. *Animal Behaviour* **83**: 301–309.

- Tieleman, B. I., Williams, J. B., Ricklefs, R. E., and Klasing, K. C. (2005). Constitutive innate immunity is a component of the pace-of-life syndrome in tropical birds. *Proceedings of the Royal Society of London B: Biological Sciences* **272**: 1715–1720.
- Tobler, M. and Schlupp, I. (2007). Influence of black spot disease on shoaling behaviour in female western mosquitofish, *Gambusia affinis* (Poeciliidae, Teleostei). *Environmental Biology of Fishes* **81**: 29–34.
- Valentinčič, T. and Caprio, J. (1994). Consummatory feeding behavior in intact and anosmic channel catfish *Ictalurus punctatus* to amino acids. *Physiology and Behaviour* **55**: 857–863.
- Valentinčič, T., Wegert, S., and Caprio, J. (1994). Learned olfactory discrimination versus innate taste responses to amino acids in channel catfish, *Ictalurus punctatus*. *Physiology and Behaviour* **55**: 865–873.
- Valentinčič, T. and Caprio, J. (1997). Visual and chemical release of feeding behavior in adult rainbow trout. *Chemical Senses* **22**: 375–382.
- van Boven, M. and Weissing, F. J. (2004). The evolutionary economics of immunity. *The American Naturalist* **163**: 277–294.
- van Der Veen, I. T. (2005). Costly carotenoids: a trade-off between predation and infection risk? *Journal of Evolutionary Biology* **18**: 992–999.
- Verzijden, M. N. and Rosenthal, G. (2011). Effects of sensory modality on learned mate preferences in female swordtails. *Animal Behaviour* **82**: 557–562.
- von Frisch, K. (1938). Zur psychologie des Fische-Schwarmes. *Naturwissenschaften* **26**: 601–606.
- von Frisch, K. (1941). Über einen Schreckstoff der Fischhaut and seine biologische Bedeutung. *Zeitschrift Fur Vergleichende Physiologie* **29**: 46–145.
- Vielma, A., Ardiles, A., Delgado, L., and Schmachtenberg, O. (2008). The elusive crypt olfactory receptor neuron: evidence for its stimulation by amino acids and cAMP pathway agonists. *Journal of Experimental Biology* **211**: 2417–2422.
- Vilhunen, S. (2006). Repeated antipredator conditioning: a pathway to habituation or to better avoidance? *Journal of Fish Biology* **68**: 25–43.
- Vonlanthen, P., Bittner, D., Hudson, A. G., Young, K. A., Müller, R., Lundsgaard-Hansen, B., Roy, D., Di Piazza, S., Largiader, C. R., and Seehausen, O. (2012). Eutrophication causes speciation reversal in whitefish adaptive radiations. *Nature* **482**: 357–362.

- van Oosterhout, C., Harris, P. D., and Cable, J. (2003). Marked variation in parasite resistance between two wild populations of the Trinidadian guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Biological Journal of the Linnean Society* **79**: 645–651.
- van Oosterhout, C., Joyce, D., Cummings, S., Blais, J., Barson, N., Ramnarine, I., Mohammed, R., Persad, N., and Cable, J. (2006). Balancing selection, random genetic drift, and genetic variation at the major histocompatibility complex in two wild populations of guppies (*Poecilia reticulata*). *Evolution* **60**: 2562–2574.
- van Oosterhout, C., Mohammed, R. S., Hansen, H., Archard, G. A., McMullan, M., Weese, D. J., and Cable, J. (2007). Selection by parasites in spate conditions in wild Trinidadian guppies (*Poecilia reticulata*). *International Journal of Parasitology* **37**: 805–812.
- van Oosterhout, C., Potter, R., Wright, H., and Cable, J. (2008). Gyro-scope: an individual-based computer model to forecast gyrodactylid infections on fish hosts. *International Journal for Parasitology* **38**: 541–548.
- van der Sluijs, I., Gray, S. M., Amorin, M. C. P., Barber, I., Candolin, U., Hendry, A. P., Krahe, R., Maan, M. E., Utne-Palm, A. C., Wagner, H.-J., and Wong, B. B. M. (2011). Communication in troubled waters: responses of fish communication systems to changing environments. *Evolutionary Ecology* **25**: 623–640.
- Warburton, K. and Lees, N. (1996). Species discrimination in guppies: learned responses to visual cues. *Animal Behaviour* **52**: 371–378.
- Ward, M. N., Churcher, A. M., Dick, K. J., Laver, C., Owens, G. L., Polack, M. D., Ward, P. R., Breden, F., and Taylor, J. S. (2008). The molecular basis of color vision in colorful fish: four long wavelength sensitive (LWS) opsins in guppies (*Poecilia reticulata*) are defined by amino acid substitutions at key functional sites. *BMC Evolutionary Biology* **8**: 210.
- Weadick, C. J. and Chang, B. S. W. (2007). Long-wavelength sensitive visual pigments of the guppy (*Poecilia reticulata*): six opsins expressed in a single individual. *BMC Evolutionary Biology* **7**: S11.
- Werner, E. E. and Peacor, S. D. (2003). A review of trait-mediated indirect interactions in ecological communities. *Ecology* **84**: 1083–1100.
- Whittington, I. D. (1996). Benedeniine capsalid monogeneans from Australian fishes: pathogenic species, site-specificity and camouflage. *Journal of Helminthology* **70**: 177–184.
- Willing, E. V. A., Bentzen, P., van Oosterhout, C., Hoffmann, M., Cable, J., Breden, F., Weigel, D., and Dreyer, C. (2010). Genome-wide single nucleotide polymorphisms

