Consequences of host personality and environmental change for parasite infections in freshwater fish

Thesis submitted for the degree of

Doctor of Philosophy

at the University of Leicester

Saman Yaqub

Department of Neuroscience, Psychology and Behaviour

University of Leicester

December 2018

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Abstract

The study of consequences of interaction between host personality and parasite infections can have significant ecological implications. To develop an understanding on the role of host personality on susceptibility of developing infection and disease progression following exposure to parasite infection a novel experimental study was conducted. The model host parasite system used in thesis was consisted of three-spined sticklebacks as host and *Schistocephalus solidus* as parasite. The results of this study show parasite infection can influence voluntary food intake as it progresses in host. In addition, personality type of host can affect the probability of developing infection. Whereas, no evidence of any significant interaction between host personality and parasite infections found on host-parasite biology. These results highlight that personality of animal can influence its fitness and parasite infection can have the potential to affect food intake by causing energetic drain. Furthermore, a novel experimental study demonstrated the significant effects of presence of conspecifics (social context) on personality. This result show that presence of conspecific can influence animal personality.

The second part of the thesis investigated the importance of food availability and rising water temperature on aspects of the stickleback-*Schistocephalus* host-parasite relationship. While increasing temperature is known to influence parasite growth in this system, the potential interaction with food availability is unknown. The results of this novel study confirmed significant effects of increasing temperature on parasite growth with no additional effects of host food intake levels. Furthermore, the larger parasites establishing in fish held under the warmer temperature produced more eggs through *in vitro* culture techniques. In addition, elevated temperature also found to significantly influence level of neurotransmitters in the brains of sticklebacks. As animal are exposed to multiple stressors in wild, therefore the effects of multiple stressors i.e. pollutant (copper) and rising temperature on the parasite fitness were investigated. The results showed no significant effects of multiple stressors on parasite fitness. The final part of this thesis covers details of preliminary laboratory work conducted to develop *Pimephales promelas-Ligula intestinalis* as a model host-parasite system to investigate relevant question in the field of experimental parasitology and behavioural ecology.

The overarching conclusion of the thesis is that environmental rise in temperature can potentially influence not only the host-parasite interaction/biology but underlying neurophysiological pathways controlling behaviour. Further study of interaction between personalities (consistent behaviour) and parasite infections with larger sample size can examine the subsequent effects of this interaction on host-parasite biology.

Acknowledgements

I am extremely grateful to Almighty Allah for His countless blessings over the past 4 years which were very challenging. My PhD journey was a great learning experience and I want to thank everyone who helped me during my PhD.

I would like to start with kind regards and thanks to my funding body Abdul Wali Khan University, Mardan (AWKUM), Pakistan, for sponsoring my PhD studies. I am thankful to my supervisory team, Dr Iain Barber and Dr Will Norton for their supervision. Thanks to Iain for accepting me as a PhD student under his supervision, for his feedback on my thesis and his valuable comments on my project. I am thankful to Will for taking me under his supervision after Iain. Without Will's feedback, I would not be able to put up this thesis together. I want to thank Ceinwen for her help in laboratory, Carl for his help in aquariums, Dr Andrew Young for his help in using HPLC machine and fixing that machine whenever it stopped working and Hector for his help in collecting brain samples. I was blessed to be a part of two research group during my PhD, and I am thankful to everyone in Norton lab and Barber lab for their help.

I am very much thankful to all my friends that I made along my PhD journey. Without their help, support and understanding I would not be able to bring my work to completion. I am thankful to Nawshin Dastagir and her family for being very kind to me. I owe all my understanding of statistical analyses to Aisha Amer. I will never forget our long and non-stop discussions on statistics on the way back from university to home and at home all night until 3 to 4 a.m. Thanks to Huraira for being my friend and for all those meetings over numerous cups of tea and for motivational talks that kept me going. I am grateful to Hajara (Alfa) for her kindness, friendship and all those walks we had along the River Soar with some serious and deep conversations about life and science. Thanks very much for listening. I am very thankful to Rubab, I will never forget the laughter fits we had wherever we travel in the UK, especially when waiting for our train at London underground, at bus stops and at home over innumerable cups of tea – Pakistani tea. I guess you came at the very right time in my life and have turned on the funny side of my personality which I have lost somewhere during tight, intense,

stressed and busy schedules of lab work. I am grateful to Mehwish Marwat for all her help and support.

I cannot thank my family enough for their support, help, patience and understanding over the last 4 years. Without their support and believe in me, I would never be able to come to UK to complete a doctorate research studies. I am very grateful to the superhero of my life – Miss Ajaib Sultana, who let me follow my dream and have always wished me the very best in life. I want to thank my siblings (Shafaq and Farrukh) for their constant love, support and prayers. I am grateful to Ehtesham for being a phenomenal human being, for believing in me and for all his support. Zargham, you are a star, I know that your prayers and best wishes are always with me and kept me going.

In the end, I want to thank myself for all the hard work I did to complete my PhD project. My PhD journey faced some serious setbacks, starting from numerous failed attempts of developing parasite life cycle in laboratory, moving of supervisor to another university, to putting up all experimental work into a thesis. All these challenges helped me to understand that everything happen for a reason and I have become a more patient, courageous, determined, dedicated, resilient and persevered person than before.

Dedication

I dedicate this research work to my father, Muhammad Yaqub, to all my Teachers and to my motherland – Pakistan …

List of Contents

List of tables

List of figures

Chapter 1.

General Introduction

1.1 Introduction

1.1 Parasites and host behaviour

Parasites exist in intimate, continuous partnerships with their host organisms, which directly or indirectly fulfil their metabolic needs (Smyth, 1994). There are two main different types of parasite: ectoparasites, which remain on the surface of host bodies, and endoparasites, which are completely confined within the host body (Goater et al., 2014). In aquatic animals, monogeneans and arthropods are the prime examples of ectoparasites, whereas, protists, myxozoans, flukes, acanthocephalans, nematodes and tapeworms have primarily adapted to the endoparasitic mode of life (Goater et al., 2014). Endoparasite typically exhibit complex, indirect life cycles, which involve obligate intermediate hosts for life cycle completion (Smyth, 1994, Goater et al., 2014). These parasites develop into successive larval stages into intermediate host(s) and use trophic transmission to reach their definitive host, to complete their life cycle and produce eggs. Parasites affect host morphology, biology and fitness through a number of mechanisms, including physical deformation (Barber and Svensson, 2003), altered growth and reproduction by influencing main physiological pathways in the body (Lafferty and Kuris, 2009, Trubiroha et al., 2011) and by influencing the probability of survival (Robar et al., 2010). A number of trophically-transmitted parasite species induce phenotypic changes in their intermediate hosts (Moore, 2002). These phenotypic changes include morphological and behavioural changes, which can have direct consequences on the probability of transmission of parasites from intermediate hosts to definitive hosts by altering patterns of predation (Lafferty and Morris, 1996, Hammerschmidt et al., 2009, Seppälä et al., 2004, Seppälä et al., 2008). Host behaviour and parasite infection are likely to be linked at a range of phenotypic, ecological and evolutionary levels and mechanisms (Hart, 1990). After encountering a host, the parasite has to overcome host behavioural defences and immune responses to establish infection. Parasites are likely to affect host fitness negatively, to facilitate their own growth and development (Hart, 1994). Once they have overcome host behavioural defences and immune responses, parasites are then able to establish and potentially change host behaviour. The influence of parasite infections on the behaviour of hosts has been studied in detail over the last few

decades (Moore, 2002, Moore, 2013, Barber et al., 2000). However, far less is known about, how consistent differences in the behaviour (i.e. 'personality') of individuals in a host population influences the outcome of their interactions with parasites. The following paragraphs review the literature on animal personality, social contexts and parasitism and identify outstanding questions that will be addressed in the thesis.

1.2 Animal personality, social context and parasitism

1.2.1 Interaction between host personality and parasite infections

The concept of animal personalities describes consistent differences in the behaviour of individuals within populations or species over time and across contexts (Reale et al., 2007). Generally, five different behavioural traits or extreme phenotypes used to quantify the personality of individuals in a population in ecological situations: (i) the shyness-boldness continuum, including individual responses to predation; (ii) the exploration-avoidance continuum, including individual responses to novel food or object; (iii) activity in a novel environment; (iv) aggressiveness, individual aggressive responses towards conspecifics; (v) sociability, individual responses towards conspecifics (Reale et al., 2007). Consistent differences between individuals in any of these axes has the potential to influence subsequent interactions with parasites.

An individual's personality can potentially interact with parasite infections in a number of ways (Figure 1.1). In the encounter phase (exposure risk) personality is likely to affect the probability of acquiring infection by affecting the risk or chance of exposure. Within populations, the risk of infection exposure is often linked to differences in personality, with bolder individuals having higher infection rates (Wilson et al., 1993b, Aalvik et al., 2015). For example, in Siberian chipmunks *Tamias sibiricus*, the pattern of activity and exploration predicts an individual's risk of acquiring tick infections (Boyer et al., 2010).

Following exposure to a parasite, the likelihood of infections establishing, and the subsequent progression of disease, depends on the host's physiological and immunological condition and state (i.e. energy reserves) (Albert et al., 2001, Barber et al., 2008). Different personalities may also be likely to exhibit differences in metabolic rate (Mathot et al., 2015), or other aspects of their

physiology, including levels of circulating hormones, which might play role in establishment of infections. For example, in wild caught three-spined sticklebacks *Gasterosteus aculeatus*, variations in expression of genes responsible for the stress cortisol hormone were found to significantly correlate with personality type (Aubin-Horth et al., 2012). Furthermore, in rainbow trout *Oncorhynchus mykiss*, activity and aggression were significantly affected by circulating cortisol levels (Øverli et al., 2002) and humans with type D personality (distressed personality – negative emotions) are likely to be at a greater risk of developing cardiovascular disease, due to higher levels of cortisol alongside other factors (Sher, 2005).

The next stage following development of infection is disease progression. The particular way in which disease progresses and can cause severe and intense behavioural, physiological and immunological changes in the host is termed as the infection phenotype (Barber et al., 2017).

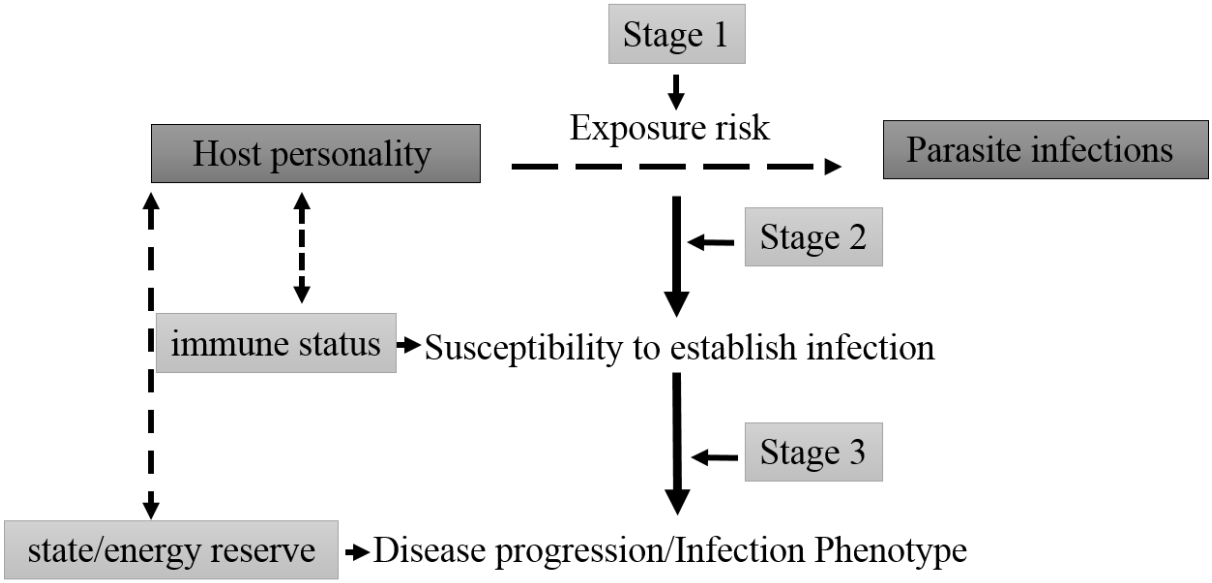

Figure 1.1 Schematic diagram showing the three different stages of host-parasite interaction that may be impacted by individual personality differences. (1) Stage 1; role of personality type in determining risk to parasite exposure (2) Stage 2; the role of personality differences on susceptibility of establishing parasite infections (mediated through links between personality and immunocompetence) and (3) Stage 3; the role of personality differences on the disease progression/infection phenotype (mediated through differences in energetic status linked to personality type).

Individuals with higher growth and fecundity rates are likely to show correlated variation in physiological and behavioural characters that support their lifestyle.

An example of this would be higher food intake rates or greater risk taking during foraging (i.e. boldness) to fulfil greater energy needs of individuals with higher metabolic rates (Biro and Stamps, 2008, Careau and Garland, 2012). According to this view, the personality and energetics of individuals are likely to be linked (Careau and Garland, 2012). From an adaptive perspective, differences in personality arise due to differences in the state of organisms as result of growth-mortality trade-offs (Stamps, 2007, Sih et al., 2015). In a recent study, three-spined sticklebacks quantified for boldness in a non-foraging context were individually tested in maximum voluntary food intake trials. Bold sticklebacks had significantly higher voluntary food intake (Jolles et al., 2016). The food intake rates, productivity and life-history traits (growth and/or fecundity) are expected to be correlated with personality traits such as boldness, activity or aggression in wide range of taxa (Biro and Stamps, 2008). Individual differences in metabolic rates can promote individual differences in behaviour patterns that either consume energy (e.g. courtship activity) or provide net energy (e.g. foraging behaviour) (Biro and Stamps, 2010). The role of individual life-history and metabolic trade–offs, and the mechanism by which they drive consistent variation in suites of behaviour have been reported in studies (Careau et al., 2008, Careau and Garland, 2012). From this point of view that, life-history and metabolic trade-offs can drive consistent variations in suites of behaviour, individual food intake rates are likely to play a crucial role in individual fitness in the context of personality. As parasites depend on the host's energy reserves (i.e. state) to fuel their growth (Barber et al., 2008) and personality is likely to be linked with food intake rates. Furthermore, parasites that impose a metabolic burden on hosts may have value in uncovering the interaction between host personality / food intake rates and parasite infection.

In recent years, the interaction between host personality and parasite infections has gained some attention. The effect of parasitic manipulation of the trematode *Coitocaecum parvum* and *Microphallus spp.* on the behaviour *Paracalliope fluviatilis*, its amphipod host has been studied in the context of behavioural syndromes. The time spent in light by infected individuals was lower compared to uninfected amphipods (Coats et al., 2010). Furthermore, amphipods infected with *Mirophallus spp.,* showed strong correlation between behaviours compared

to non-infected amphipods. This study suggested that, infection can act as a stressor which increases behavioural correlations similar to other studies in which predation causes behavioural correlations (Bell and Stamps, 2004, Dingemanse et al., 2007). Intertidal gastropods *Littorina littorea* infected with the trematode parasite *Cryptocotyle lingua* were found to be more cautious in behaviour with no significant effect on repeatability of behaviour (Seaman and Briffa, 2015). However, to date no studies have investigated the effects of interaction between host personality type and parasite infection on subsequent host parasite biology and their ecological and evolutionary significance.

1.2.2 Social context, personality and host-parasite interaction

One of the main factors that can influence animal behaviour is the social environment in which it occurs. Yet the consequences of social context on the expression of animal behaviour are often underestimated in the laboratory studies (Webster and Ward, 2011). The presence of conspecifics (social context) is likely to influence the expression of animal behaviour by two ways, i.e. either through conformity and/or facilitation effects. Conformity effects arise when the variation in the expression of a given behaviour between individuals within a social group is restricted by the presence of conspecifics. For example, in three-spined sticklebacks, foraging individuals can restrict variation in the expression of preferences for food patches, and individuals prefer social conformity instead of the private (Webster and Hart, 2006). Facilitation effects arise when the presence of conspecifics changes the likelihood and frequency of the expression of a behaviour. For example in the common toad *Bufo bufo*, activity levels were higher among individuals when held in groups than when held alone (Griffiths and Foster, 1998).

Studies investigating the consequences of social context on the expression of behaviour patterns that are consistent over time and across context (i.e. animal personalities) are limited (Webster and Ward, 2011). How inter-individual difference are maintained or changed across different social contexts is an important area of evolution and behavioural ecology, since animals experience a variety of different social contexts in their lifetime. Individual behavioural differences are noticeable under social conditions (Laskowski and Bell, 2014, van

Oers et al., 2005, Webster and Ward, 2011). For example, in zebra finches *Taeniopygia guttata,* the focal bird tends to be more exploratory in the presence of its partner compared to when it is tested alone in exploratory trials (Schuett and Dall, 2009b). In great tits *Parus major*, social context had an effect on the exploratory behaviour by increasing the boldness of individuals (van Oers et al., 2005), whereas in zebra finches individuals were bolder in an asocial context (Mainwaring et al., 2011). In addition, juvenile pumpkinseed fish *Lepomis gibbosus,* showed consistent inter individual differences within both context, however there was no correlation found across contexts (Coleman and Wilson, 1998).

Social context potentially influences the interaction between individual and their physical environment which can subsequently affect individual fitness (Magnhagen and Bunnefeld, 2009, Magnhagen and Staffan, 2005, Schuett and Dall, 2009b, Webster et al., 2007). For example, the survival of asocial lizards was higher in a low-density population, whereas social females produce more offspring (Cote et al., 2008) In mud crabs, *Panopeus herbstii,* behavioural plasticity in boldness is modulated by perceived predation risk. In the presence of conspecifics, crabs showed bolder personality compared to those tested individually, with reduced activity levels in the presence of predatory cues, compared to no predation cues (Belgrad and Griffen, 2017). The set of behavioural phenotypes produced by a single individual in a given set of environment is called behavioural reaction norm (BRN) (Smiseth et al., 2008) and the extent to which the behavioural phenotype change as response to variation in external stimuli is called contextual plasticity (Stamps, 2016). Often individual personalities vary showing behavioural plasticity in different contexts. For example, in a study that demonstrated the role of previous social experience on leadership and risk-taking in three-spined stickleback showed, shy fish tend to behave more boldly in the presence of a bolder partner, showing relative personality' (Jolles et al., 2014).

Parasitism is an ecological factor and likely to influence the inter-individual differences in behaviour in a number of ways (see section 1.2.1). The studies that have investigated the effect of social context on personality primarily focussed on aggression (Verbeek et al., 1996) foraging (Marchetti and Drent, 2000) and

sex differences (Schuett and Dall, 2009a). Other factors that potentially influence inter-individual differences in behaviour could be natural, sexual selection pressure due to environmental variability, predation and parasitism (Dingemanse et al., 2004, Bell and Sih, 2007, Barber and Dingemanse, 2010, Dingemanse et al., 2010, David et al., 2011, Poulin, 2013). However, the effects of parasitism on inter-individual differences in behaviour are under investigated.

1.3 Host-parasite interactions in changing environment

Parasites play a crucial role through their various impacts on hosts, which can include subtle, sub-lethal and lethal effects. Parasites can affect behavioural, physiological, morphological and reproductive aspects of host biology, which ultimately influence ecological processes including food web dynamics and affect the structure of communities. Parasites with multi-host life cycles typically rely on trophic transmission, and their presences is a likely indicator of quality of food web, biodiversity and environmental stress in ecosystem (Marcogliese, 2004). It is therefore important to study the effects of changing environment on host-parasite interaction as this can be a potential indicator of ecosystem health.

Host-parasite interactions may be greatly affected by environmental stressors, including elevated temperature and pollutants (Lohmus and Bjorklund, 2015, Scharsack et al., 2016, Sures et al., 2017b). However, predicting the consequences of changing environments for parasite infections is challenging. In some cases, anthropogenic stressors can increase levels of parasitism, for example by decreasing the resistance of host organisms, or by increasing the density of intermediate and definitive hosts and thereby increasing the number of infective stages. On the contrary, environmental pollutants that act as toxins may cause direct or indirect mortality either to host populations, or to the parasites themselves, thus decreasing levels of parasitism (Lafferty, 1997). Climate change, pollutants and eutrophication are the most significant anthropogenic threats to the natural environment (Poulin, 2007, Budria, 2017).

Ecological conditions can be greatly affected by anthropogenic activities which are likely to modify the transmission rate of parasites. Parasites affect the growth, behaviour, nutritional status and fecundity of individuals and influence trophic interactions in food webs and biodiversity (Cable et al., 2017). Anthropogenic

pollutants in aquatic environments can potentially affect the impact of parasites. Pollutants may increase levels of parasitism by (a) increasing host susceptibility, and (b) increasing the abundance of intermediate hosts/vectors. Pollutants may decrease levels of parasitism if (a) infected animals have higher mortality under stressed environment, (b) parasites are more susceptible to the pollutant than the host, or (c) pollutants negatively affect the survival of intermediate hosts (Lafferty and Kuris, 1999). Pharmaceutical wastes and agricultural chemicals can alter the susceptibility of parasitic infection by affecting the physiological and immunological conditions of the host (Morley, 2009). For example, the susceptibility of the amphibian host *Rana sylvatica* to its trematode parasite *Echinostoma trivolvis* increased after exposure to a herbicide contaminant atrazine at a concentration of 30 µg/L (Koprivnikar et al., 2007). Accumulation of metals in tissues of both hosts and parasites provides valuable information about the quality of the environment. A number of endoparasite have been used in research including trematodes, cestodes, nematodes and acanthocephalans. Intestinal helminths are bio indicators for heavy metal contamination in aquatic habitats (Sures, 2003). Parasites respond to pollution in a number of ways, as pollutant sinks, indicators of ecosystem health and as bio indicators of pollutants (Sures et al., 2017b) and they may act as driving agents for key ecological processes (Sures et al., 2017a).

Animals are potentially exposed to a number of abiotic and biotic factors in their natural environment. Chemical (anthropogenic) and natural stressors (predation, parasitism and competition) have the potential to interact and affect living species either synergistically or antagonistically, in different ways (Holmstrup et al., 2010, Laskowski et al., 2010) and multiple stressors are often more damaging than single stressor (Sih et al., 2004c). Multiple stressors that animals face in natural environment could be anthropogenic stressors and natural stressors including, parasites, predation and competition (Christensen et al., 2006, Coors and De Meester, 2008). There are three possible mechanisms by which multiple stressors could interact to affect animal health (Coors and De Meester, 2008). In the first mechanism, multiple stressors could interact additively and produce a larger effect when add on together. For example, in male fathead minnows, *Pimephales promelas;* five estrogenic chemicals, estradiol, ethynylestradiol,

nonylphenol, octylphenol and bisphenol add on together in an additive manner to produce female egg yolk protein precursor VTG, compared to when they are applied alone (Brian et al., 2005). When the multiple stressors produce less effect then expected it is called an antagonistic effect (Holmstrup et al., 2010). On the contrary, the effect which is bigger than expected from simple additive effects is called a synergistic effect (Marcogliese et al., 2010). For example, limb deformities in the wood frog *Rana sylvatica* increase as a result of synergistic interactions between trematode infection and agricultural waste (runoff) exposure, where individually trematode exposure have relatively little effect (Kiesecker, 2002). Changing environments can potentially influence host-parasite interactions. Studies have started to investigate the detrimental effect of changing environments including temperature, pH, and pollutants on the host parasite interactions (Martin et al., 2010, Mitchell et al., 2005, Macnab and Barber, 2012, Macnab et al., 2016, Franke et al., 2017). However, far less is known about how multiple anthropogenic stressors impact existing host-parasite relationships.