- reveal population history and adaptive divergence in wild guppies. *Molecular Ecology* **19**: 968–984.
- Wilson, A. D. M., Krause, S., James, R., Croft, D. P., Ramnarine, I. W., Borner, K. K., Clement, R. J. G., and Krause, J. (2014). Dynamic social networks in guppies (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology* **68**: 915–925.
- Wisenden, B. D., Vollbrecht, K. A., and Brown, J. L. (2004). Is there a fish alarm cue? Affirming evidence from a wild study. *Animal Behaviour* **67**: 59–67.
- Wisenden, B. D., Pogatschnik, J., Gibson, D., Bonacci, L., Schumacher, A., and Willett, A. (2008). Sound the alarm: learned association of predation risk with novel auditory stimuli by fathead minnows (*Pimephales promelas*) and glowlight tetras (*Hemigrammus erythrozonus*) after single simultaneous pairings with conspecific chemical alarm cues. *Environmental Biology of Fishes* **81**: 141–147.
- Wong, B. B. M., Candolin, U., and Lindström, K. (2007). Environmental deterioration compromises socially enforced signals of male quality in three-spined sticklebacks. *The American Naturalist* **170**: 184–189.
- Woolhouse, M. E., Dye, C., Etard, J. F., Smith, T., Charlwood, J. D., Garnett, G. P., Hagan, P., Hii, J. L., Ndhlovu, P. D., Quinnell, R. J., Watts, C. H., Chandiwana, S. K., and Anderson, R. M. (1997). Heterogeneities in the transmission of infectious agents: implications for the design of control programs. *Proceedings of the National Academy of Sciences* **94**: 338–342.
- Worden, B. D., Parker, P. G., and Pappas, P. W. (2000). Parasites reduce attractiveness and reproductive success in male grain beetles. *Animal Behaviour* **59**: 543–550.
- Wyatt, T. D. (2010). Pheromones and signature mixtures: defining species-wide signals and variable cues for identity in both invertebrates and vertebrates. *Journal of Comparative Physiology A: Neuroethology* **198**: 685–700.
- Xavier, R., Faria, P. J., van Oosterhout, C., Johnson, M. B., and Cable, J. (2015). Evidence for cryptic speciation in directly transmitted gyrodactylid parasites of Trinidadian guppies. *PLoS ONE* **10**: e0117096.
- Yokoyama, S. (2000). Molecular evolution of vertebrate visual pigments. *Progress in Retinal and Eye Research* **19**: 385–419.
- Yokoyama, S. and Radlwimmer, F. B. (2001). The molecular genetics and evolution of red and green color vision in vertebrates. *Genetics* **158**: 1697–1710.
- Yunker, W. K., Wein, D. E., and Wisenden, B. D. (1999). Conditioned alarm behaviour in fathead minnows (*Pimephales promelas*) resulting from association of chemical

- alarm pheromone with a nonbiological visual stimulus. *Journal of Chemical Ecology* **25**: 2677–2686.
- Zuk, M. and McKean, K. A. (1996). Sex differences in parasite infections: patterns and processes. *International Journal for Parasitology* **26**: 1009–1023.
- Zuk, M. (2009). The sicker sex. *PLoS Pathogens* **5**: e1000267.
- Zylberberg, M., Klasing, K. C., and Hahn, T. P. (2012). House finches (*Carpodacus mexicanus*) balance investment in behavioural and immunological defences against pathogens. *Biology Letters* **9**: 20120856.