1.3.1 Temperature change and host-parasite interactions

The increased anthropogenic production of greenhouse gasses is a leading cause of global climate change. The greenhouse gasses mainly trap thermal energy in the lower layer of atmosphere which increases temperature (McMichael et al., 2006). Temperature change as a result of global warming affects host-parasite interactions (Poulin, 2006). The mechanisms by which this occurs can be diverse. Temperature changes can affect host susceptibility to infection, for example, offspring of *Daphnia magna* held at higher temperature and under a restricted diet show more resistance to infections of the bacterial parasite *Pasteuria ramosa* (Garbutt et al., 2014). Similarly, climatic temperature affects the pathology and dynamics of host-parasite interactions. A rise of 3˚C in temperature results in the release of the parasite *Ribeiroia ondatrae* nine months early from its intermediate snail host with a four times higher mortality of the infected snail host. As a consequence, the overlap between parasite and its amphibian definitive host reduces with less parasitic load and reduced pathology of 67% (Paull and Johnson, 2014). Temperature changes can also affect the host's thermal preferences due to the influence of parasite-induced changes in

host thermal choice. This was demonstrated in an experimental study in which plerocercoids of the cestode parasite *Schistocephalus solidus* grew and developed more rapidly in a *Gasterosteus aculeatus* host, which preferred to stay in warmer temperatures when infected (Macnab and Barber, 2012). Elevated temperatures have broad ecological consequences by affecting host population dynamics. Microphallid trematodes use amphipod, *Corophium volutator* as a second intermediate host and mud snails as first intermediate host. To test the effects of climate change on the transmission of parasite from snail to amphipod studied in a three month mesocosm experiment. The plant and animal community were exposed to four different treatments of; mean temperature of water 18˚C (lower range) and 22˚C (higher range) with <4% (low) and 31% (higher) trematode prevalence in snail population respectively. There was significant impact of snail parasitism and temperature on amphipods with higher levels of parasitism at higher temperature and complete removal of amphoipods.(Larsen and Mouritsen, 2014).

One of the critical factors that determine parasite growth and reproduction is host nutrition. Therefore, the host's nutritional status is potentially the key factor that is important for survival of both host and parasite. For example, the spore production of the microsporidian parasite *Vavraia culicis* was elevated as the nutrition of its host *Aedes aegypti* increased (Bedhomme et al., 2004). In ectotherms, the metabolic burden rises at elevated temperatures. For instance, in juvenile cobia (*Rachycentron canadum)*, fish grew faster at 33˚C when fed to satiation, whereas at 27˚C the growth rate was found to be equal or better when food was restricted, with a significant lower growth rate at 21˚C in all treatments except starvation (Sun and Chen, 2009). However, the effect of different food intake levels at elevated temperatures on the outcome of host-parasite interaction is still under investigated.

In the stickleback-*Schistocephalus solidus* model system, the parasite relies on host-derived nutrients for growth and development (Barber et al., 2008). Some recent studies (Macnab and Barber, 2012, Franke et al., 2017) have demonstrated that, when food is provided *ad libitum*, elevated temperatures can have opposite consequences for hosts and parasites in the stickleback-*Schistocephalus solidus* host-parasite system. At 20˚C, plerocercoids grew more

quickly compared to the hosts, which showed higher growth at 15˚C. Furthermore, the larger size achieved by parasites at the higher temperature is expected to lead to a proportional increase in egg production by the adult parasites (Dorucu et al., 2007). On the other hand, since climate change can affect the quality and quantity of available food to living organisms (Woodward et al., 2010) it is unclear how altered food availability would impact the growth of hosts and parasites under changing thermal regimes.

1.3.2 Neurophysiological changes in response to stressors

Animals are exposed to a range of environmental and biological stressors. These stressors may include anthropogenic stressors, parasites, competition and predation (Christensen et al., 2006, Coors and De Meester, 2008). In response to stressors, various physiological pathways activated in the body including the hypothalamic-pituitary-adrenal and monoamine neurotransmitter systems (Winberg et al., 1997). The chemical substances namely, dopamine (DA), norepinephrine (NE) and serotonin (5-HT) are produced to control various physiological pathways and function in body are classified as monoamine neurotransmitters. Studies have shown the role of different brain monoamine neurotransmitters on the animal's behaviour by influencing different signalling pathways. For example, the monoamine neurotransmitters norepinephrine (NE), dopamine (DA) and 5-hydroytryptamine (5-HT) influence feeding in teleost fishes (de Pedro et al., 1995, de Pedro et al., 1998, Kulczykowska and Sánchez Vázquez, 2010). Dopamine and serotonin also affect fish swimming and social behaviour (Winberg et al., 1993, Øverli et al., 2004). In goldfish the neurotransmitters serotonin (5-hydroxytryptamine: 5-HT), dopamine (DA) and norepinephrine (NE) influence feeding (de Pedro et al., 1998, de Pedro et al., 1995) and they also affect locomotion in the arctic charr, *Salvelinus alpinus* (Winberg et al., 1993).

Abiotic environmental stressors, for example elevated temperature in juvenile common carp *Cyprinus carpio*, can result in higher levels of monoamine metabolites (Boeck et al., 1996). The biotic stressors, for example parasitism has been reported to influence neurotransmitter levels in animals, For example, in a study of three-spined sticklebacks infected with *Schistocephalus solidus* levels

of 5-HIAA:5HT were elevated in the hypothalamus and brainstem of infected fish, and 5-HT and NE in the telencephalon of control sticklebacks (Øverli et al., 2001). In another study, the parasite infections of trematode parasite; *Euhaplorchis californiensis* enhances dopamine activity and reduces serotonin activity in the hypothalamus and hippocampus regions of Californian killifish *Fundulus parvipinnis* brain (Shaw et al., 2009) with increased locomotory behaviour.

Temperature change can affect various aspects of host-parasite interactions ranging from growth, behaviour, susceptibility and disease emergence and progression (Paull and Johnson, 2011, Macnab and Barber, 2012, Garbutt et al., 2014, Paull and Johnson, 2014), particularly the severity of parasite infection. Under changing temperature which can have serious effects on the severity of parasite infection, the effects of infection on the neurophysiological mechanism (amount of neurotransmitters) is poorly understood and require further investigation.

1.3.3 Copper toxicity and host-parasite interactions

Anthropogenic activities resulting from industrial processes release a wide variety of environmental pollutants in aquatic environments, which constitutes a major threat to aquatic ecosystems and the organisms that they contain. One such group of pollutants are the heavy metals, which are often toxic to aquatic organisms (Wong and Pak, 2004). The toxic effects of copper on the shore crab (*Carcinus maenas*), common limpet (*Patella vulgate*) and blue mussel (*Mytilus edulis*) were investigated with exposure to copper concentrations for 7 days. The effect of 6.1µg/L copper was significant on lysosomal stability, neurotoxicity, metabolic impairment, physiological status and induction of protective metallothionein proteins. The concentration of 68.1 µg/L copper produced cellular and neurotoxic effects in *C. maenas* (Brown et al., 2004). In addition, the juvenile striped bass *Morone saxatilis* showed, exposure to copper for 7 days had significant effects on tissue specific transcription for immune system genes, with a four-fold increase of mRNA at 42 ppb (42µg/L) Cu and a ten-fold increase at 160 ppb (160µg/L) Cu on transcription of metallothionein in the spleen (Geist et al., 2007). Copper (Cu) which is a naturally occurring element, is also produced by a range of anthropogenic activities. It is an essential micronutrient that can

lead to harmful effects if available in excess. In crustaceans, Cu is uptaken either by food intake or through the permeable body surfaces and is then transported in the haemolymph upon binding to metabolites (Marsden and Rainbow, 2004). For example, in developmental stages of *Mesocyclops pehpeiensis* (a freshwater copepod), exposure to heavy metals reduces the nauplius development compared to controls in both acute and chronic toxicity tests (Wong and Pak, 2004). In another study, exposure to a higher concentration of cadmium increased the mortality of copepods such as *Diaptomus forbesi* (Ghosal and Kaviraj, 2002). Furthermore, the effects of acclimation of different concentration of copper studied on *Daphnia magna*. The results showed that, there was two-fold increase in acute copper toxicity in multiple generation as acclimation concentration increases (Bossuyt and Janssen, 2003). For example, the endoparasite trematode *Posthodiplostomum minimum* uses an aquatic snail as its first intermediate host and the bluegill sunfish *Lepomis macrochirus* as its second intermediate host. Exposure of the snail hosts to copper solutions showed a ~64% mortality in response to 44.6 µg/L copper and ~87% to 89.2 µg/L copper. Free living stages of the parasite *Posthodiplostomum minimum* showed a toxic effect of copper (9-h LC50-32 µg/L and 12-h LC50-26.0 µg/L) (Soucek and Noblet, 1998).

For parasites with complex, multi-host life cycles, the environmental conditions and host can change at every single stage. Host-parasite interactions in aquatic environments are not only exposed to different environmental pollutants but also global temperature changes, potentially impacting the dynamics of host parasite interactions. Temperature change has already been shown to have a significant effect on the growth of parasites in fish hosts (Franke et al., 2017, Macnab and Barber, 2012), making it important to investigate the combined effects of stressors on the parasite fitness as it progresses through its life cycle. Studies of disease progression in intermediate hosts under altered heavy metal conditions are scarce. In *Cyclops strenuus* infected with procercoids of *Bothriocephalus acheilognathi*, tapeworms exposed to 100 µg/L Cadmium showed decreased copepod survival (Khalil et al., 2014) suggesting the detrimental effect of heavy metal exposure and parasitism on copepod mortality. The effects of multiple environmental stressors on parasite fitness are under investigated.

1.4 Three-spined stickleback- *Schistocephalus solidus* model system

The model host-parasite system used in this research consisted of the three-spined stickleback as the host and *Schistocephalus solidus* as the parasite. The three-spined stickleback-*Schistocephalus solidus* system is an excellent experimental model to study host-parasite interactions (Barber and Scharsack, 2010). Growth and energetics of the fish and parasite in this host-parasite interaction are well documented (Barber et al., 2008, Arnott et al., 2000, Barber, 2005). This model system has also been used to study the consequences of environmental perturbation on host parasite interactions (Franke et al., 2017, Macnab and Barber, 2012, Macnab et al., 2016). Furthermore, the effects of growth and ontogeny of *S. solidus* within the first intermediate hosts (copepods) on fitness in three-spined sticklebacks are well documented (Benesh and Hafer, 2012).

1.4.1 The three-spined stickleback Gasterosteus aculeatus

Three-spined sticklebacks are small fish in the Family Gasterosteidae that are widely distributed in waters of the northern hemisphere (Wootton, 1976). Their length ranges from 4-8 cm, with three distinctive dorsal spines and a variable number of bony plates. The male in this species possess a bright red throat during the spawning season. It builds a fibrous nest for the female to lay eggs in. A typical female usually lay 90-450 eggs which hatch in 5-20 days. Sticklebacks usually feed on invertebrates, mainly crustaceans, small worms and insect larvae (Maitland, 2000). Three-spined sticklebacks have been used extensively as a model animal in laboratory studies in different fields of biology, including evolutionary ecology, reproductive biology, parasitology, molecular genetics and ecotoxicology. The ability to breed them in the laboratory by facilitating natural spawning and by using *in vitro* fertilization (IVF) protocols, and their small size make, them an ideal species for research (Barber et al., 2000, Huntingford and Ruiz-Gomez, 2009, Barber and Nettleship, 2010, Barber, 2013).

Three-spine sticklebacks harbours different types of parasites (Barber, 2007, Zander et al., 2002) both ecto and endoparasite. The commonly found ectoparasites in sticklebacks is Gyrodactylus (Platyhelminthes). These parasites affect fins, gills and skin of their stickleback host (Özer et al., 2004). As a host,

three spine sticklebacks can play vital role in the completion of multi-host parasite life cycle. Among endoparasite, commonly occurring parasites are Diphyllobothrum sp., (Barber, 2007) *Diplostomum spathaceum, Diplostomun gasterostei*, (Kalbe et al., 2002) and *Schistocephalus solidus* (Barber and Scharsack, 2010).

In this thesis, sticklebacks have been used as a second intermediate host of cestode parasite *Schistocephalus solidus* in experimental studies.

1.4.2 Schistocephalus solidus

Schistocephalus solidus is diphyllobothriidean cestode that needs to undertake phases of development in each of three separate hosts in order to complete its life cycle (Smyth, 1990, Barber and Scharsack, 2010). The first intermediate host (IH1), a cyclopoid copepod, becomes infected after ingesting the free-swimming larval form, the coracidium. On development of infection, the parasite grows into a procercoid in the haemocoel of the copepod, with the formation of a cercomer indicating infectivity to the next host (Smyth and McManus, 1989). The second intermediate host (IH2), is the three-spined stickleback. It is the only obligate host (Barber and Scharsack, 2010) and becomes infected after ingesting copepods infected with a procercoid containing a developed cercomer. In the digestive tract of the fish, the procercoid sheds its outer layer in fish stomach, and bores through the intestinal wall to make its way to the body cavity for further development into the plerocercoid larva form (Hammerschmidt and Kurtz, 2007). At this stage it is likely to attain the sexually developed stage called progenesis (Dorucu et al., 2007). Furthermore the age of *S. solidus*, a correlation between size and ontogeny, can affect the infection probability of the fish host (Benesh and Hafer, 2012). Plerocercoids rely on their hosts' energy reserves for growth (Barber and Svensson, 2003) and become infective once they attain a size of 50 mg in stickleback hosts (Tierney and Crompton, 1992). In the definitive host the parasite starts laying eggs within 48 hours of arrival in the host intestine. These eggs are transported out of the body into water via the faeces of the fish (Barber and Scharsack, 2010). After incubation the eggs hatch into free swimming coracidia. However, in laboratory controlled conditions the definitive host can be replaced by *in vitro* culture techniques (Smyth, 1946, Smyth, 1954).

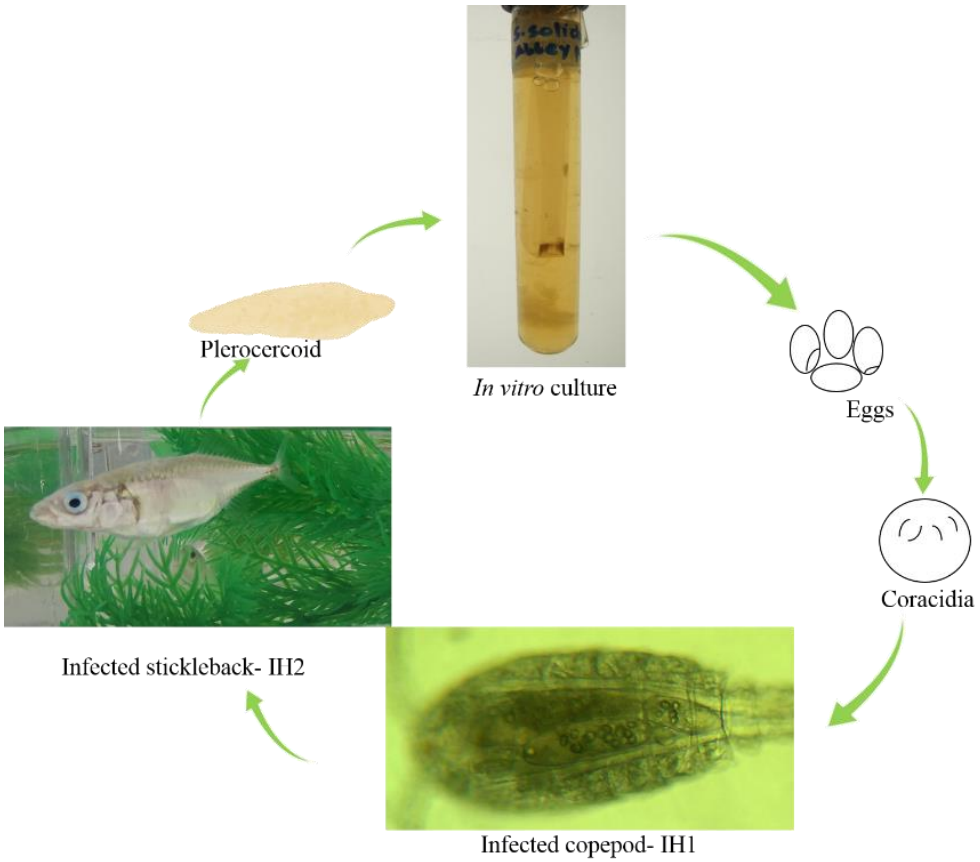

Figure 1.2 The life cycle of *Schistocephalus solidus* (Images by Yaqub. S)

1.5 Aims and objectives of the thesis

The overall aim of thesis was to investigate the consequences of host personality and environmental change for parasite infections in freshwater fish. In the first part of the thesis, the effects of social context and parasitism were investigated on the personality and behaviour. This was followed by investigating the effects of interactions between personality and parasite infections on the subsequent host-parasite biology.

The second half of thesis investigated the effect of environmental change for parasite infections in fish. Two main environmental factors i.e. changing temperature and heavy metal pollutant (copper) were used as an environmental change factors. This section commenced by studying the effects of temperature change under altered food intake ration of host on subsequent host parasite biology. In addition, the effects of temperature change were investigated on the neurochemical physiology of fish. Then, the consequences of pre-exposure to

environmental pollutant i.e. copper in the first intermediate host (copepod) were investigated on the fitness (i.e. growth) of *S. solidus* in the second intermediate host (fish) at two different temperatures. Four separate experimental studies were undertaken, and the results of these studies are described in the following chapters of the thesis.

1.5.1 Chapter 2

There is an increasing interest in the causes and consequences of personality variation among individuals in natural populations, but often empirical studies test the personality traits of individuals in isolation from other conspecifics, which may not reflect their natural ecological situation. In this chapter individuals were tested for their personality traits in the presences of their conspecifics to answer following questions.

1) Are consistent differences in behavior of individuals maintained across social contexts?
2) Does parasitism affect behavior in sticklebacks?

1.5.2 Chapter 3

The parasite infection can have the potential to affect the behavior of their hosts, but the interaction between host personality (consistent behavior) and parasite infections is still under investigated. In this chapter, the consequences of interaction between host personality and parasite infections on the infection phenotype were investigated. The hypothesis tested in this chapter include:

1) Does personality exist in the laboratory bred three-spined sticklebacks?
2) Does personality affect the probability of developing infection following exposure to *S. solidus*?
3) Does interaction between personality and parasitism affect the subsequent host-parasite biology?
4) Does parasite infections and personality affect the voluntary food intake in sticklebacks?
5) Does infections of *S. solidus* affect behaviour in sticklebacks?

1.5.3 Chapter 4

Natural environments are under anthropogenic threats in the form of climate change, pollutants and eutrophication. The effects of global temperature change are multidimensional and can have the potential to affect the availability of food. The effects of temperature change under altered food intake ration of host can have considerable effects on subsequent host parasite biology. To investigate this hypothesis an experimental study was carried out in chapter 4 of this thesis.

1.5.4 Chapter 5

Temperature change have the potential to affect the host-parasite biology with more pronounced effects on parasite fitness. Parasites can affect the neurophysiology of their host by causing chronic stress. Under temperature change, the consequences of parasite infections on neurophysiology is under investigated. The hypothesis tested in the chapter 5 include:

1) Do parasite infections of *S. solidus* affect neurotransmitter levels of sticklebacks?
2) Does temperature change influence neurotransmitter levels in sticklebacks?
3) Does interaction between temperature and parasite infections affect neurotransmitters in sticklebacks?

1.5.5 Chapter 6

Elevated temperature favors enhanced parasite growth in the second intermediate host. In nature, animals are exposed to number of environmental perturbation and can have considerable ecological implications. The effects of multiple stressors on the fitness of multi-host parasite are poorly understood. To investigate effects of multiple environmental stressors on parasite fitness as it progresses from one intermediate (copepod) host to the second intermediate host (fish) during its life cycle are poorly understood. The research question addressed in this chapter were

1) Does pre-exposure to environmental pollutant affect susceptibility to develop infection in fish host?

2) Does pre-exposure to environmental pollutant in copepod host and temperature in fish host affect parasite fitness?

1.5.6 Chapter 7

This chapter includes the research work undertaken to develop the fathead minnow as an experimental host for investigating the impact of experimentally-induced *Ligula intestinalis* infections on host behaviour and personality.

Chapter 2.

Personality, social context and parasitism in three-spined sticklebacks

2.1 Abstract

The effects of conspecific presence (i.e. the social context) can impact the behavioural responses of individual animals and have potential ecological implications. In addition, the consequences of parasite infections for the behaviour of host animals are also increasingly recognised. However, how the effects of parasite infections on behaviour of host organisms interact with the presence and absence of conspecifics is less well understood. In this chapter, results of an experimental study examining the effects of social context on personality and effects of parasitism on behaviour are reported. First, individual three-spined sticklebacks *Gasterosteus aculeatus* were quantified for their individual behavioural responses in the asocial and social context respectively. Individual fish were then either exposed to experimental parasite infections, or were sham-exposed, by being fed either a copepod infected with infective larvae of the parasite *Schistocephalus solidus*, or a non-infected copepod. After ten weeks of exposure / sham-exposure, each individual fish was then re-tested for their individual behavioural response. The results showed significant effects of social context on personality and individual were consistently more active in the presence of conspecifics compared to their absence. Further, parasitism showed significant effects on the mean personality scores whereas there was no effect of parasitism noticed on the behaviour.

2.1 Introduction

From both a behavioural ecological and an evolutionary perspective, it is important to understand the extent to which consistent inter-individual behavioural differences are maintained or changed across different contexts. One of the main modulating factor that can influence individual behavioural responses is the presences of conspecifics, i.e. the social context frequently impacts observed patterns of behaviour (Webster and Ward, 2011). The social context can have significant effects on individual behavioural responses (van Oers et al., 2005, Schuett and Dall, 2009a). For example, in zebra finches *Taeniopygia guttata* focal birds tended to be more exploratory with its partner as compared to when they were tested alone for exploratory trials (Schuett and Dall, 2009a). In great tits, *Parus major*, the social context had an effect on the exploratory behaviour, by increasing the boldness of individuals (van Oers et al., 2005). To investigate whether temporally consistent differences in the behaviour of individuals are maintained across social contexts, it is therefore important to examine the behaviour, and the consistency of behavioural responses, of test subjects across both asocial and social contexts. However, many studies designed to study consistent individual differences in behaviour (i.e. 'personality') are undertaken in an asocial context, despite the fact that the subjects of such studies in nature often live in groups.

Another ecological factor that has the potential to cause inter-individual differences is parasitism (Barber and Dingemanse, 2010, Poulin, 2013). It is well-documented that parasites can influence a range of host behaviours, including foraging, predatory and shoaling behaviour (Barber et al., 2000, Morgan, 2003, Moore, 2013). Furthermore, parasites have the potential to generate state-dependent personality differences in infected individuals of a population, and can potentially act as a selective agent by influencing host behaviour, temporal consistency, host reaction norms and behavioural syndromes (Barber and Dingemanse, 2010, Poulin, 2013). To investigate whether parasitism act as selection agent, it is therefore important to examine the effects of parasites on behaviour.

Parasites are known to affect a host's social behaviour. Many studies have examined how shoaling behaviour in fish is influenced by parasite infections. Experiments measuring the shoaling preference of social western mosquitofish *Gambusia affinis* showed that social individuals shoaled more and swam less than asocial individuals. Asocial individuals were more likely to prefer large shoals during the short time that they interacted (Cote et al., 2012). Parasites tend to affect social interaction of infected hosts. For example, in rainbow trout *Oncorhynchus mykiss* infected with eye-dwelling metacercariae of the trematode *Diplostomum spathaceum,* shoaling behaviour was significantly lower than in control fish (Seppala et al., 2008). Similarly, female western mosquitofish, *Gambusia affinis* infected with the trematode *Uvulifer* sp. had significantly reduced shoaling tendencies (Tobler and Schlupp, 2008). European minnows, *Phoxinus phoxinus,* infected with *Ligula intestinalis* also showed increased neighbour distance in dynamic schools (Barber and Huntingford, 1996). Altered shoaling behaviour is likely reason for infected host to be more susceptible to predation to reach definitive host. In this study, the European minnows were naturally infected with parasite infection. However, the effects of controlled laboratory infection can give more insight and therefore important to examine the effects of parasitism on shoaling behaviour in experimentally infected fish.

The main questions that were addressed in this chapter are: (1) Are consistent differences in the behaviour of individuals maintained across different social contexts? (2) How does parasitism affect behaviour across different social contexts? (3) Does experimental infection affect shoaling behaviour in sticklebacks?

2.2 Methods

2.2.1 Fish supply and husbandry

A total of twenty adult, laboratory-bred sticklebacks produced from parental stock originating from Clatworthy Reservoir, Somerset U.K. (N51˚07'29'' W3˚36'29'') were randomly selected from stock tanks. Each individual fish was blotted dry and measured (standard length, *SL*, to 0.1 mm) and weighed (wet mass, *M,* to 0.001 g) before being transferred into holding aquaria and maintained at a

temperature of 12˚C ± 1˚C, under a 12L: 12D photoperiod throughout the experimental period.

Two glass holding aquaria (60L×40W×30H cm) used for keeping fish throughout the experimental period. Each glass aquarium further divided into 10 compartments; each compartment (19.3 L×11.3W×20H cm) contained a bio-filter for aeration, filtration and enriched with a gravel substratum. One fish kept in each compartment. Fish were maintained for one week in holding aquaria to acclimatise them to the new environment, and were fed daily *ad libitum* and to excess with bloodworms (*Chironomus* spp. larvae) throughout the experiment.

2.2.2 Overview of experimental approach

The experiment divided into five phases (Figure 2.1). In the first phase of experiment, sticklebacks were tested for two behavioural tests (i.e. activity in novel environment and exploration of novel object to quantify their personality in asocial and social context respectively. The fish were then either sham-exposed (i.e. controls, N= 6) or experimentally exposed to infective stages of the parasite *Schistocephalus solidus* (N= 12) and allowed to grow for 10 weeks. Individual difference in behaviour quantified in both contexts at 10 weeks post-exposure or sham-exposure. Fish were then tested for their shoaling behaviour against a shoal of three infected fish. At the end of study, fish were sacrificed, and infection status was recorded.

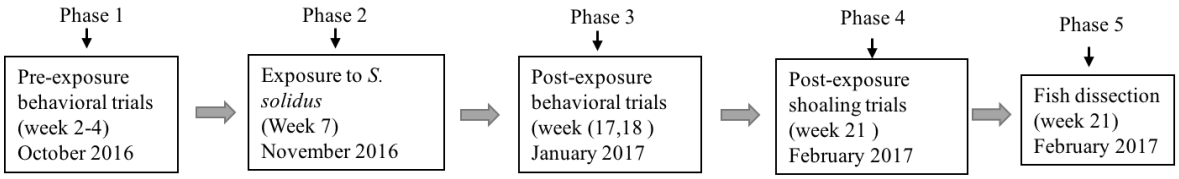

Figure 2.1 Schematic diagram summarising the experimental study designed to examine the effect of social context and experimentally induced parasite infection of *S. solidus* on the behaviour of three-spined sticklebacks.

2.2.3 Behavioural screening tank setup

A square Plexiglas tank (40W×40L×15H cm) including an internal acclimation zone (10W×10L×15H cm) with a sliding door on one side was used (Figure 2.2.a). When measuring activity in a novel environment, three stone mounts (8 cm height, 2-3 cm radius) were used to make the environment novel, whereas,

in the exploration of novel object trials two different novel objects including a plastic ball (green and white in colour) and a Universal container (30ml) filled with wet sand were used. The stone mounts differed from the novel objects in both size, colour and outer texture.

For behavioural testing in the social context (i.e. in the presence of conspecifics), the original tank (40W×40L×15H cm) was placed inside a larger square tank (dimensions: 50W×50L×20H cm). Ten conspecific sticklebacks were introduced into the larger tank to act as stimuli for the focal test fish (Figure 2.2.b), and the inner tank was perforated with 2-3mm diameter holes to allow the transmission of any olfactory cues. To ensure conspecifics distributed evenly around the tank, eleven dividers (3W×50L×100Hmm with 2-3mm diameter holes) were used (Figure 2.3).

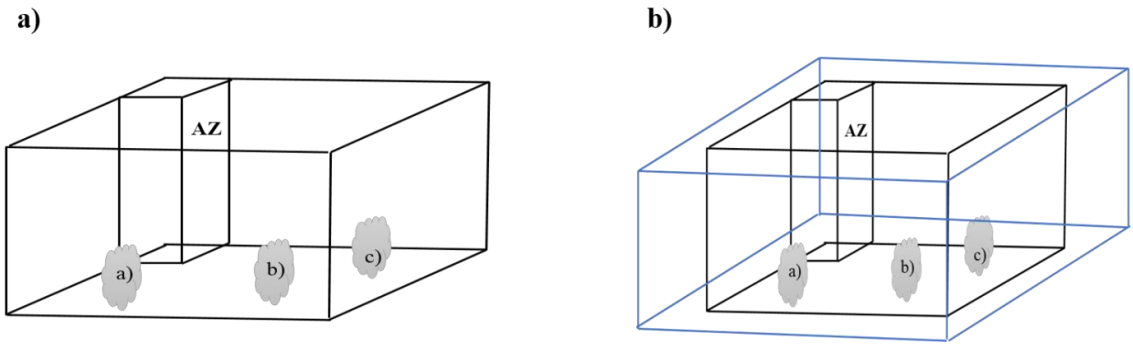

Figure 2.2 Diagrammatic illustration of the experimental tanks to assess temporal consistency in behavior of three spined sticklebacks in two experimental arenas. (a) The square tank used for behavioral screening of activity in novel environment (A-NE) and exploration of novel object (E-NO). AZ is acclimation zone and grey shaded area (a), (b) and (c) were stone mounts in A-NE, whereas in E-NO three stone mounts were replaced with single novel object for asocial context. (b) Additional square tank with square tank (a) inside for testing behavioral consistency in social context.

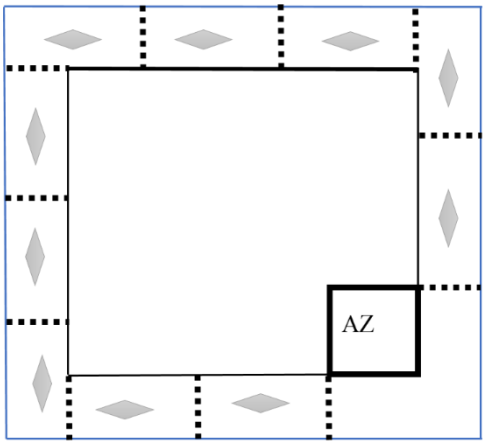

Figure 2.3 View of the experimental setup in social context ( ) represents individual fish used as conspecific in a social context.

2.2.4 Behavioural assay

After one week of acclimation in the holding aquarium, all fish were tested for two personality traits (i) activity in a novel environment, and (ii) exploration of a novel object. Each fish was quantified first in the asocial context, and then in the social context. Each behvioural test was conducted twice during pre-exposure phase, to test consistency of behaviour. All individuals were tested for the activity in novel environment (NE) on the first day and for second behaviour, exploration of novel object (NO) the next day. The next trial was conducted after a gap of four days. Ten weeks after parasite exposure or sham-exposure, all individuals were tested again for activity in novel environment twice, with a four-day gap between replicate trials. Fish failed to show any consistency in exploring a novel object in the asocial context, therefore fish were tested for one personality trait, i.e. activity in the novel environment (NE) only during the post-expoure behavioural trials (phase 3).

Prior to each behavioural screening test, individual fish were transferred gently by hand net from the home tank to a 1-L static (off-system) aquarium (18L×11W×8H cm) to reduce any stress. After a few minutes, fish were transferred by net into the acclimation zone (AZ) in the experimental arena with door closed for 300 seconds to reduce any stress caused by handling and transfer before start of behavioural recording. After a settling period of five minutes, the door of the acclimation zone was gently and remotely opened, and the behaviour (activity in NE and/or exploration of NO) was recorded for five

minutes using a webcam (Logitech c920HD) connected to a laptop computer, for subsequent analysis.

2.2.5 Data collection from behavioral recordings

Recorded videos were replayed by using Windows Media Player on a Windows desktop computer, and five different variables were scored manually for activity in the novel environment. Detail of variables is explained in Table 2.1. All these five variables were scored for the two trials in each context. For exploration of the novel object, five variables including latency to leave the acclimation zone, time spent in the less exploratory zone, time spent in the more exploratory zone, number of visits in the less exploratory zone and number of visits in the more exploratory zone were scored manually. The size of the less and more exploratory zone were adapted from published literature (Castanheira et al., 2013). All videos were scored by the same observer, and to standardise data recording a template sheet was used for each fish. Video replayed and the test i.e. activity in novel environment (Figure 2.4.a) and exploration of novel object (Figure 2.4.b) the variables (Table 2.1) were marked on the template sheet with fish identity number. After marking template sheets for all recorded films, the scores were transferred into the data sheet randomly. Furthermore, once the data transferred into Excel sheet than for the first time the scores were arranged in the proper order i.e. context, test type, fish identity and trial number.

Table 2-1 Detail of the five variables that were scored to test the activity of three spined sticklebacks in the novel environment in both asocial and social context. Variables were scored in each of two trials to test the temporal consistency of behavior.

No.	Variable	Abbreviation
1	Latency to leave acclimation zone	LE-AZ
2	Number of squares (grid) moved in peripheral zone	F-PZ
3	Number of times squares crossed in peripheral zone	No-PZ
4	Number of squares (grid) moved in inner zone	F-IZ
5	Number of times squares crossed in inner zone	No-IZ

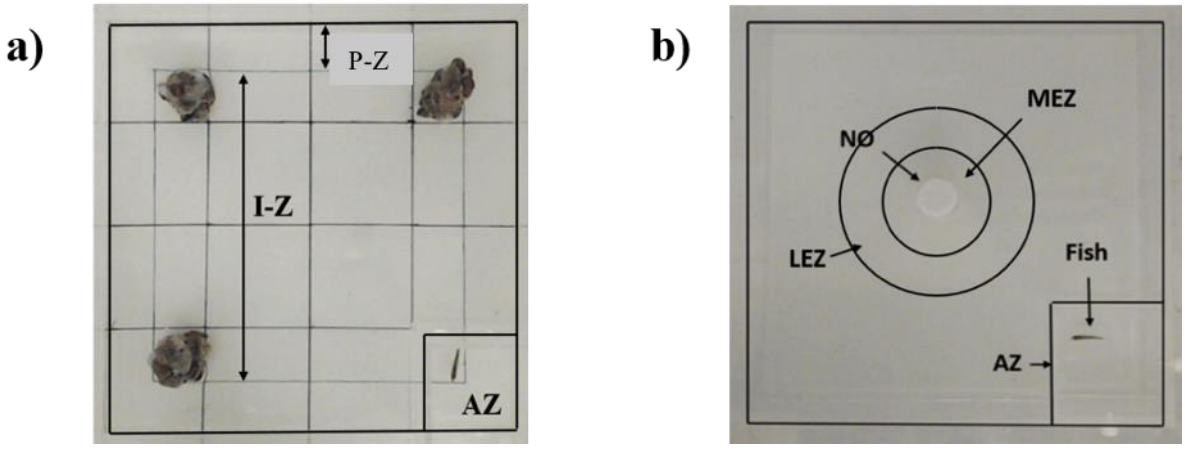

Figure 2.4 The experimental arena used to measure behaviour in the study (a) View of the experimental set-up used to quantify activity of sticklebacks in a novel environment. AZ: acclimation zone; P-Z: the peripheral zone in the novel environment; I-Z: the inner zone in the novel environment; with three stone mounts used to make the experimental tank novel. (b) View of the experimental setup used to test exploration of the novel object. AZ: acclimation zone; NO: the novel object; LEZ: less exploratory zone (4 cm radius); MEZ: more exploratory zone (8 cm radius).

2.2.6 Experimental infections

Plerocercoids of *S. solidus* were recovered from wild-caught infected sticklebacks of River Soar origin (Leicestershire, UK) and cultured using established *in vitro* techniques (Smyth, 1947) to obtain embryonated eggs. Eggs were kept at 20˚C in the dark and exposed to light to stimulate hatching, and the emergent, free-living coracidia were used to infect lab-reared copepods *Cyclops strenuus*. Exposed copepods were reared for five weeks to allow the parasites to develop into infective procercoids (i.e. with a hooked cercomer), and were

screened for infective status under a compound microscope (4×/0.1 and 10×/0.25 magnification). Twelve lab-bred sticklebacks (Clatworthy Reservoir origin) were each fed one infected copepod, and six fish were sham-exposed by feeding them with non-infected copepods. A group of ten sticklebacks were selected randomly from the stock tanks and were exposed on the same day and in a similar manner to generate fish for the infected shoal to be used in the shoaling behavioural trials. The infected shoal was used to test the importance of visual cues and infection status on shoaling behaviour.

The experimental work was carried out under home office project license number (PPL) 70/8148 and personal license number (PIL) I16D5C5DA.

2.2.7 Post-exposure shoaling trials

Fourteen weeks after being exposed to parasites, all individuals (n = 18, exposed = 12, sham-exposed (i.e. control) = 6) were allowed to interact with a stimulus shoal composed of three individuals that had been experimentally infected with *S. solidus* to test the effect of experimental infections on shoaling behaviour.

A rectangular experimental tank (34.5L×19.5W×20H cm) was divided into three compartments. A 2 L plastic cylinder with both ends open (9 cm diameter) was used as an acclimation zone (AZ), and a 10cm wide area (I-S) at one end of tank was used to keep the infected shoal fish to test shoaling behaviour (Figure 2.5).

The shoaling tendency of focal fish was measured in terms of their latency to enter, and the amount of time they spent within the 5cm area ('shoaling area') next to the stimulus shoal (i.e. within the I-Z). Each individual was transferred into the acclimation zone and was left undisturbed for the next 300 seconds. Following acclimation, the door was lifted gently and the video was recorded for the next 300 seconds by using a webcam (Logitech c920HD) connected to a laptop computer.

Recorded videos were replayed and the latency to enter the 5cm area and amount of time spent in the 5cm area next to compartment holding stimulus shoal (I-S) was scored manually.

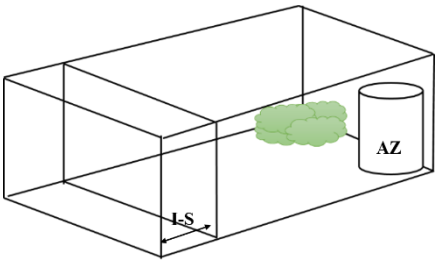

Figure 2.5 The experimental arena used to measure shoaling behaviour of fish in the study. AZ: acclimation zone; I-S: 10 cm wide compartment to keep infected shoal fish; green shaded area: plants used in study to minimize stress/novelty for fish.

2.2.8 Fish dissection

At the end of the study, all test fish were euthanized by overdose of Benzocaine anaesthetic. Any fish containing *S. solidus* plerocercoids were recorded as infected and all plerocercoids were weighed individually (to 0.001g). A total of six parasite-exposed fish developed infections, giving an infection rate of 50% (6/12).

Two fish died following the pre-exposure phase of experiment, therefore 18 fish were used in the study. In the post-exposure phase, only activity in novel environment was quantified, as exploration of novel environment failed to show any temporal consistency in the asocial context in sticklebacks during the pre-exposure phase. In the post-exposure phase, behavioural data for one individual fish was excluded due to an experimental malfunction limiting the analysis to 17 individual fish.

2.2.9 Statistical analysis

2.2.9.1 Exploration of a novel object

A multivariate technique – principal component analysis (PCA) – was employed to extract a single behavioural axis from the five correlated variables used to measure aspects of exploration of the novel object. The principal component (PC) scores were then used for further analysis of the behavioural trait. The Spearman's rank correlation analysis used on the PC scores of trials 1 and 2 to test temporal consistency.

2.2.9.2 Activity in novel environment

Principal component analysis (PCA) was also used to extract a single behavioural axis from the five correlated variables recorded to measure activity in a novel environment. The principal component (PC) scores were then used for further analysis. The Spearman's rank correlation analysis used on the PC scores of trials 1 and 2 to test temporal consistency. There was no temporal consistency found between trail 1 and 2.

Therefore, activity in the peripheral and inner zones was calculated by dividing number of times squares crossed by number of squares. As a result, three variables – latency to leave acclimation zone, activity in peripheral zone and activity in inner zone – were used in further statistical analysis. A Spearman's rank correlation analysis was used to test temporal consistency.

The average (of trial 1 and 2) of activity in peripheral zone and inner zone was calculated and used as mean activity scores further analysis. The mean scores of the variables (which were consistent over time) used to rank fish from higher to lower activity scores. The first six fish which showed higher mean scores were classified as bold and the other six scored as shy. This classification of fish on the basis of their mean scores was adapted from already published data (Ward et al., 2004).

2.2.9.3 Cross-contextual consistency

A mixed model ANOVA was used, with social context as a fixed factor, fish number (identity) as random factor and the activity score in the peripheral zone of arena (trial 1 & 2 both context) as response continuous variable for the data obtained in pre-exposure phase of experiment. Normality of data checked by using Kolmogorov-Smirnov test and data was $\log_{10}$ transformed where necessary to meet the assumptions of normality.

2.2.9.4 Effect of S. solidus on activity in social context

A t-test used to examine differences in the mean activity score of infected and sham-exposed sticklebacks in the social context.

2.2.9.5 Effect of experimental infections on shoaling behavior

A one-way analysis of variance (ANOVA) was used to test the effect of infection status on the shoaling behavior.

All analyses were carried using Minitab 17 Statistical Software, IBM SPSS Statistics 24, and Microsoft Excel 2013 for Windows.

2.3 Results

2.3.1 Personality in asocial and social context, pre-exposure to *S. solidus*

2.3.1.1 Exploration of novel object in sticklebacks

The first principal component accounted for 57% of the total observed variation (Table 2.2) in exploration of the novel object in the asocial context, and was selected as the index of exploratory behavior. There was no significant correlation found between trial 1 and 2 (r_s = 0.095, p = 0.709, n = 18; Figure 2.6.a).

The first principal component explained 66% of the total observed variation for exploratory behavior in the social context (Table 2.2). A significant correlation found between the exploratory behavior (PC scores) between trial 1 and 2 (r_s = 0.490, p = 0.039, n= 18; Figure 2.6.b).

Table 2-2 Results of principal component analysis on various measure of exploratory behavior in sticklebacks. Variables used were latency to leave acclimation zone (L-AZ), time spent in less exploratory zone (T-LEZ), time spent in more exploratory zone (T-MEZ), number of visits to less exploratory zone (V-LEZ) and number of visits to more exploratory zone (V-MEZ).

	Asocial context		Social context	
	PC1	PC2	PC1	PC2
L-AZ	0.202	-0.974	-0.027	-0.997
T-LEZ	0.431	0.006	0.501	-0.066
T-MEZ	0.410	0.098	0.451	0.168
V-LEZ	0.551	0.178	0.526	-0.113
V-MEZ	0.550	0.102	0.517	-0.019
Eigenvalue	2.8657	0.9266	3.3186	1.0258
Proportion	0.573	0.185	0.664	0.025
Variation	0.573	0.758	0.664	0.869

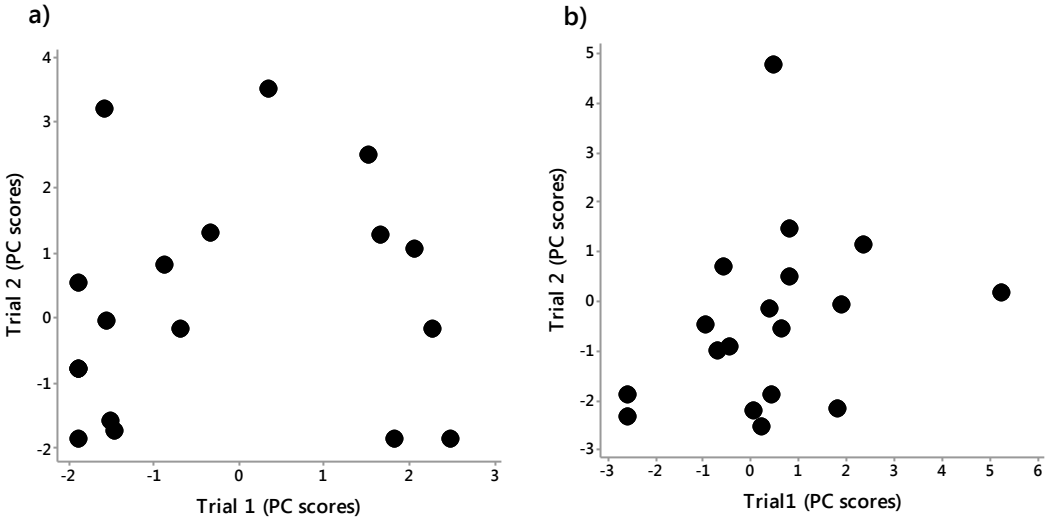

Figure 2.6 Scatterplots showing relationship between principal component scores (PC scores) in (a) asocial context and (b) social context for exploration of novel object test between trail 1 and 2 in sticklebacks.

2.3.1.2 Activity in novel environment in sticklebacks

2.3.1.2.1 Asocial context

There was no significant temporal consistency in the latency to leave the acclimation zone (r_s = 0.08, p = 0.72, n =18), or activity in the inner zone, (r_s =

0.34, p = 0.15, n = 18). However, individuals behaved consistently in the peripheral zone across the two trials, (r_s = 0.56, p = 0.01, n = 18; Figure 2.7.a).

2.3.1.2.2 Social context

In the presence of conspecifics in the experimental arena there was no significant temporal consistency found in terms of latency to leave the acclimation zone, (r_s = -0.13, p = 0.59, n = 18) or in activity in the inner zone of arena (r_s = 0.45, p = 0.055, n = 18). Whereas, there was significant temporal consistency in the levels of activity in the peripheral zone of the arena (r_s = 0.54, p = 0.02, n = 18; Figure 2.7.b.).

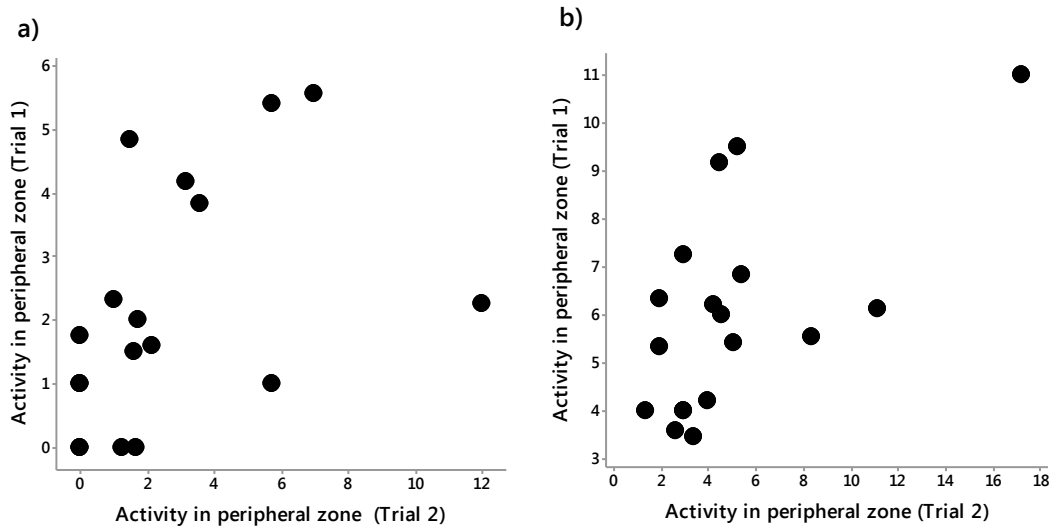

Figure 2.7 Scatterplots showing relationship for activity in peripheral zone of experimental arena between trial 1 and trial 2 in (a) asocial context and (b) social context for activity in novel environment in three spined sticklebacks.

2.3.1.2.3 Cross-contextual activity in novel environment

Sticklebacks were significantly less active in the novel environment in the asocial context than in the social context (Mixed Model ANOVA, $F_{(1, 17)}$ = 25.49, p = 0.000). However, there was no significant effect of fish identity ($F_{(1, 17)}$ = 1.34, p = 0.272) and there was a significant interaction between context and fish identity ($F_{(1, 17)}$ = 2.70, p = 0.006). In other words, the rank order of behaviour between fish was not maintained across social contexts. Number of individuals was 18 and number of observations were n = 72 (Figure 2.8 and Table 2.3).

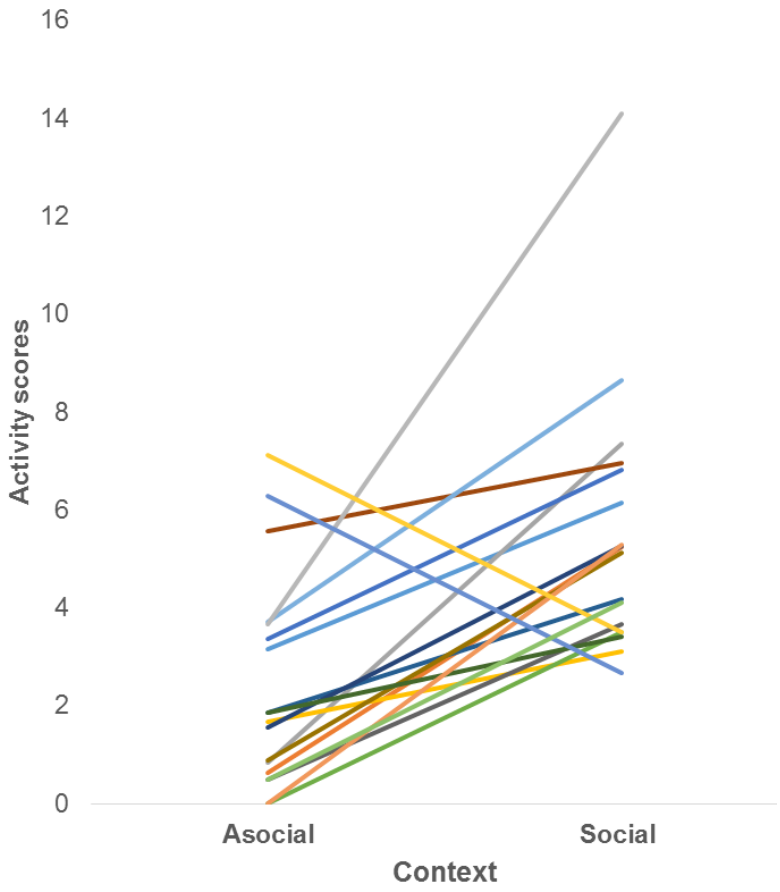

Figure 2.8 Reaction norm plot showing the activity scores between asocial and social context, and each line represent a single individual (n= 18).

Table 2-3 The activity scores (mean) of fish in asocial and social context, showing how they were ranked and classified as bold intermediate and shy based on their activity scores in the peripheral zone of arena in activity in novel environment test.

Fish no	Mean activity score $_{AS}$	Rank	Classification	Mean activity score $_{S}$	Rank	Classification
1	3.15	12	Intermediate	6.14	13	Bold
2	0.62	5	Shy	5.24	10	Intermediate
3	0.83	6	Shy	7.36	16	Bold
4	1.66	9	Intermediate	3.11	2	Shy
5	3.37	13	Bold	6.81	14	Bold
6	0.00	1	Shy	3.50	4	Shy
7	1.86	11	Intermediate	4.16	8	Intermediate
8	5.56	16	Bold	6.96	15	Bold
9	0.50	3	Shy	3.66	6	Shy
10	0.88	7	Intermediate	5.12	9	Intermediate
12	1.55	8	Intermediate	5.27	11	Intermediate
13	1.85	10	Intermediate	3.41	3	Shy
14	3.70	15	Bold	8.64	17	Bold
15	0.00	2	Shy	5.27	12	Intermediate
17	3.65	14	Bold	14.1	18	Bold
18	7.12	18	Bold	3.50	5	Shy
19	6.27	17	Bold	2.66	1	Shy
20	0.50	4	Shy	4.10	7	Intermediate

2.3.2 Personality in asocial and social context, following exposure to *S. solidus*

The overall infection rate was 50%, with six out of 12 exposed fish developing *S. solidus* infections. The data obtained from the behavioural trials for activity in novel environment post-exposure, was first analysed irrespective of infection status to see the temporal consistency in the peripheral and inner zone of experimental arena.

The data which showed temporal consistency i.e. activity in the inner zone in the social context only, was further used to see the effect of infection on the activity in novel environment.

2.3.2.1 Activity in novel environment irrespective of infection status

2.3.2.1.1 Asocial context

In the absence of conspecifics, there was no consistency found in latency to leave the acclimation zone (r_s = -0.25, p = 0.29, n = 18) activity in the peripheral zone, (r_s = 0.21, p = 0.39, n = 18) or activity in inner zone (r_s = 0.43, p = 0.07, n = 18).

2.3.2.1.2 Social context

In the presence of conspecifics there was temporal consistency found in activity in the inner zone, (r_s = 0.59, p = 0.01, n = 17; Figure 2.9), whereas there was no significant temporal consistency found in the latency to leave the acclimation zone, (r_s = 0.20, p = 0.43, n = 17), or activity in the peripheral zone, (r_s = 0.14, p = 0.57, n = 17).

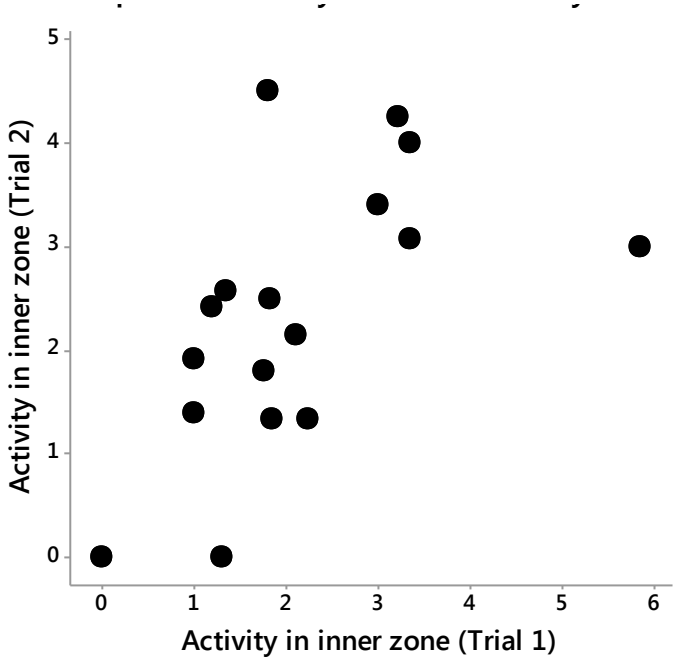

Figure 2.9 Scatterplot showing the relationship between activity in the inner zone of the experimental arena quantified in trial 1 and trial 2, under the social context, for activity in novel environment in three spined sticklebacks.

2.3.3.2 Effect of *S. solidus* on the activity of sticklebacks in a novel environment

The sticklebacks showed significant temporal consistency in the inner zone of the experimental arena, therefore only this variable was used in further analysis.

Infection was associated with a significant difference in the mean activity scores in the inner zone of experimental arena, with higher scores among infected fish (n = 5) compared to sham-exposed (control) fish (n = 6) (t_7 = 3.19, p = 0.015; Figure 2.10 & 2.11).

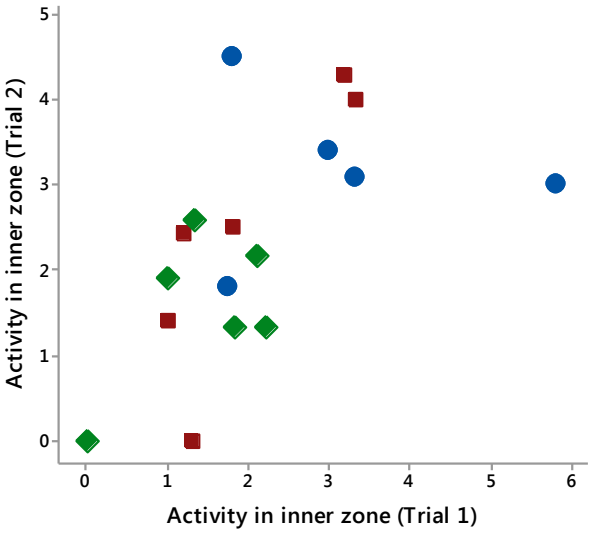

Figure 2.10 Scatterplot showing relationship between trial 1 and trial 2 of activity scores in the inner zone of the arena, under the social context, of sticklebacks 10 weeks following sham-exposure or exposure to the parasite *S. solidus.* Blue circles: experimentally-infected fish; red squares: parasite exposed, but non-infected fish; green diamonds: sham-exposed controls.

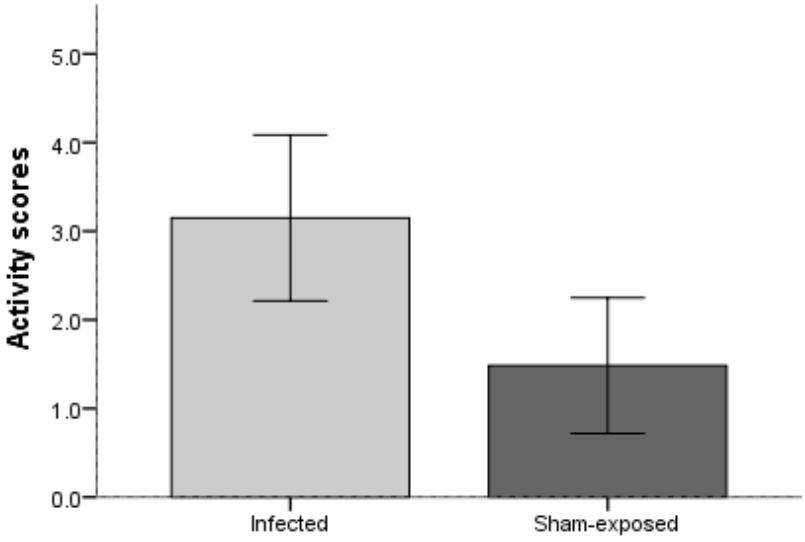

Figure 2.11 Bar chart showing the difference in the activity scores (Mean, ±1SD) of sticklebacks infected with infections of *S. solidus* and sham-exposed (control) in the presences of social context.

2.3.3 Effects of experimental *S. solidus* infections on shoaling behaviour

2.3.3.1 Shoaling behavior

There was no significant temporal consistency found in shoaling behaviour in terms of time spent in shoaling area, (r_S = 0.355, p = 0.148; Figure 2.12.a) and latency to enter shoaling area (r_S = 0.154, p = 0.542; Figure 2.12.b).

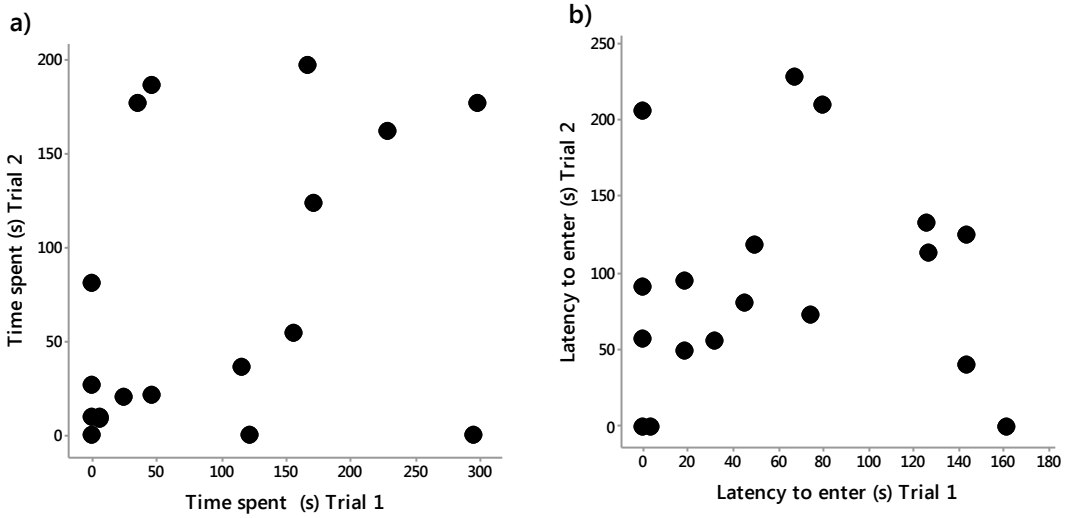

Figure 2.12 Scatterplots showing relationship between shoaling behaviour variables in three spine sticklebacks in trials I and II. (a) Time spent within 5cm of the stimulus shoal, and (b) latency to enter 5cm area.

2.3.3.2 Effect of infections on shoaling behavior

There was no significant effect of infection status on latency to enter (($F_{(2, 15)}$ = 0.22, p = 0.809; Figure 2.14) or the time spent ($F_{(2, 15)}$ = 0.12, p = 0.866; Figure 2.13) in the shoaling area.

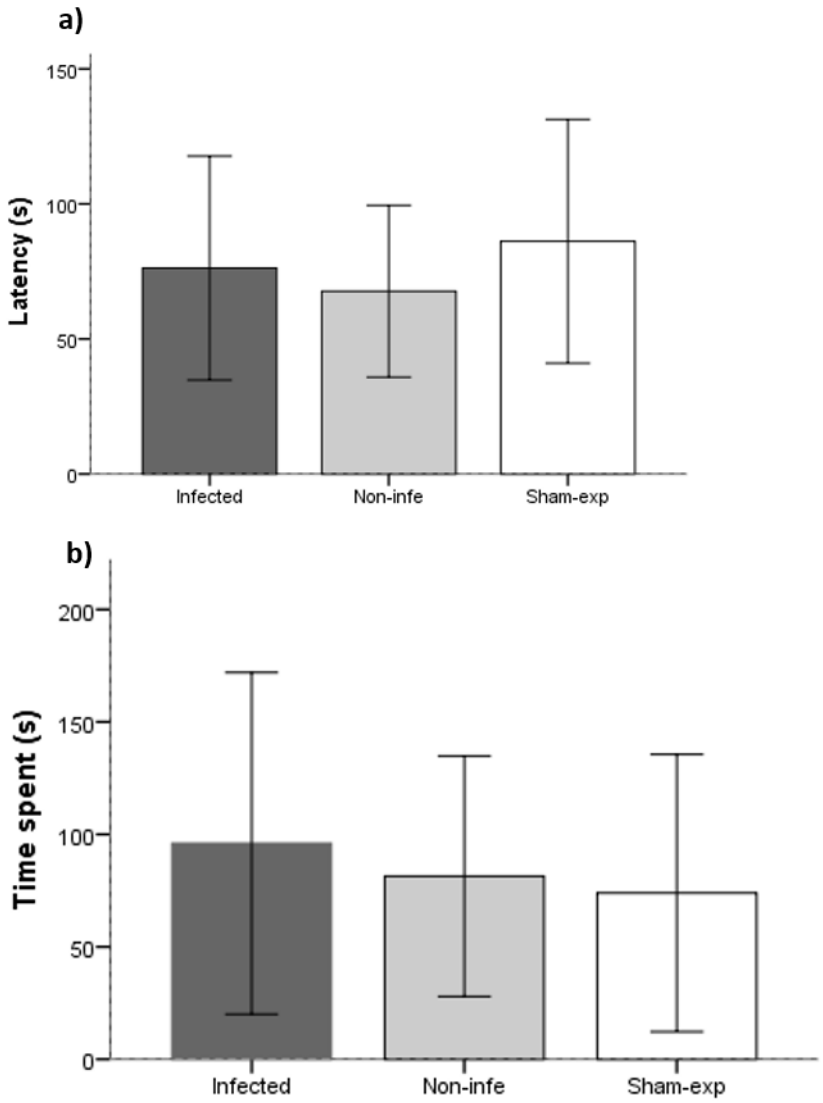

Figure 2.13 Bar charts showing the difference in the (a) latency to enter, (b) time spent in shoaling area of sticklebacks infected with *S. solidus*, sham-exposed (control) and exposed but non-infected.

2.4 Discussion

The main aims of this study were to investigate the effects of social context on personality, the role of parasitism in affecting behaviour of its host and the effects of experimental infections on the shoaling behaviour. The results of this study show that presence of conspecifics (i.e. the social context in which behaviour occurs) significantly affects personality, showing that fish were more active in the social context compared to asocial context. The parasite infections of *Schistocephalus solidus* showed significant effects on the activity of infected fish with infected fish having higher mean activity scores compared to sham-exposed (control) fish. Furthermore, parasite infections showed no significant effects on the shoaling behaviour of sticklebacks.

2.4.1 Temporal consistency in behaviour

Overall, individuals showed significant temporal consistency in the exploration of the novel object only in the presence, and not in the absence, of conspecifics. This shows that social context can influence temporal consistency of behaviour for exploring novel object in sticklebacks. The existence of temporal consistency for novel object exploration in this study is similar to another study in which rainbow trout *Onchorhyncus mykiss* were tested for their temporal consistency by using their latency to approach a novel object (Frost et al., 2007). The absence of any temporal consistency for exploring the novel object in the absence of conspecifics is similar to another study on juvenile gilthead sea bream *Sparus aurata* (Castanheira et al., 2013).

The measurement of individual activity following transfer into a novel environment is frequently used as a test of one axis of personality, and has identified individual differences within populations (Sih et al., 2004a, Bell, 2005, Reale et al., 2007). In the present study, three-spined sticklebacks showed repeatability in activity in the peripheral zone in both the presence and the absence of conspecifics in pre-exposure behavioural trials. The repeatability of activity over time between trial I and II is consistent in both context were similar to finding of a study (Ward et al., 2004). However, in this present study only one behaviour – activity in peripheral zone was consistent between two contexts. Whereas the variable, latency to

leave acclimation zone and activity in inner zone showed no consistency in any context. Furthermore, sticklebacks showed temporal consistency in the inner zone of experimental arena for activity in novel environment only in the post exposure behavioural trials in the presence of conspecifics.

2.4.2 The influence of social context

Social context showed significant effects on expression of consistent individual differences in behaviour (i.e. personality). Sticklebacks showed significant temporal consistency in the exploration of novel object in social context. This result is similar to a study in cichlid fish *Oreochromis mossambicus*, in which male fish showed higher exploration and less neophobia in the presence of familiar conspecifics than in the presence of unfamiliar conspecifics or when alone (Galhardo et al., 2012). One likely reason of the result of this present study (i.e. effects of social context on personality) can be facilitation effects of conspecifics on exploring novel objects (Webster et al., 2007). One other reason of lack of temporal consistency in behaviour (i.e. exploration of novel object) in the asocial context (i.e. absence of conspecifics) could be the design of experimental study. The novel object test was carried out on the following day of activity in novel environment, where individuals may have not recovered from the previous behavioural trials, similar to (Castanheira et al., 2013) in which the novel object behavioural test was carried out following feeding recovery test behaviour. Therefore, for future work while designing the experiment considering the recovery break time for individuals possibly lead to different outcome. In order to avoid any carryover effects from previous behavioural tests, it is important to test animals in a randomised way (Bell, 2012).

Activity in the peripheral zone of the novel environment was significantly higher in the social context compared to the asocial context, evidencing the effects of social context on behaviour. In three spined sticklebacks, boldness is context-specific in grouped fish (McDonald et al., 2016). Species that are social, often have higher activity and exploration in the presence of conspecifics and seek out actively towards conspecifics. For example, sticklebacks were found to be more active, and perch *Perca fluviatilis* were bolder in risk taking behaviour, when tested in the presence of conspecifics than when tested alone (Magnhagen and

Bunnefeld, 2009, Webster et al., 2007), supporting the results of the present study.

Furthermore, the rank order based on activity scores in asocial and social context were different, which shows plasticity in individual's behaviour. The phenomenon of behavioural plasticity has been observed in other studies. For example, in one year old perch, *Perca fluviatilis*, the risk-taking behaviour of individuals was assessed when fish were held in groups, and when alone. Fish typically showed more shy behaviour when tested alone, but the boldest individuals remained bold in both social treatments (Magnhagen and Bunnefeld, 2009). In other words, the bold personality was more influential in group of a shoaling perch. Furthermore, in a group, consensus decision about foraging suppress the individual personality in sticklebacks (McDonald et al., 2016). Bold individuals were stable in personality both in groups and alone, however the shy personality showed plasticity and individual crabs, *Panopeus herbestii* with shy personality became bolder in group compared to alone (Belgrad and Griffen, 2017). One likely reason of plasticity in individual's behaviour may be due to the facilitation effect of conspecifics. From evolutionary perspective, this change in personality may be adaptive response of personalities to adjust to the new environmental conditions.

2.4.3 The effects of parasite infections on behaviour

The results of this study showed that the activity of individuals in the inner zone of novel environment was repeatable, but only in the presence of conspecifics. However, there was no consistency found in activity in peripheral zone and latency to leave acclimation zone in asocial context and social context. Environmental factors are often responsible for generating or maintaining personality in animals. For example, in three spined sticklebacks exposure to predators during early life can generate personality (Bell and Sih, 2007), whereas fluctuations of less than 3˚C within in day temperature can influence the activity, boldness and aggression of coral reef fish (Biro et al., 2010). One likely explanation of the results of this present study could be, that post-exposure trials were carried out after 10 weeks of pre-exposure behavioural trials. The individuals over this time were fed bloodworm *ad libitum*, and some individuals were infected with *S. solidus* infections may have grown differentially partly due

to parasite burden and partly due to their metabolic rates as a result their personality/behaviour changes.

Another possible explanation of these results could be the side effect or manipulation of parasite on host behaviour. Considerable literature is available on the manipulative effects of parasites on host behaviour (Moore, 2013). Interestingly, parasite infections showed a significant effect on the mean activity scores of sticklebacks in the presence of conspecifics. Infected fish showed relatively higher mean activity compared to sham-exposed (control). The possible explanation could be that parasite infections have the potential to influence their host behavioural responses (Barber and Dingemanse, 2010, Poulin, 2013). The number of fish developed infection was low, however, and further research work with larger sample sizes will give more insight.

The results of this study shows that there is no effect of infection status on the shoaling behaviour of three spined sticklebacks. These results are not consistent with the findings of other studies, where infection status have significantly lowered the shoaling preference and/ or tendencies. For example, in non-infected and infected *Gambusia affinis* females with parasite infections of black spot disease *Uvulifer sp*. (Diplostomatidae), preferred to shoal with a group of four conspecifics instead of no stimulus when there was a choice between the two, with a reduced tendency of shoaling in infected fish (Tobler and Schlupp, 2008). Furthermore, European minnows, *Phoxinus phoxinus,* infected with *Ligula intestinalis* showed increased neighbour distance (Barber and Huntingford, 1996). In rainbow trout *Oncorhynchus mykiss* infected with *Diplostomum spathaceum* the shoaling behaviour found significantly lower than control fish (Seppala et al., 2008). One likely reason of these results could be the social interactions of three spined sticklebacks as this species is facultative social (Coolen et al., 2003) compared to other sticklebacks. As age, kinship and familiarity can all play a role in shoaling in three spined sticklebacks and study showed, sticklebacks of sub adult age preferred to shoal with familiar sibling, inbred adult fish showed no preference, and outbred prefer to shoal with unfamiliar kin (Frommen et al., 2007). In this present study, fish were adults and the stimulus shoals used consisted of unfamiliar infected shoal. Although infected

fish comprising the stimulus shoal were from same population and of same age, they were kept separately from one another, so during shoaling trials it is likely they were perceived as an unfamiliar shoal. One other reason could be the infectivity of infected individuals of stimulus shoal and shoal size. In this study, the infected stimulus shoal consisted of three individuals. Sticklebacks with infection of *S. solidus* showed increased dorsal profile by more than 40% (Barber, 1997) and heavily infected sticklebacks could appear as different species to focal fish. However, in this study the plerocercoids of *S. solidus* recovered from infected stimulus shoal were consisted of 1% to 6% of body mass, which appear to have no effect on the shoaling behaviour.

2.5 Conclusion

This study clearly showed the effect of conspecifics on the personality in three-spined sticklebacks. The infection of *Schistocephalus solidus* showed significant effects on the behaviour in social context. Furthermore, parasitism showed no effect on the shoaling behaviour.

Chapter 3.

How does host personality affect subsequent aspects of host-parasite biology?

3.1 Abstract

The effects of parasites on the behavior of their hosts have been widely documented and can have significant ecological importance. In addition, the consequences of parasite infections for the cross-contextual and temporal consistency in behavior (i.e. personality) have also been subject to recent study. However, little is known about the consequences of host personality for the biology of subsequent host-parasite interactions. In this chapter, an experimental study was designed to examine the implications of personality differences among prospective host three-spined stickleback fish, *Gasterosteus aculeatus* for the outcome of infections by the parasite *Schistocephalus solidus*. The personality of individual host fish was quantified by screening their activity levels in a novel environment and their tendency to explore a novel object, before exposing some of them to infective stages of the parasite, while others were sham-exposed. Over the 8 weeks' post-exposure period, the voluntary food intake of all fish was quantified on a daily basis. At the end of the study, the infection status of all fish was determined, and basic health and immune status indices were calculated. The key result was that personality exists in lab-bred three spined sticklebacks. Furthermore, infection status was found to affect the voluntary food intake of host fish as infection progressed in the 4^{th} week of the study. However, individual personality did not significantly influence the voluntary food intake and parasitism did not influence post exposure activity behaviour of sticklebacks.

3.2 Introduction

Animal personality has the potential to interact with parasite infections in many ways (see section 1.2) and studies have started to explore the consequences of host personality and parasite infections. For example, in rodents the levels of ectoparasite infections was found to be higher in more exploratory chipmunks (*Tamias minimus*) compared with those individuals that were less exploratory (Bohn et al., 2017). Furthermore, in Atlantic cod *Gadus morhua*, fish with infections of the trematode *Cryptocotyle lingua* showed larger monthly home ranges and were typically found in deeper water compared to non-infected fish (Aalvik et al., 2015). One interpretation of the results of these studies is that personality may influence either the probability of encounter, or the susceptibility of acquiring infections following an encounter, with more exploratory animals often having higher infection levels. These observational studies highlights the role of personality in acquiring infection following exposure. Whereas the role of personality on developing infection is less well understood.

This chapter first describes an experimental study designed to investigate how consistent individual differences in behavior (i.e. personality) influence the susceptibility of three-spined sticklebacks to infection following exposure to infective stages of the parasite *Schistocephalus solidus.* The second part of the study investigates the impact of parasite infections on the subsequent behavior of the stickleback hosts. Following the establishment of an infection in a host, the interaction between host and parasite infection can often lead to physiological, morphological and behavioral changes, known as the infection phenotype (Barber et al., 2017). To elucidate the infection phenotype following the interaction of host personality and parasite infection, three-spined sticklebacks were reared for eight weeks' post exposure. Parasites have the ability to cause mortality in their hosts (Robar et al., 2010). They can also reduce host fitness, mainly by affecting host's energy reserves – state, and reproductive success (Lafferty and Kuris, 2009, Barber and Dingemanse, 2010). Furthermore, personality and energetics of individuals are likely to be linked (Careau et al., 2008) as parasites rely on host metabolic needs to nourish and grow (Barber et al., 2008). Parasites may influence the food intake rates by two ways, firstly

directly by affecting appetite or stomach capacity of parasitized individuals and secondly by influencing foraging behavior or competitive ability (Barber et al., 2000). Therefore, over a period of 8 weeks, the food intake rates of fish that had either been exposed to *S. solidus* or a control, sham-exposure, were recorded on a daily basis to see the effect of both personality and parasite infection, on host food intake.

The consequences of interaction between host personality and parasite infection and their effects on the subsequent host-parasite biology are poorly understood. Hence, a comprehensive experiment was conducted to answer following questions. (1) Does personality (i.e. consistent individual differences in behaviour) exist in the lab-bred and reared three-spined sticklebacks? (2) Does personality affect the susceptibility of developing infections in sticklebacks following exposure to infections of *S. solidus*? (3) Does the interaction between personality and infection affect the outcome of host-parasite interactions? (4) Do personality and/or *S. solidus* infections affect the voluntary food intake in sticklebacks? (5) Does parasite infection affect host behaviour?

3.3 Methods

3.3.1 Fish supply and husbandry

Forty laboratory-bred three-spined sticklebacks (standard length: 40.2 ± 2.18 mm) of River Welland origin (Leicestershire, UK) were randomly selected from stock that had been generated through *in vitro* breeding techniques. Each fish was blotted dry, measured (standard length, SL_0, to 0.1 mm) and weighed on an analytical balance (wet mass, M_0, to 0.001 g) before being transferred individually into one of four holding aquaria (each 60L × 40W × 30H cm) and maintained at 15˚C ±1°C under a 12L:12D photoperiod. Each glass aquarium was divided into ten individual compartments; each compartment (19.3L × 11.3W × 20H cm) consisted of one bio-filter to provide filtration and aeration, a glass watch (for holding food during the feeding trials) and was enriched with a gravel substratum and a plastic plant for shelter. One fish was placed into each compartment. Prior to the start of the experimental study, the fish were kept for one week to acclimatise them to the new environment, and they were fed daily with bloodworm *ad libitum* and to excess in the first phase of experiment.

3.3.2 Experimental approach

The experimental study was divided into five phases (Figure 3.1). The fish were initially screened for consistent inter-individual variations in behaviour to allow their personalities to be scored and ranked (Phase 1). This was followed by a controlled exposure to infective stages of *S. solidus* (Phase 2). The voluntary daily food intake of all individuals was then recorded each day over an eight week post-exposure period (Phase 3). The behaviour of all fish was then re-screened to test for infection-related changes (Phase 4) before all fish were terminated and dissected, to confirm infection status and quantify host and parasite fitness parameters (Phase 5).

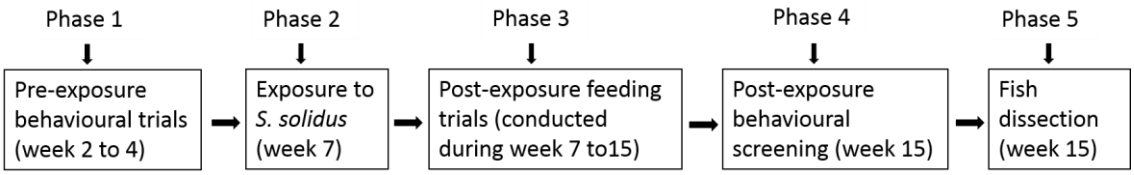

Figure 3.1 Schematic diagram summarising the experimental study designed to examine the effect host personality and *S. solidus* infections on subsequent host-parasite biology.

3.3.3 Behavioural screening

A square Plexiglas aquarium tank (40W×40 L×15Hcm), consisting of an acclimation zone (10W×10L×15 Hcm) with a sliding door on one side (Figure 3.2), was used as experimental arena. For activity in novel environment three stone ornaments (8 cm height, 2-3 cm radius) were used to make the environment novel, whereas, for exploration of novel object two different objects including plastic ball (green and white in colour) and Universal tube (30 ml) filled with wet sand were used (see figure 2.5.b).

All fish were tested for two personality traits: (i) activity in novel environment, and (ii) exploration of novel object. Each behavioural test was conducted three times, to test for temporal consistency of behaviour. All individuals were screened for their activity in novel environment (NE) on the first day and for the second

behaviour, exploration of novel object (NO) the next day, whereas the next trial was conducted after the gap of four days.

Individual fish were caught using a hand net and transferred carefully into a 1 L static tank (18L×11W×8H cm) to settle, to reduce and standardise stress. After a few minutes individual fish were transferred by net into the acclimation zone (AZ) of an experimental arena for five minutes to allow recovery from handling and transfer. After 300 seconds the door of the acclimation zone was gently and remotely opened and the behaviour (activity in NE, or exploration of NO) was recorded using a webcam (Logitech c920HD) connected to a laptop computer for five minutes in the experimental arena for subsequent analysis.

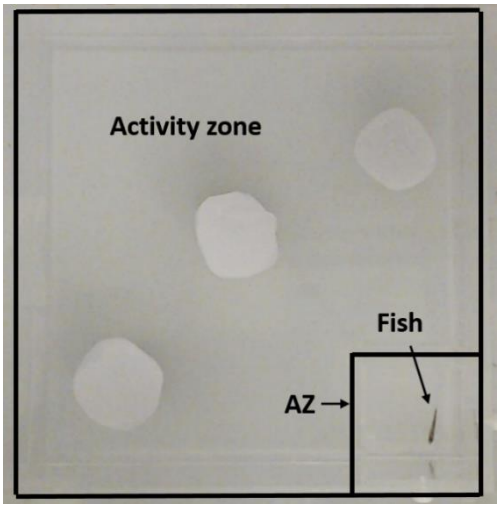

Figure 3.2 The experimental arena used to measure behaviour in the study **(a)** View of the experimental set-up used to quantify activity of sticklebacks in a novel environment. AZ: acclimation zone; NE Act-Z: the activity zone in the novel environment; with three stone mounts used to make the experimental tank novel.

3.3.4 Experimental infections

Schistocephalus solidus plerocercoids were recovered from naturally-infected sticklebacks collected from the River Soar (Leicestershire, UK), and cultured using standard *in vitro* techniques (Smyth, 1947) through to the adult, egg-producing stage. Eggs were incubated for 21 days at 20˚C in the dark before being exposed to light to stimulate hatching. Newly-hatched coracidia – the free swimming, infective stages of *S. solidus* – were collected and used to infect lab-bred and reared copepods *Cyclops strenuus abyssorum.* Parasite-exposed copepods were reared for five weeks, to allow infections to take place and the

parasites to develop sufficiently to be infective to the stickleback second intermediate hosts. The exposed copepods were screened for the presence of infective procercoid parasites under a compound microscope (4×/0.1 and 10×/0.25 magnification). A single infected copepod, with multiple infections of between 4-6 noticeable procercoids, was then fed to each of the parasite-exposed group of sticklebacks, whereas sham-exposed (control) sticklebacks received identical treatment, but were fed a non-infected copepod (N=28 exposed, N=11 sham exposed (control) fish).

3.3.5 Quantification of food intake during experiment

Voluntary food intake was measured each day from the day following parasite exposure or sham exposure, and continued for 56 days. Frozen bloodworms (*Chironomus* spp. larvae) were defrosted and blotted dry, and an excess (approximately 150-280 mg wet mass) of bloodworms was weighed (to 0.001 g) on an analytical balance before being transferred into each well of a 24-well plate, which had been labelled with fish identity. The measured quantity of blood worms was carefully pipetted into the watch glass in each individual fish-holding compartment. Each fish was then left undisturbed for 10 minutes, after which the remaining food was removed using a plastic pipette, blotted dry and re-weighed allowing the mass of food consumed by each fish to be calculated by subtraction. These trials were conducted between 1400 h and1800 h each day. At the end of the trial, each fish was provided with three additional bloodworms (7-8 mm length), in case the fish had not eaten during the 10 minute trials.

3.3.6 Terminal behavioural screening and dissection

Fifty-six days after parasite exposure or sham-exposure, all fish were screened for a single personality trait (activity in novel environment, as detailed in section 3.2.3). After behavioural screening, fish were blotted dry, measured (SL_{56}, to 0.1 mm) and weighed (M_{56}, to 0.001 g) and euthanized by an overdose of Benzocaine anaesthetic. Fish were dissected, and the mass of various organs weighed (each to 0.0001 g) to quantify fish health indices. Any fish containing *S. solidus* plerocercoids were recorded as being infected, and each plerocercoid was weighed individually (to 0.0001 g).

The mass of each plerocercoid recovered from each infected fish was recorded and total parasite mass (M_P) calculated. Fish containing *S. solidus* plerocercoids was recorded as infected. The parasite index of plerocercoids calculated as (M_p / M_{56}) *100. For infected fish, all health indices were calculated excluding parasite mass (i.e. fish mass, $M_F = M_{56} - M_P$). The mass of the spleen (M_S) was weighed to provide an insight into the level of immune activation of fish, and was used to calculate spleen somatic index ($SSI = (M_s/M_F *100)$). Specific growth rate (SGR) of individual fish over the course of the study was calculated as $(\ln(M_{56}) - \ln(M_0))/d) *100$, where d is the length of the intervening period, in days. The body condition factor (BCF) was calculated as $(M_{56} - M_P) / SL_{56}^3) *10^5$.

3.3.7 Data collection from behavioral recordings

Recorded videos were replayed by using Windows media player on the Windows desktop computer and both the latency to leave the acclimation zone and the time spent in activity zone while moving (in seconds) were recorded manually for activity in novel environment (Figure 3.2.a).

For the exploration of novel object (NO), the experimental arena was divided into the acclimation zone, the more exploratory zone (MEZ, close to the novel object) and less exploratory zone (LEZ, next to the MEZ). The novel object was placed in the middle of the experimental arena and the variables quantified were latency to leave the acclimation zone (L-AZ) and the time spent in more exploratory zone.

3.3.8 Statistical analysis

3.3.8.1 Personality in sticklebacks

Spearman rank correlations were used to test the rank-order consistency of activity in novel environment and exploration of novel objects between separate trials.

3.3.8.2 Probability of developing infection following exposure

A Chi-square test was used to test for significant association between personality 'type' (bold, intermediate and shy) and developing an *S. solidus* infection following exposure to infective parasites.

The p values obtained was 0.096, therefore two extreme personality types (i.e. bold and shy) were used for further Chi-square analysis.

3.3.8.3 Effect of personality and infection on voluntary food intake

The data on each individual's voluntary food intake over the 55 days of the study were grouped into eight weekly mean values, which were used for further analysis. The Kolmogorov Smirnov test of normality, and Levene's test of equal variance, were used to test the assumptions for parametric tests. The data was $\log_{10}$ transformed to meet the assumptions of parametric tests. Following $\log_{10}$ transformation the voluntary food intake value for week 7 met the minimum criteria of equal variance (0.096) and used for further analysis.

Two separate repeated measure ANOVA analyses, with voluntary food intake over time as the dependent repeated measure (calculated as a weekly mean, from week 1 to week 8) and personality and infection status as independent factors. The data that failed to meet the assumption of parametric test were $\log_{10}$ transformed. The Mauchly's test was used to test the assumption of sphericity. Where the assumption of sphericity was violated, the Greenhouse-Geisser correction was used. In the case of a significant interaction effect, to test the main effect an ANOVA test was used.

3.3.8.4 Effect of personality, infection and interaction on infection phenotype

A two-way ANOVA was used to test the effects of personality, infection status and their interaction on stickleback specific growth rate (SGR), body condition factor (BCF), and splenosomatic index (SSI) as an indicator of immune activation. The specific growth rate data was normalized by using $\log_{10}$ transformation, and Levene's test used to test equal variance. A Kruskal Wallis test was used to test

the effect of host personality type (bold, intermediate and shy) on total parasite mass and parasite index.

3.3.8.5 Effect of infection on personality

The difference in activity between the post-exposure trial score and the pre-exposure trial (mean value of trials 1 and 2) was calculated. A one-way non-parametric ANOVA i.e. Kruskal Wallis test, with infection status as the independent factor and the difference in pre-post activity scores as dependent factor, was used to test the effect of infection on behaviour.

3.4 Results

3.4.1 Personality in three-spined sticklebacks

3.4.1.1 Exploration of novel object

There was no temporal consistency in the rank order of individuals in terms of either their latency to leave the acclimation zone (trial 1 v 2, r_s = -0.094, p = 0.568, n = 39; trial 2 v 3, r_s = -0.007, p = 0.966, n = 39; trial 1 v 3, r_s = -0.045, p = 0.784, n = 39) or the time spent in the more exploratory zone (trial 1 v 2, r_s = 0.260, p = 0.110, n = 39; trial 2 v 3, r_s = -0.058, p = 0.728, n = 39; trial 1 v 3, r_s = -0.007, p = 0.966, n = 39) during the exploration of novel object trials.

3.4.1.2 Activity in Novel Environment

The results show that there was no significant rank order consistency in latency to leave the acclimation zone (trial 1 v 2 r_s = 0.231, p = 0.175, n = 39; trial 2 v 3 r_s = -0.172, p = 0.309, n = 39; trial 1 v 3, r_s = 0.303, p = 0.072, n = 39). There was significant rank order consistency in the amount of time spent moving in the activity zone between trials 1 and 2, and between trails 1 and 3(trial 1 v 2, r_s = 0.685, p = 0.000, n = 39; trial 1 v 3, r_s = 0.319, p = 0.048, n = 39; Figure 3.3.a & b), whereas there was no significant rank order consistency between trials 2 and 3, (r_s = 0.194, p = 0.236, n = 39; Figure 3.3.c).

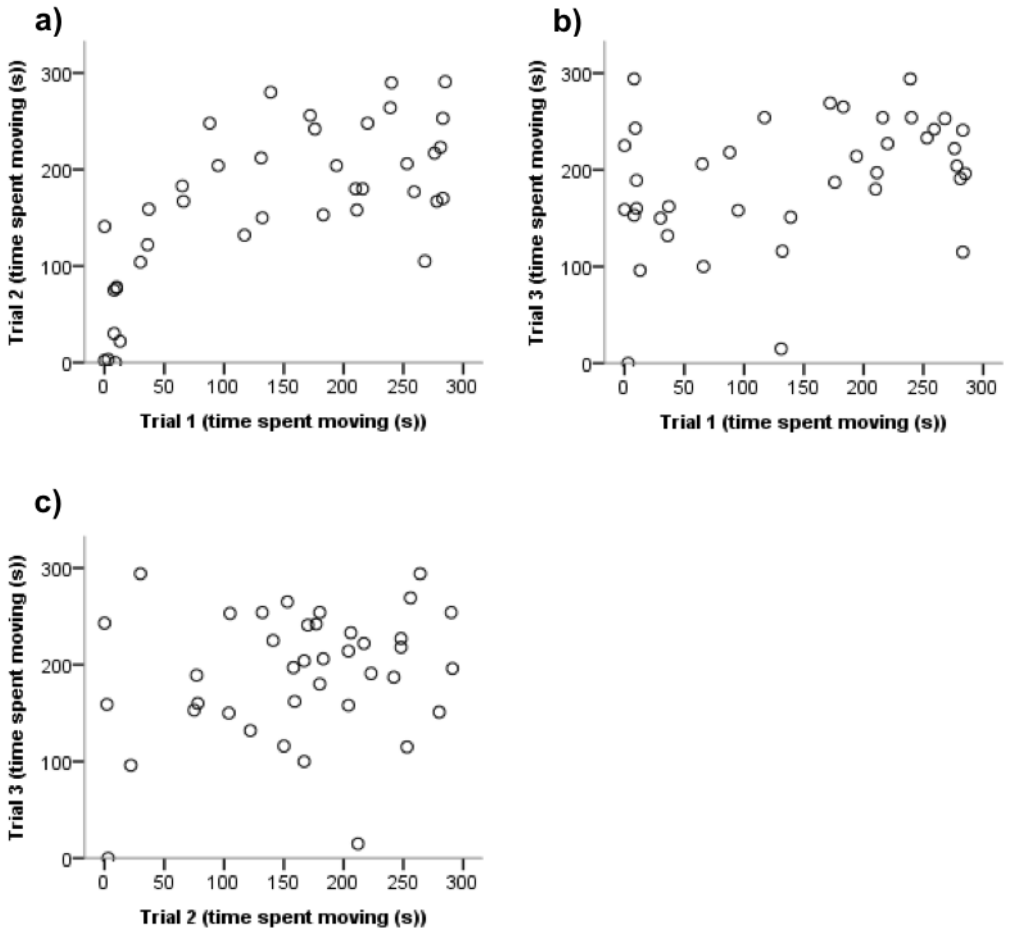

Figure 3.3 Scatterplots showing relationship between amount of time spent (in seconds) between trials in the activity zone of the experimental arena to quantify activity of sticklebacks in the novel environment (a) trial 1 v 2, (b) trial 1 v 3, (c) trial 2 v 3.

3.4.2 Associations between personality type and developing *S. solidus* infections following exposure

The results show that there was no significant association between personality type (i.e. bold, intermediate and shy) and developing an *S. solidus* infection following exposure to infective stages of the parasite ($\chi^2 = 4.684$, $p = 0.096$, $n = 28$; Figure 3.4.a).

The results show that there was significant effect of personality type (i.e. bold and shy) on developing infections following exposure to *S. solidus* infections ((χ^2) = 4.253, $p = 0.039$, $n = 21$; Figure 3.4.b). Fish with shy personality showed the highest infection development rate.

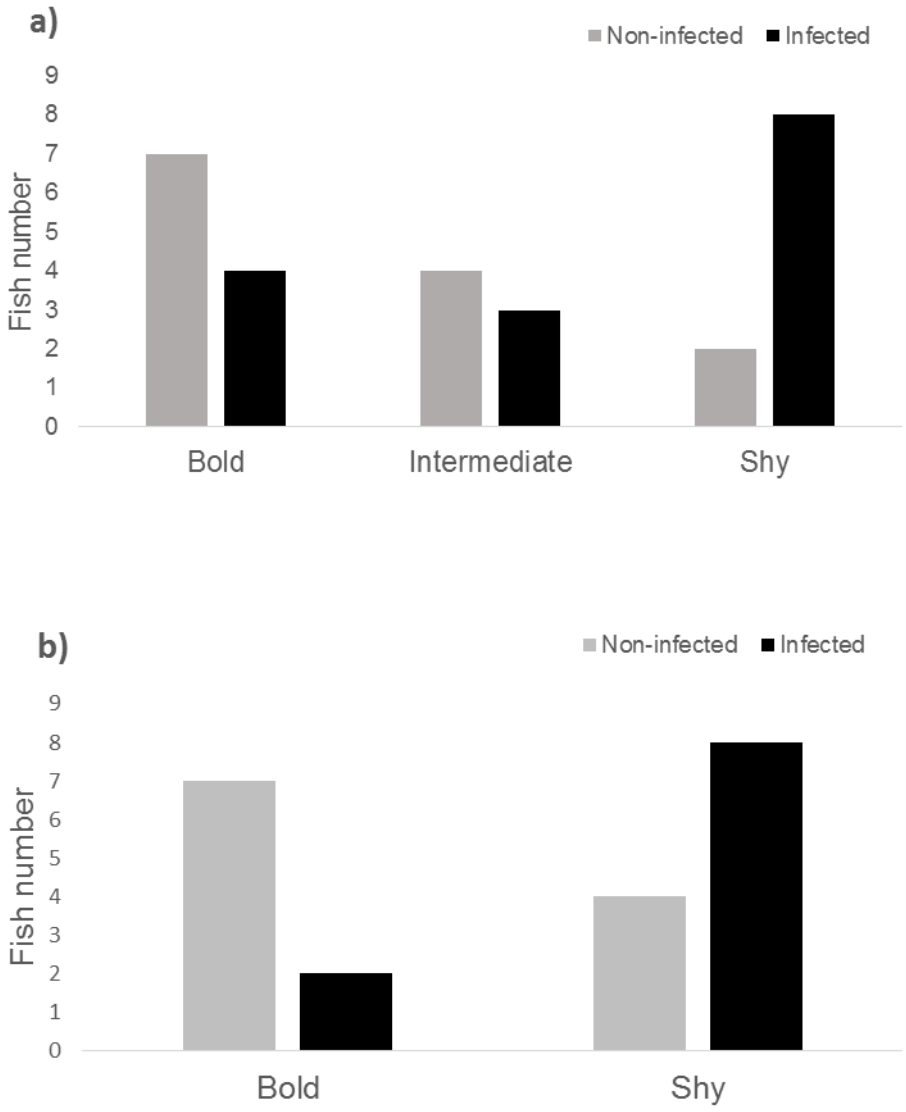

Figure 3.4 A histogram showing the effect of personality type of sticklebacks on susceptibility to develop infection following exposure to infections of *S. solidus*) (a) bold, intermediate and shy fish (b) bold and shy fish only.

3.4.3 Effect of personality and infection on voluntary food intake

3.4.3.1 Effect of personality on voluntary food intake

The result show that overall the voluntary food intake of individual fish was significantly affected by the week of study ($F_{(3.25, 117.02)} = 11.67$, $p = 0.000$; Figure 3.5). The significant differences in voluntary food intake between weeks are shown in table 3.1 and figure 3.5.

There was an initial sharp increase in voluntary food intake from low levels in week 1 then steady decrease in voluntary food intake over the course of the study.

The results show no significant effect of personality type on the voluntary food intake of sticklebacks ($F_{(2, 36)} = 1.35$, $p = 0.270$ $n = 39$; Figure 3.5). Furthermore, there was no significant interaction between personality type and week on voluntary food intake ($F_{(6.5, 117.02)} = 1.056$, $p = 0.395$).

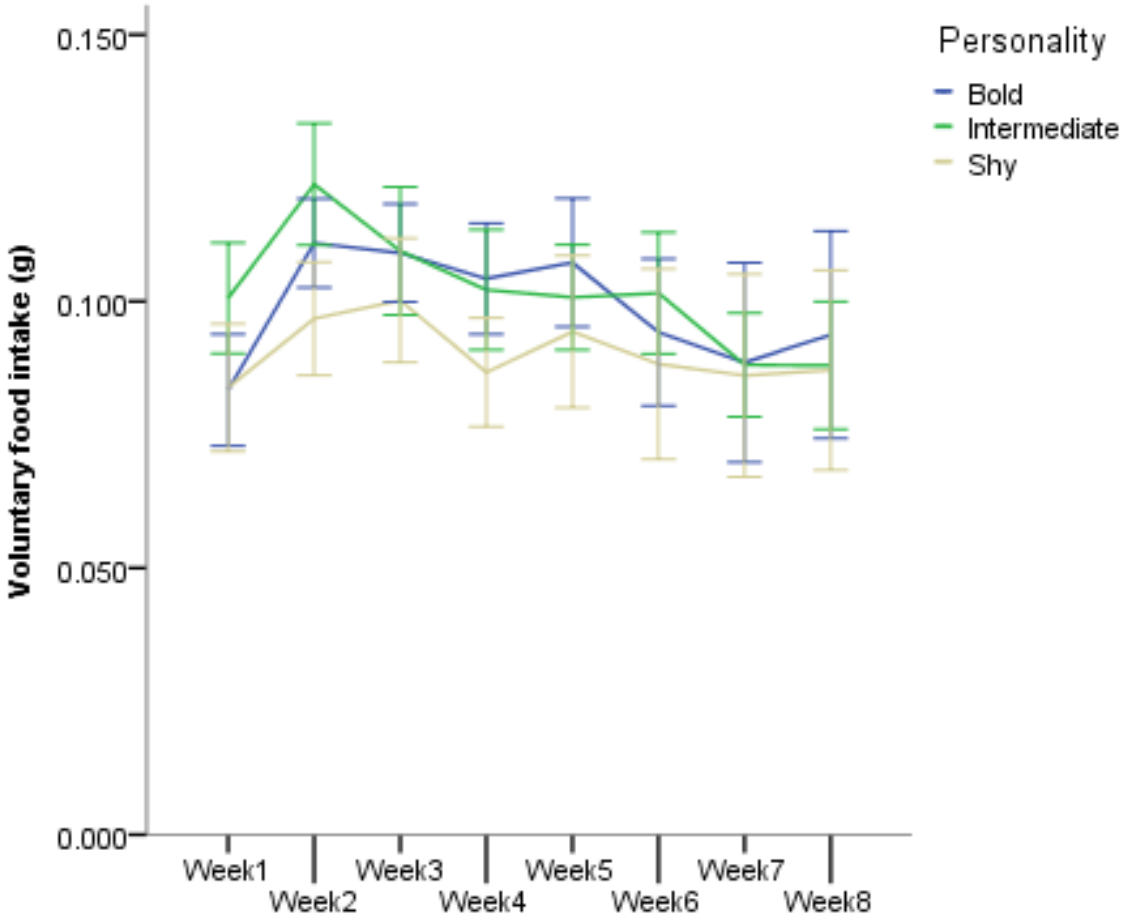

Figure 3.5 Line chart (mean values with error (±2) SE) showing the effect of personality type (bold: blue; intermediate: green; yellow: shy) on the voluntary food intake (mean (g)) over the period of eight weeks in three-spined sticklebacks.

Table 3-1 Table showing the result of repeated measure ANOVA with voluntary food intake per week as dependent variable and personality type (bold, shy and intermediate) as an independent variable.

Source	Week	df	Mean Sq	F	Sig.
Week	Week 1 vs. Week 2	1	0.357	**84.58**	**0.000**
	Week 2 vs. Week 3	1	0.008	1.09	0.301
	Week 3 vs. Week 4	1	0.058	10.81	**0.002**
	Week 4 vs. Week 5	1	0.006	1.99	0.166
	Week 5 vs. Week 6	1	0.043	5.63	**0.023**
	Week 6 vs. Week 7	1	0.064	6.18	**0.018**
	Week 7 vs. Week 8	1	0.002	0.18	0.674

3.4.3.2 Effect of infection status on voluntary food intake

The result show that the voluntary food intake of fish varied significantly over time, being affected by the factor 'week' ($F_{(3.67, 132.15)} = 13.87$, $p = 0.000$). The result show no consistent significant difference in voluntary food intake between the three infection status groups ($F_{(2, 36)} = 1.18$, $p = 0.31$; Figure 3.6). However, there was a significant infection status x week interaction effect on the voluntary food intake of fish ($F_{(7.34, 132.15)} = 2.52$, $p = 0.017$; Table 3.2) with higher voluntary food intake rates being exhibited by infected fish than non-infected fish in the later weeks, compared to earlier weeks of study where no significant effects of infection found on the voluntary food intake levels.

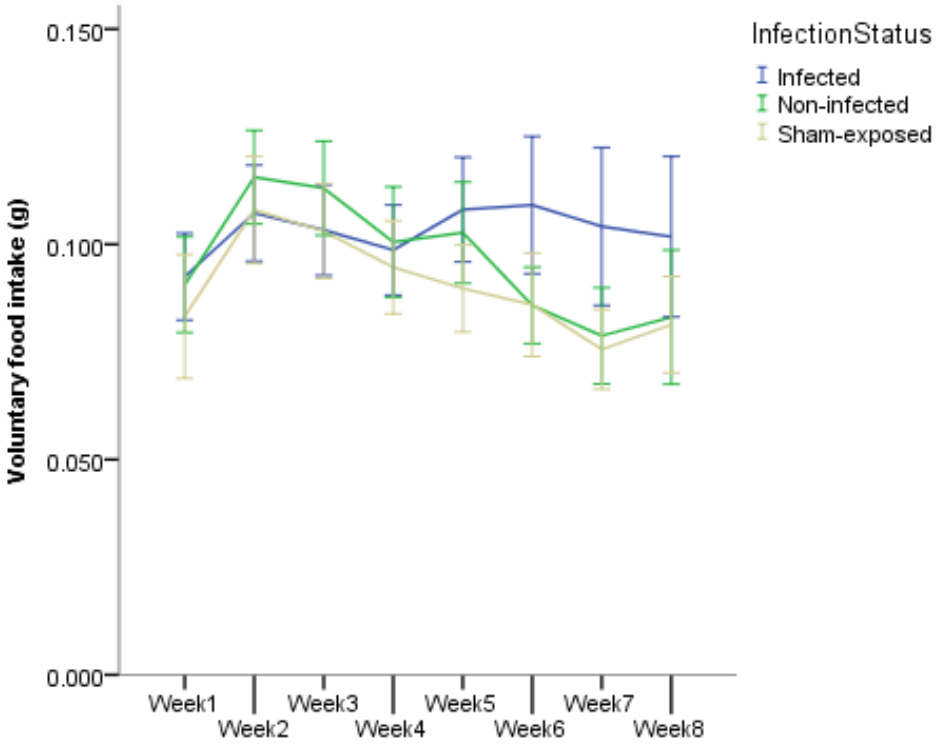

Figure 3.6 Line chart showing the effect of infection status (infected: blue; exposed non-infected: green; yellow: sham-exposed) on the voluntary food intake of three-spined sticklebacks post eight weeks' exposure to *S. solidus* infection.

Table 3-2 Table showing the result of repeated measure ANOVA with voluntary food intake per week as dependent variable and infection status (control, infected, non-infected) as an independent variable.

Source	Week	df	Mean Square	F	Sig.
Week	**Week 1 vs. Week 2**	1	0.388	89.53	**0.000**
	Week 2 vs. Week 3	1	0.009	1.095	0.302
	Week 3 vs. Week 4	1	0.057	10.47	**0.003**
	Week 4 vs. Week 5	1	0.003	1.147	0.291
	Week 5 vs. Week 6	1	0.047	6.31	**0.017**
	Week 6 vs. Week 7	1	0.070	6.58	**0.015**
	Week 7 vs. Week 8	1	0.004	0.361	0.551
Week*infection	Week 1 vs. Week 2	2	0.014	3.116	0.056
	Week 2 vs. Week 3	2	0.000	0.036	0.965
	Week 3 vs. Week 4	2	0.005	0.843	0.439
	Week 4 vs. Week 5	2	0.012	4.56	**0.017**
	Week 5 vs. Week 6	2	0.019	2.51	0.094
	Week 6 vs. Week 7	2	0.002	0.223	0.801
	Week 7 vs. Week 8	2	0.008	0.676	0.515

3.4.4 Effect of personality and infection on host growth and health indices

There was no effect of personality type ($F_{2, 30} = 0.672$, P = 0.518, n = 39; Figure 3.7.a) or infection status ($F_{2, 30} = 0.344$, P = 0.712, n = 39; Figure 3.7.a) on the specific growth rate of fish achieved during the study, and no significant interaction between the two factors ($F_{2, 30} = 1.114$, P = 0.368, n = 39; Figure 3.7.a).

There was no effect of personality type ($F_{2, 30} = 2.542$, P = 0.096, n = 39; Figure 3.7.b) on fish body condition factor, and no significant interaction between infection status and personality ($F_{2, 30} = 0.357$, P = 0.837, n = 39; Figure 3.7.b) with significant effect of infection status ($F_{2, 30} = 4.247$, P = 0.024, n = 39; Figure 3.7.b)

There was no effect of personality ($F_{2, 30} = 0.579$, P = 0.566, n = 39; Figure 3.7.c) on splenosomatic index. However, infection status had a significant effect on the splenosomatic index of fish ($F_{2, 30} = 9.383$, P = 0.001, n = 39; Figure 3.7.c), with higher splenosomatic index in infected fish compared to exposed-non-infected and sham-exposed (control) fish. There was no significant interaction between infection status and personality ($F_{2, 30} = 0.341$, P = 0.848, n = 39; Figure 3.7.c) on the splenosomatic index.

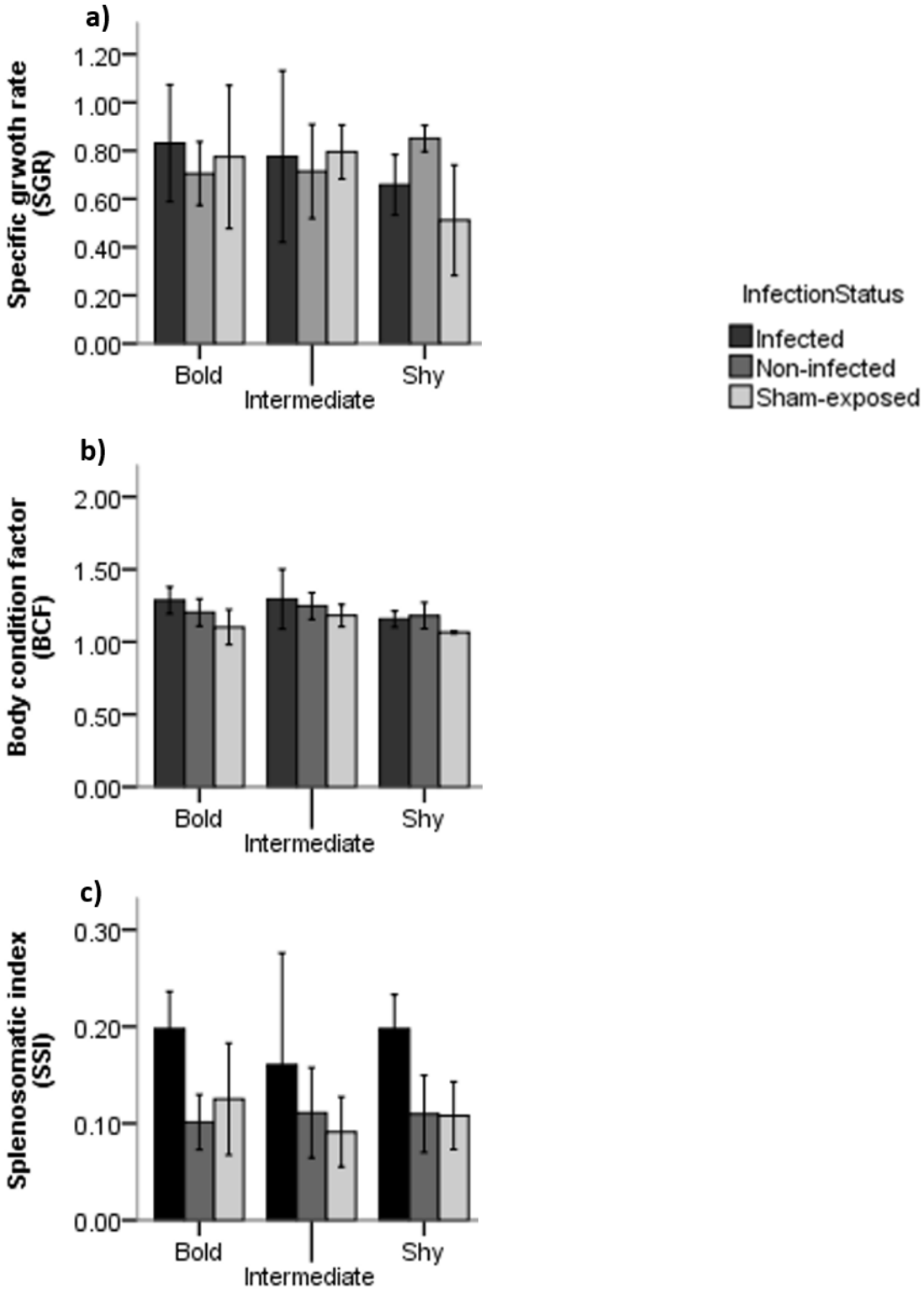

Figure 3.7 The effect of S. solidus infection and personality type on measurements of growth and immune status of sticklebacks; (a) specific growth rate (SGR), (b) body condition factor (BCF) and (c) splenosomatic index (SSI). Bar height shows the mean, and error bars ± 2 SE. Data for sham exposed (light grey bars) infected (black bars) and exposed, non-infected (dark grey bars) individuals that had been classified as bold, intermediate or shy following initial behavioural screening.

3.4.5 Effect of personality on parasite growth

Personality type (i.e. bold, intermediate or shy) did not influence the total mass of parasites that developed (χ^2 (2) = 3.019, p = 0.221, n = 15; Figure 3.8.a), nor the parasite index (χ^2 (2) = 3.206, p = 0.201, n = 15; Figure 3.8.b) among the fish experimentally infected with *Schistocephalus solidus*.

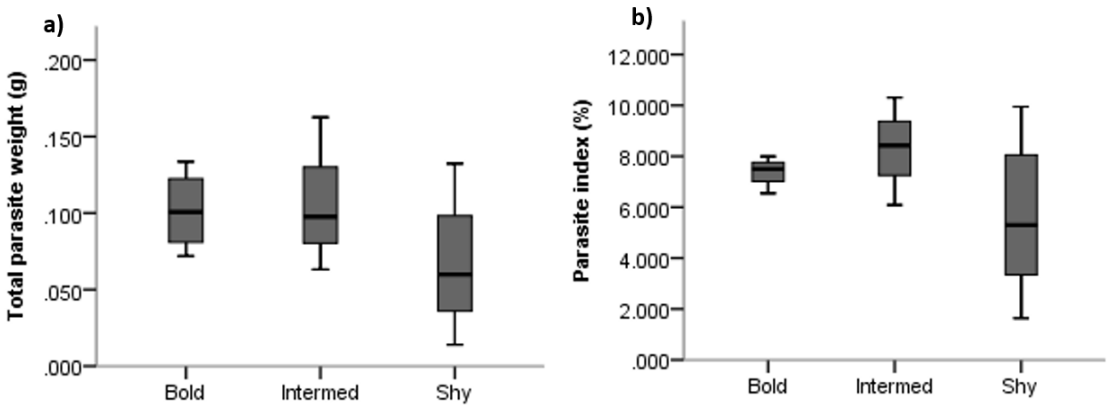

Figure 3.8 Boxplots showing the effect of personality type (bold: intermediate: shy) on (a) total parasite mass (g) and (b) parasite index (%) of *Schistocephalus solidus* recovered from sticklebacks eight weeks' post exposure.

3.4.6 Effect of infection of *S. solidus* on personality of sticklebacks

There was no significant effect of infection status (sham-exposed, experimentally infected and exposed-non-infected) found in change in activity between first and last screenings of the activity in novel environment of sticklebacks ($F_{(2, 38)}$ = 1.324, p = 0.279, n = 39; Figure 3.9).

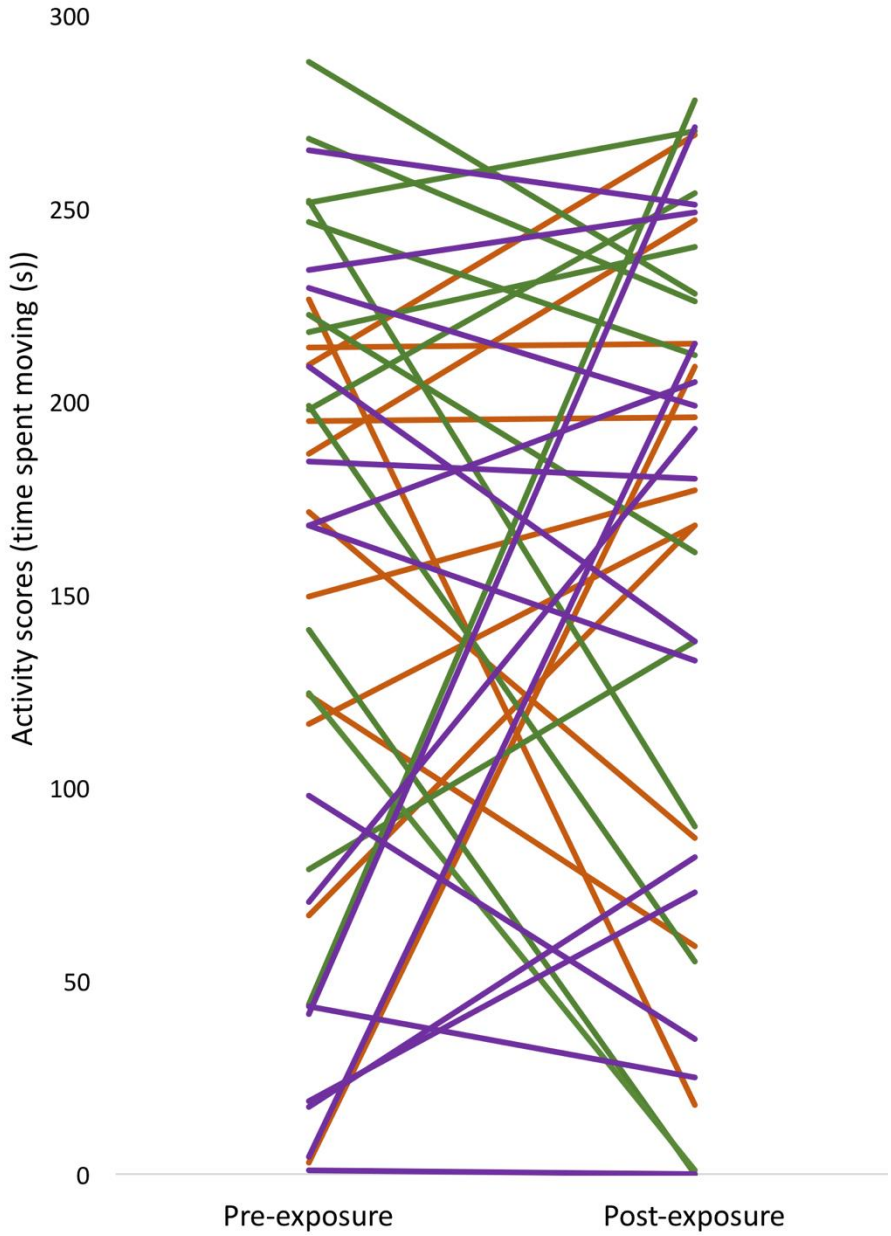

Figure 3.9 A reaction norm plot showing the difference in the activity scores (time spent moving (s)) of three-spined sticklebacks pre-and post-exposure to infections of *S. solidus* in the three groups. (a) green lines: sham-exposed (control); (b) purple lines: exposed non-infected; and (c) orange lines: infected.

3.4 Discussion

The main aim of the study was to investigate the implications of individual difference in personality on subsequent parasite infections, growth, body condition and immune activation of host. In addition, the effects of personality and infection status on host voluntary food intake and infection-induced changes in behaviour of host were examined. The sticklebacks were tested to quantify temporal consistency in behavior (personality). The effects of personality on probability of developing parasite infections, voluntary food intake of host and on parasite fitness were tested. Furthermore, the effect of parasite infection on voluntary food intake and post exposure activity behavior of host were investigated.

3.4.1 Temporal consistency in behavior

The result of this study showed that individual variation in novel object exploration shows a general lack of consistency in three spined sticklebacks. These results are consistent with finding of the studies conducted on juvenile gilthead seabream *Sparus aurata* (Castanheira et al., 2013) and Mozambique tilapia *(Oreochromis mossambicus)* (Galhardo et al., 2012). Whereas, there was significant temporal repeatability of behavior for activity in novel environment in three spined sticklebacks like other studies (Sih et al., 2004b, Bell, 2005, Reale et al., 2007). However, the repeatability for activity in novel environment was consistent over two trials (trial 1 and 2), whereas the third trial showed no consistency with the second trial. This lack of consistency could be attributed to habituation over time, and individuals becomes less responsive to the repeated stimulus (Reale et al., 2007). However, to mitigate this problem, the position of the objects (stone mounts) were changed in each behavioral trail.

3.4.2 Association between personality type and the likehood of developing *S. solidus* infections folowing exposure

The results showed that overall there was no effect of personality on the susceptibility of infection when three different personality groups (i.e. bold, intermediate and shy) were considered in analysis. However, when the two extreme personality groups (i.e. bold and shy) were included in analysis showed

significant effects of personality on susceptibility of developing infection, where shy fish developed more infections compared to bold fish.

In the present study one of the reason that no significant effects of personality type (three groups) on infection susceptibility could be the limited sample size of the experiment. In this present study the overall sample size of study (n =39) shrink down to further small sample sizes when fish were classified according to their personality scores into three different personality groups. Future studies with big sample size (of each personality group) will be insightful. In addition, in laboratory controlled conditioned for future work , exposure of fish to single procercoid and its effects on susceptibility of developing infections will help in developing better understanding of evolutionary ecology of personality in face of parasitism.

The results showed (when two extreme personality groups i.e. bold and shy considered) that, bold sticklebacks were less susceptible to infection following exposure to *S. solidus* compared to shy sticklebacks. These results are not consistent with other studies where bold individuals end up having more infections (Wilson et al., 1993a, Aalvik et al., 2015). These previously published studies undertaken in wild and mainly focused on the effect of personality on encounter rate, which limits to standardized post-encounter mechanisms. However, in the present experimental study, I was able to look at the effect of personality on susceptibility of developing infection following encounter / exposure in laboratory controlled conditions. One possible explanation that might explain the results of this present study that bold personality develop less infections compared to shy personality could be due to variation in the internal circulating hormones (i.e. cortisol) which might have an impact on the behaviour and immunocompetence of host. For example, in three spined sticklebacks, the expression of genes encoding the stress hormone cortisol is significantly related to personality type. The expression of glucocorticoid receptors (GR1 & GR2) were positively correlated with behavioral syndrome of boldness and aggression. (Aubin-Horth et al., 2012). This present study does not provide any evidence of physiological state i.e. circulating hormones and immune status of bold and shy individuals. However, a detailed look at the immune responses of fish that vary

in terms of their personality at the time of exposure and following experimental exposure with bigger sample size would be valuable in determining the role of personality type on the establishment of infection in fish.

3.4.3 Voluntary food intake in three spined sticklebacks

3.4.3.1 Lack of association between personality and voluntary food intake

According to pace-of-life theory, personality traits – including activity, boldness and aggressiveness – are positively correlated with fecundity and growth (Careau et al., 2008, Biro and Stamps, 2008). In three spined sticklebacks, bolder individuals with a higher risk-taking behavior under predation threat had higher food consumption of prey compared to shy sticklebacks (Ioannou et al., 2008). However, the results show that there was no significant relationship between personality type and the voluntary food intake levels among sticklebacks in this study. One possible reason for this could be the design of the experimental study. Published data has shown that food deprivation can increase risk-taking behavior (Killen et al., 2011). Risk-taking behavior is considered as feature of boldness. However, in this study the food provided to fish daily was provided *ad libitum*, meaning that food deprivation did not occur. Furthermore, in holding aquariums there was no possibility that fish could show any risk taking or competitive behvaiour, as fish were held individually and food was provided in excess on daily basis. Hence, this is likely that individuals could not show their true personality traits (i.e. quantified in pre-exposure phase) which may have influence the voluntary food intake.

Furthermore, the voluntary food intake trials were carried out after three to four weeks of personality trials. It may possible that, over the period individuals being kept in individuals holding compartments have changed their personality – plasticity. The extent to which the behavioural phenotype change as a response to the variation in the external stimuli called contextual plasticity (Stamps, 2016). Aggressive individuals towards conspecifics tend to take greater risk when exposed to predators (Riechert and Hedrick, 1993). It is likely that individual housing of fish may influence the expression of their personality, which

consequently influence their food intake leading to finding of no significant effects of personality on food intake.

3.4.3.2 Effect of infection on voluntary food intake

Parasite infections have the potential to reduce host fitness, by affecting the 'state' of their hosts, for example in terms of their reproductive outcome or energy reserves (Lafferty and Kuris, 2009, Barber and Dingemanse, 2010, Barber et al., 2008). The results of this study showed that infection status influence food intake in sticklebacks as infection progresses over time. The infected fish showed higher levels of voluntary food intake in the later weeks of study compared to the weeks in the start of study. The finding that infection status showed no statistically significant effects on voluntary food intake are consistent with another study on *S. solidus* effects on the meal size of three spined sticklebacks. The infection showed no clear effect on the meal size of sticklebacks (Wright et al., 2006). One likely reason of these results could be the experiment design of the study. As parasites may influence the food intake rates by two ways, first – directly by affecting appetite or stomach capacity of parasitized individuals and secondly – indirectly by influencing foraging behavior or competitive ability (Barber et al., 2000). Here, in this case firstly, there was no opportunity for the fish to show competitive ability as fish were held individually. Secondly the food provided to fish were *ad lib* and time given to fish to eat was 10 minutes, which may likely not enough to eat. However, to overcome this each fish was provided with three additional bloodworms to reduce the chances of starvation. One other reason could be, stomach capacity of parasitized individuals – which results in lowering the food intake in infected individuals. These results are in contrast to study in larvae of rainbow smelt *Osmerus mordox,* where the parasitized larvae of the cestode *Proteocephalus* sp. ate significantly less than non-infected fish (Sirois and Dodson, 2000). However, the parasites recovered from infected fish in the present study reported in this thesis (2 to 10% of body weight) which is not enough to affect the stomach capacity of parasitized individuals. Whereas, the cut-off for an effect of parasite on food intake is 15% parasite weight (Wright et al., 2006).

However, there is significant interaction effect between infection status and week on the voluntary food intake of sticklebacks in the 4th week of study. Furthermore,

there is a clear trend that infection status influence the voluntary food intake in later weeks of the study that infected fish showed increased voluntary food intake rates compared to sham-exposed (control) and exposed-non-infected (fig 3.7). This is an indication that parasite growth is influencing the food intake rate to meet the growth of parasite as parasite rely on host energy reserves to nourish and grow (Barber et al., 2008). Hence, clear trend is noticeable in the food intake of infected sticklebacks.

3.4.4 Lack of association between personality or infection status and aspects of host performance

The result shows there was no significant association between personality or infection and host specific growth rate or body condition factor. However, infection had significant effect on relative size of the spleen, with bigger spleens – which can indicate recent immune activation (Handy et al., 2002, Zapata et al., 2006) (Kalbe and Kurtz, 2006) – being found among infected fish compared to sham-exposed control and exposed yet non-infected fish.

Personality differences can arise from growth – mortality trade off (Stamps, 2007, Biro and Stamps, 2008) and energetics of animals is often linked to their personality (Careau et al., 2008). Individuals with higher growth and fecundity rates potentially show certain adaptive behaviors to fulfill energy requirements, for example by exhibiting higher food intake rates and a tendency to take risks (Careau et al., 2008, Biro and Stamps, 2008). According to this concept, individuals showing more bold personality may be expected to have higher food intake levels and greater growth rates. In a recent study of three spined sticklebacks, the bolder sticklebacks ate more compared to shyer individuals (Jolles et al., 2016). In this present study, the personality showed no effects on the voluntary food intake, since the growth rates are linked to food intake rate. Therefore, no significant effects of personality found on stickleback's growth through their influence on food intake rates. The voluntary food intake trials during experiment lasts for 10 minutes' duration, it may possible that, fish kept

individually and during 10 minutes' food intake trials, fish had not eaten enough which is required. In a study in three spined sticklebacks the feeding trials lasts for 60 minutes (Wright et al., 2006). For further studies, the time for food intake trials should be reconsidered and fish should be given more than 10 minutes to eat.

The infection status failed to produce any significant difference in the fish specific growth rate and body condition factor. Parasites rely on host energy reserves to fulfill their requirements (Barber and Dingemanse, 2010). The fish infected with *S. solidus* infection typically show reduced specific growth rate (Barber and Svensson, 2003) and body condition. However, in this study the case is different. The plerocercoids recovered fish at the end of study were smaller in size (individual plerocercoids mass < 50 mg), it is likely that sue to low infectivity of infections no significant effects of infection were noticed host growth and body condition.

3.4.5 No association between host personality and parasite growth

The parasite take nourishment from its host energy reserve to fulfill its metabolic requirements (Barber et al., 2008) and host energy reserves are linked with personality type (Careau et al., 2008). The personality type of the host fish did not influence the parasite growth in terms of parasite index (PI) or the total plerocercoid mass developed by infected fish. There is enough literature available on the host manipulation by parasite infections (Moore, 2002, Moore, 2013), however there is no published literature available on the consequences of host personality type on parasite growth. One likely possible explanation of these results could be as parasite rely on host energy reserves (Barber et al., 2008), and there was no differences observed in the food intake levels across personality groups in this study. Hence the differences in the parasite index and total parasite mass were significantly not different among personality groups. However, there is clear trend noticebale that shy fish showed lower parasite mass and parasite index as expected. Lower sample size hinder to get significant difference between personality groups. Future studies with bigger sample size will give more insight.

3.4.6 Effect of infection on personality following development of infection

The results show that there was no consistent effect of infection status on activity in the novel environment. The possible reason could be experimental design. The post exposure behavioral trial for activity was conducted only one time, therefore it is difficult to examine the effects of infection on the repeatability of behavior.

One other reason could be the infectivity of the plerocercoids recovered from infected sticklebacks. The plerocercoid of *S. solidus* become infective once it attain the size of 50 mg (Tierney et al., 1996) and ready to infect the definitive host and likely to affect the behaviour of sticklebacks. However, the mass of the individual plerocercoid recovered from multiply infected fish was not up to infective stage (i.e. plerocercoid mass ≥ 50 mg) with few exception. Hence, they did not influence the behaviour of their host and no infection-induced behavioural changes were observed.

3.5 Conclusion

This experimental study demonstrates that lab-bred three spined sticklebacks show characteristic personality type and personality, which may be linked to the probability of developing *S. solidus* infections following an experimental parasite challenge. The infection status can influence the voluntary food intake as its progresses in the stickleback's host. However, personality showed no effects on the voluntary food intake. It is possible that the lack of significant effects may reflect the relatively benign conditions under which the fish were held. More experimental studies with bigger sample size are clearly required to understand the effects of personality on the voluntary food intake and on infection phenotype in a more competitive environment.

Chapter 4.

How does food availability affect host-parasite interactions in changing environment?

4.1 Abstract

The effects of anthropogenic global warming on host-parasite interactions have gained much attention due to their considerable ecological implications. In addition, the effects of multiple stressors related to global temperature change has also been subject to recent study. However, the consequences of interaction between anthropogenic temperature change and available food on the subsequent host-parasite biology is poorly understood. In this chapter, the three-spined stickleback – *Schistocephalus solidus* model host-parasite system was used to test the hypothesis that, whether the interaction between temperature and food intake of host can affect the subsequent host-parasite biology. Sticklebacks were either exposed to controlled parasite infections or were sham-exposed by feeding them infected or non-infected copepods respectively. Fish were then held for eight weeks at either 20°C or 15°C, and were fed a daily ration of either 8% or 16% of their body weight, which was recalculated every two weeks. At the end of the study, the infection status of each fish, the mass of the parasites recovered from each infected fish and a range of fish health indices were determined. The parasites recovered from infected fish were then cultured by using a standard *in vitro* culture technique, to determine any carryover effects of either temperature or host ration effects on subsequent parasite fitness. The data showed a significant effect of temperature on both host and parasite growth. The elevated temperature favours the growth of parasite, whereas host was in better body condition at lower temperature. However, once these effects were accounted for, there were no significant additional effects of host ration found on the host growth and parasite fitness.

4.2 Introduction

Changing environmental conditions potentially influence the dynamics of host-parasite interactions (Thomas and Blanford, 2003, Ferguson and Read, 2002). One of the many changing environmental conditions is temperature, and studies have suggested that changing temperature can have serious implications for host-parasite interactions. For example, *Drosophilla melanogaster* survive longer with bacterial infections when held at cooler temperatures (Linder et al., 2008). In a recent study, plerocercoids of cestode *Schistocephalus solidus* infecting three-spined sticklebacks *Gasterosteus aculeatus* grew more quickly and attained larger size when host fish were held at warmer temperature (Franke et al., 2017). Yet while the effects of thermal changes are often studied in isolation, in natural ecosystems they are unlikely to act in isolation. As a consequence, the interaction between changing environmental temperature with other environmental factors, for example the availability of food, to influence the outcome of host-parasite interactions is still not well understood.

Changing food availability might have a significant impact on how thermal regimes impact the interactions of hosts and parasites. Endoparasites depend on their host's energy reserves to fuel their growth, development and reproduction (Barber et al., 2008, Goater et al., 2014) and may also be likely to affect host food intake in proportion to their mass (Lefebvre et al., 2013). Changes in temperature can affect organisms in different ways, including food intake (influencing appetite and the metabolic cost of digestion and assimilation), behaviour, personality, and neurochemistry (Kehoe and Volkoff, 2008, Khan et al., 2015, Manciocco et al., 2015, Malavasi et al., 2013, Stamps, 2007), and are likely to influence the biology and physiology of parasites as well (Barber et al., 2016). In a recent study in the stickleback- *Schistocephalus solidus* host-parasite system showed a strong positive effect of elevated temperature on parasite growth and a positive effect of lower temperature on host growth (Franke et al., 2017). In another experimental study, the same host-parasite system was reared at two different temperature conditions, 20˚C and 15˚C. The results showed that at higher temperatures the growth of the parasite was enhanced with a lower size-corrected specific growth rate of the host as compared to the lower

temperature. These examples suggest that there may be differential effects of anthropogenic warming on host-parasite interaction, with both positive effects (enhanced growth of parasite) and negative effects (lower specific growth of host) of high temperature (Macnab and Barber, 2012).

In this experimental study (Macnab and Barber, 2012), the food provided to fish was in excess at both temperatures. Therefore, it is likely that parasite growth at the higher temperature was unhindered by food availability, whereas in natural environments experiencing elevated temperature regimes, this might not be the case. Anthropogenic environmental changes are likely to alter the quality and quantity of food available to individual animals (DeStasio et al., 1996, Woodward et al., 2010). Therefore, it may be possible that the increased rate of parasite growth at higher temperatures in experimental studies are reliant on high levels of food intake by the host, which may not reflect the most likely ecological situation. An experimental study was therefore designed to investigate the potential interaction between temperature and food availability on subsequent host growth and parasite fitness.

The host-parasite system used in this study consisted of lab-bred three-spined sticklebacks as a host, which were exposed to infective stages of the cestode parasite *Schistocephalus solidus* by feeding them with two experimentally-infected copepods (each containing a single procercoid-infective parasite stage) or were sham-exposed by feeding two non-infected copepods. Experimental fish were then held under one of two temperature treatments, 20˚C or 15˚C, for 8 weeks' post-exposure, during which they were fed a measured daily ration of either 8% or 16% of the body weight. Temperature conditions of 20˚C or 15˚C and daily food ration of 8% or 16% of body weight per day used as these condition have already been used in published studies (Macnab and Barber, 2012) and (Simmonds, 2015). The study was designed to answer the following questions: (1) Does host food intake ration interact with rearing temperature to influence the growth of hosts and parasites? (2) What are the subsequent implications of host ration and temperature change for parasite fitness, measured as egg output?

4.3 Methods

4.3.1 Fish supply and husbandry

Juvenile sticklebacks, generated from lab-bred River Welland origin parents using *in vitro* techniques (Barber and Arnott, 2000), were reared under a natural photoperiod and temperature conditions in a re-circulating aquarium system. Eighty-eight fish were transferred from their stock tanks and held individually in a custom-built re-circulating water system (Figure 4.1), one week prior to start of the experiment, to acclimatise them to the new running water conditions. During this time fish were fed daily, *ad libitum* and to excess, with frozen bloodworms (*Chironomus* spp. larvae).

Replicated large plastic trays (90 L×52 W×20 H cm) were used as water baths, and each housed up to 12 smaller plastic tanks (22 L×12 W×10 H cm; 1.7 L capacity), each of which was divided into two equal compartments to keep fish individually. The water in each compartment was continually filtered and aerated and enclosures were each enriched with a plastic plant. A re-circulating, temperature-regulated water supply was maintained using aquarium heaters. The temperature in each bath was recorded daily and maintained at mean (±SD) water temperature 19.98 ± 0.28˚C in the baths set up at 20˚C and 15.47 ± 0.30˚C in the water bath set up at 15˚C throughout the 8 week post parasite exposure period.

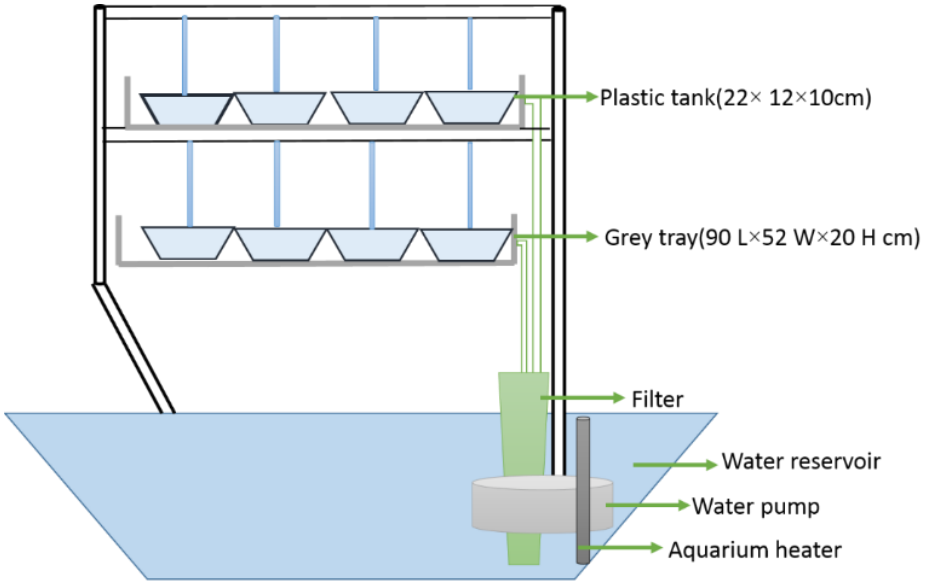

Figure 4.1 Diagram showing the details of one of the custom-built re-circulating system used to hold three-spined sticklebacks either exposed to *Schistocephalus solidus* or sham exposed throughout experiment. Two systems were used each set at different temperature (either 15°C or 20°C).

4.3.2 Experimental infection

Schistocephalus solidus plerocercoids were recovered from experimentally-infected lab-bred sticklebacks of River Welland origin and cultured *in vitro* to produce eggs (Smyth, 1954). Parasite eggs were kept in the dark at 20˚C for 10 weeks to allow embryonation, and then exposed to natural daylight which stimulated hatching, releasing infective coracidia which were added to cultures of lab-bred copepods (*Cyclops strenuus abyssorum*) maintained in two 250 ml flasks filled with 180 ml water. At 14-18 days' post exposure, copepods were screened for the presence of infective procercoid, and those with one infective procercoid with a developed cercomer (Figure 4.2) were selected for use in the fish infection studies. Individually-housed sticklebacks were then either sham-exposed by feeding two non-infected copepods, or parasite-exposed by feeding two infected copepods (each containing one infective procercoid) in 1 L static tanks (18 L×11W×8 H cm) with 700-800 ml water in three different batches.

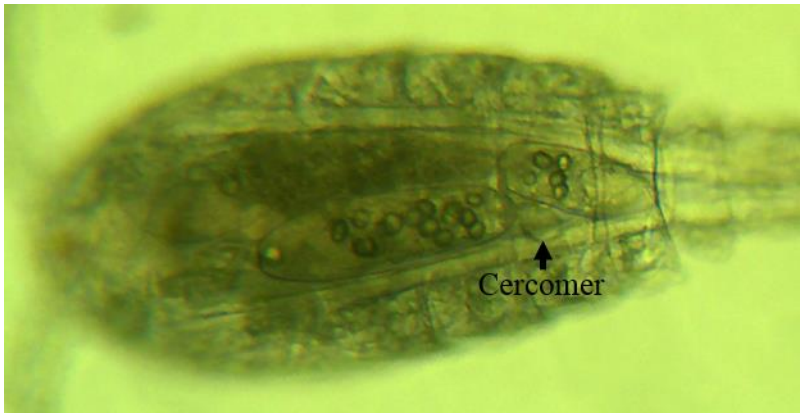

Figure 4.2 Photograph of a copepod (*Cyclops strenuus*) infected with the procercoid of parasite *Schistocephalus solidus* and developed cercomer.

Fish in the experiment were exposed to one of four different combinations of infection and temperature treatments (Figure 4.3). Fish were weighed at the start and end of the study, and every two weeks during the study, to quantify growth rates and to allow the recalculation of the absolute ration required to fulfil the 8% and 16% daily ration for each fish. Mortality was low throughout the study (6.8%) and was unrelated to infection status; three sham-exposed and three parasite-exposed fish died before the end of the study and were not included in the analysis.

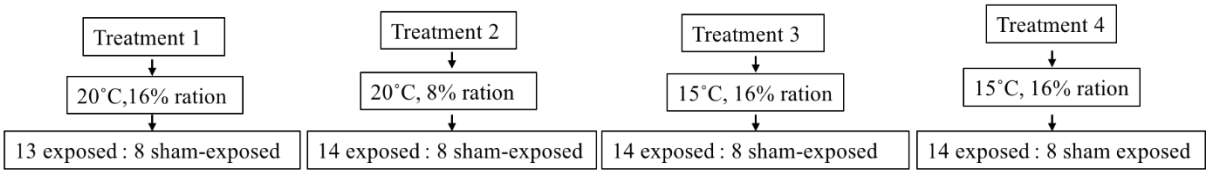

Figure 4.3 Pictogram showing detail of the treatments and number of sticklebacks exposed to *S. solidus* and sham exposed (control) for 8 weeks.

4.3.3 Fish dissection

At the end of the study, fish were measured (standard length, SL, to 0.1 mm) and weighed (wet mass, M, to 0.001 g) before being euthanized using a lethal dose of Benzocaine anaesthetic (10% of 10 g/L in 70% IMS). The mass of the internal organs was weighed separately (each to 0.0001 g). In addition, the mass of each plerocercoid recovered from each infected fish was recorded and total parasite mass (M_P) calculated. For infected fish, indices were calculated excluding

parasite mass (fish mass, M_F = M-M_P). The host health indices calculated as described in Section 3.2.6.

4.3.4 Parasite culture, egg collection and egg counts

Parasites recovered from infected sticklebacks were cultured *in vitro* using 70 ml screw-top boiling tubes filled with 50% RPMI-cell culture medium and 50% horse serum (heat Inactivated-Sigma Life Science, Sigma U.K.) and 0.05 ml penicillin an antibiotic. They were placed in a water bath set to 40˚C with 122 rpm/minute shaking. Culture tubes were checked daily to confirm parasite survival or mortality and on the eighth day, eggs were collected into a 9 cm diameter Petri dish. Harvested eggs were washed thoroughly with double distilled water (ddH_20) to remove any culture media, and the waste produced by the parasite was removed under a dissection microscope using a glass pipette. Eggs were transferred into 15 ml centrifuge tubes filled with 10 ml ddH_2O and centrifuged for 4 minutes at 1500 rpm. Following centrifugation, eggs settled at the bottom of tube, and excess liquid was removed from the tubes to leave 2 ml solution, which was shaken thoroughly to resuspend the eggs, prior to the removal of ten replicate 1.8 µl aliquots. Each aliquot was then placed on a clean cell counting slide and the total number of eggs was counted under the microscope (4×/0.1 and 10×/0.25 magnifications). From ten replicates, the highest and lowest count values were excluded and a trimmed mean egg count of the remaining eight replicates were used for further analysis (Dorucu et al., 2007).

4.3.6 Statistical analysis

All variables were tested using the Kolmogorov-Smirnov test for normal distribution and Levene's test for equal variance. For body condition factor (BCF) the data were normally distributed and so were used in a parametric analysis. The Hepatosomatic index (HSI) was non-normally distributed, therefore for infection status Kruskal Wallis test and for ration and temperature a Mann-Whitney test was used. For specific growth rate (SGR) and body condition factor (BCF) a series of ANOVA models were used to test the main effects of temperature, host ration, infection status and interaction effects of these factors on host growth, condition and immune status. A Pearson correlation analysis was used to examine the relationship between parasite mass and parasite egg output.

Two-way ANOVA was used to examine the effect of rearing temperature and host ration on parasite mass, parasite index and the number of eggs produced by parasite. A linear regression model was used to test the relationship between parasite mass and parasite egg output. To test the effects of temperature and ration on the relationship between parasite mass and eggs produced by parasite an ANOCVA model was used. The data failed to meet the assumption of ANCOVA, therefore the graphical representation of data is presented in the result section. All analyses were done using Minitab 17 statistical software for Windows, Graph pad PRISM 7 used for graphs and IBM SPSS statistics 24 for Windows.

4.4 Results

4.4.1 Effect of temperature, host food ration and infection status on host growth condition and immune status

4.4.1.1 Specific growth rate (SGR)

There was a significant effect of temperature treatment on the specific growth rate (SGR) of all fish ($F_{1, 70} = 9.55$, $P = 0.003$; Figure 4.4), with fish growing more quickly at the lower temperature. There was no significant main effect of infection treatment or host ration on specific growth rate and no significant interactions between any of these factors found on the specific growth rate of sticklebacks (Table 4.1; Figure 4.4a).

4.4.1.2 Body condition factor (BCF)

The results showed significant main effect of temperature on the body condition factor of fish ($F_{1, 70} = 9.11$, $p = 0.004$; Figure 4.4), with fish held at the lower temperature having the higher BCF values. There was no significant main effect of host ration and infection, interaction effect between host ration, infection status and temperature (Table 4.1; Figure 4.4b).

4.4.1.3 Hepatosomatic index (HSI)

Rearing temperature had no effect on the hepatosomatic index of sticklebacks (median score: 15˚C = 5.83, 20˚C = 6.436; $p = 0.071$, ($N_{15˚C}$= 41, $N_{20˚C}$=41)). Host ration had no effect on the hepatosomatic index of sticklebacks (median

score: 8% = 7.84, 16% C = 5.99; p = 0.599 ($N_{8\%}$= 44, $N_{16\%}$=38)). Infection status showed no effect on the hepatosomatic index (H= 0.10, df = 2, p = 0.953).

4.4.1.4 Splenosomatic Index (SSI)

The results show that the temperature ($F1_{, 70}$ = 5.11, p = 0.027; Figure 4.4) and *S. solidus* infection status ($F_{1, 70}$ = 43.11, p = 0.000; Figure 4.4) each had a significant effect on the splenosomatic index of sticklebacks, with higher immune activation in infected fish. There was no main effect of host ration and no significant interaction effects of factors found on the splenosomatic index of sticklebacks (Table 4.2; Figure 4.4c). However, there is triple interaction temperature, infection status and ration on splenosomatic index ($F_{2, 70}$ = 3.82, p = 0.027; Table 4.2).

Table 4-1 ANOVA table with specific growth rate (SGR) and body condition factor (BCF) as a response variable of three-spined sticklebacks either exposed to *Schistocephalus solidus* or sham exposed (control) and reared under two temperature treatment (15˚C & 20˚C) and host ration (8% & 16%) for eight weeks.

		SGR			**BCF**	
	df	*F-value*	*P-value*	*df*	*F-value*	*P-value*
Infection status	2	2.28	0.110	2	2.55	0.085
Ration	1	0.33	0.570	1	0.12	0.734
Temperature	***1***	***9.55***	***0.003***	***1***	***9.11***	***0.004***
Infection status × ration	2	2.48	0.091	2	1.60	0.209
Infection status × temp	2	0.87	0.425	2	0.00	0.996
Ration × temp	1	2.63	0.109	1	0.08	0.772
Ration × infection status × temp	2	1.75	0.182	*2*	*1.55*	*0.220*
Error	70			70		

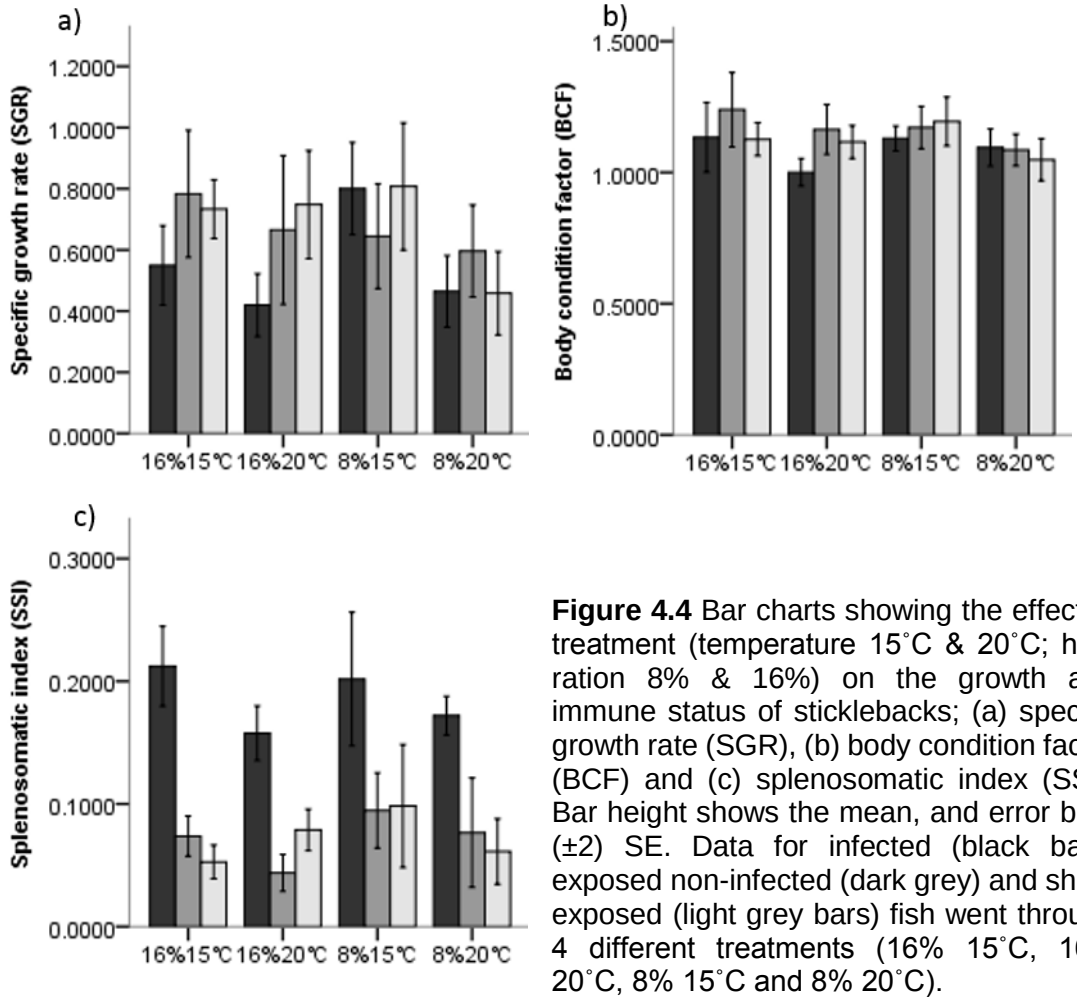

Figure 4.4 Bar charts showing the effect of treatment (temperature 15˚C & 20˚C; host ration 8% & 16%) on the growth and immune status of sticklebacks; (a) specific growth rate (SGR), (b) body condition factor (BCF) and (c) splenosomatic index (SSI). Bar height shows the mean, and error bars (±2) SE. Data for infected (black bars) exposed non-infected (dark grey) and sham exposed (light grey bars) fish went through 4 different treatments (16% 15˚C, 16% 20˚C, 8% 15˚C and 8% 20˚C).

Table 4-2 ANOVA table with splenosomatic index (SSI) a response variable of three-spined sticklebacks infected with *Schistocephalus solidus,* non-infected and sham exposed (control) and reared for eight weeks under two temperature treatment (15˚C & 20˚C) and host ration (8% & 16%).

Factors	**SSI**		
	df	*F-value*	*P-value*
Infection status	2	***43.14***	***0.000***
Ration	1	1.59	0.212
Temperature	***1***	***5.11***	***0.027***
Infection status × ration	2	0.93	0.400
Infection status × temp	2	1.53	0.224
Ration × temp	1	0.78	0.380
Ration × infection status × temp	2	3.82	0.027
Error	70		

4.4.2 Effect of temperature and host ration on parasite fitness

Temperature had a strongly significant effect on all indices of parasite fitness (total parasite mass, parasite index and mean egg output; Table 4.3, Figure 4.5 & 4.6), with parasites growing in fish held under the higher temperature produced plerocercoids with bigger mass. However, in contrast, there was no effect of host ration, and no interaction between host ration and temperature, on any aspect of parasite fitness (Table 4.3, Figure 4.5 & 4.6).

Table 4 - 3 ANOVA table showing the effect of temperature treatment (20˚C & 15˚C) and host ration treatment (8% & 16%) on total parasite mass, parasite index and eggs produced by *Schistocephalus solidus* as response variable. Significant values shown as bold and italicised.

Factor	Total parasite mass			Parasite index			Mean egg output		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Temp	***1***	***17.07***	***0.001***	***1***	***50.74***	***0.000***	***1***	***21.12***	***0.000***
Ration	1	1.55	0.229	1	1.59	0.224	1	0.00	0.958
Temp × ration	1	0.11	0.749	1	0.30	0.593	1	0.16	0.691
Total	18			18			18		

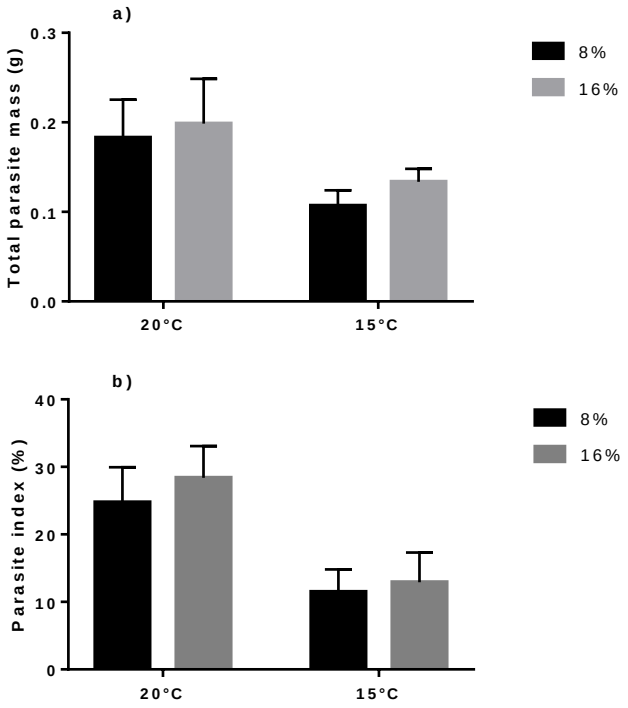

Figure 4.5 Bar charts showing the effect of temperature treatment (20˚C & 15˚C) and host ration treatment (8% & 16%) on the *Schistocephalus solidus* growth. (a) total parasite mass (g) and (b) parasite index (%) recovered from three-spined sticklebacks eight weeks' post-exposure.

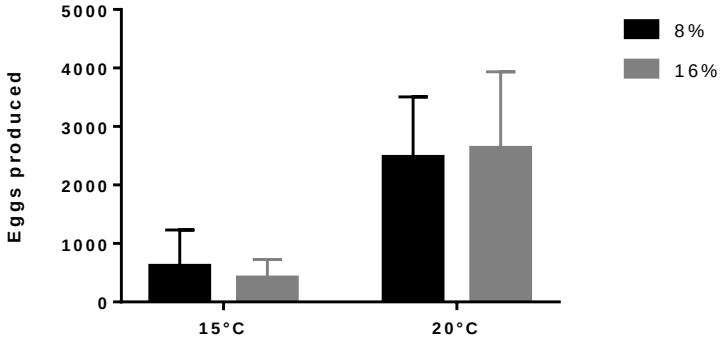

Figure 4.6 Bar charts showing the effect of temperature treatment (20˚C & 15˚C) and host ration treatment (8% & 16%) on egg production of *Schistocephalus solidus* recovered from three-spined sticklebacks eight weeks post-exposure.

4.4.2.1 Relationship between parasite mass and parasite fecundity

There was significant positive correlation between plerocercoid mass and parasite fecundity, measured as the number of eggs produced by adult parasites in culture (r_s = 0.669, p = 0.001, n = 22; Figure 4.7).

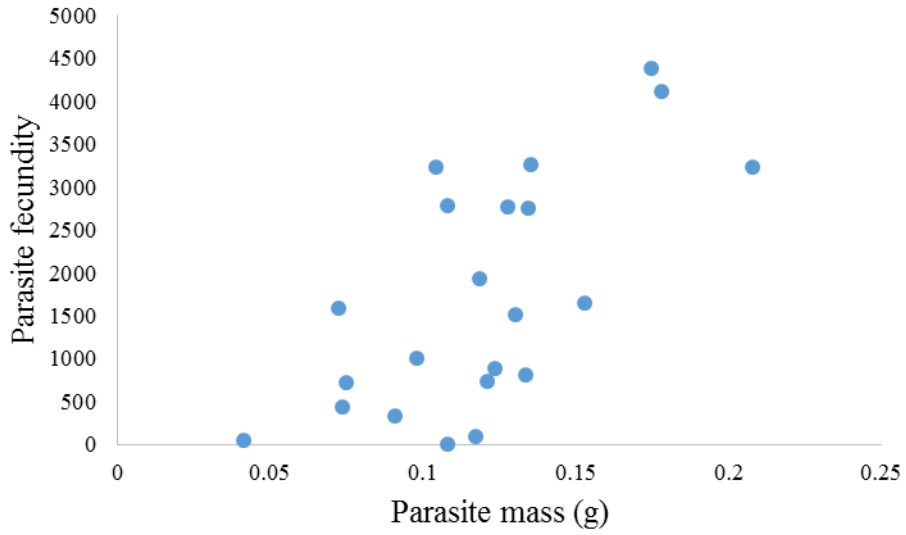

Figure 4.7 Scatterplot showing relationship between parasite mass and the eggs produce (mean) through in vitro culture technique by plerocercoids of *S. solidus* recovered from three-spined sticklebacks.

A linear regression model used to predict parasite egg output using plerocercoid mass. Plerocercoid mass accounted for 44.78% of variation in egg output, with r^2 = 44.78 %, which shows that 55.22% variation in mean egg output cannot be explained by parasite mass alone. The regression model predicts that parasite mass can significantly predict the mean egg output ($F_{(1, 20)}$ = 16.22, P = 0.001; Figure 4.7, Table 4.5).

Table 4-4 Linear regression model coefficients of regression parasite mass as predictor and parasite fecundity as response variable.

	Coefficient	*t-value*	*p-value*
Constant (intercept)	-1127	-1.51	0.146
Parasite mass (slope/gradient)	24015	4.03	0.001

4.4.2.2 Effect of temperature and ration on the relationship between parasite mass and eggs produced by plerocercoid

The small sample size hinders to carry out analysis of covariance (ANCOVA). However, there is no effect of host ration and rearing temperature noticeable on the relationship between parasites mass and eggs produced (Figure 4.8). Further, temperature showed significant effect on parasite mass, with no additional effect on the egg production, however figure 4.8 showed that fewer plerocercoids reared at higher temperature produced fewer eggs than expected.

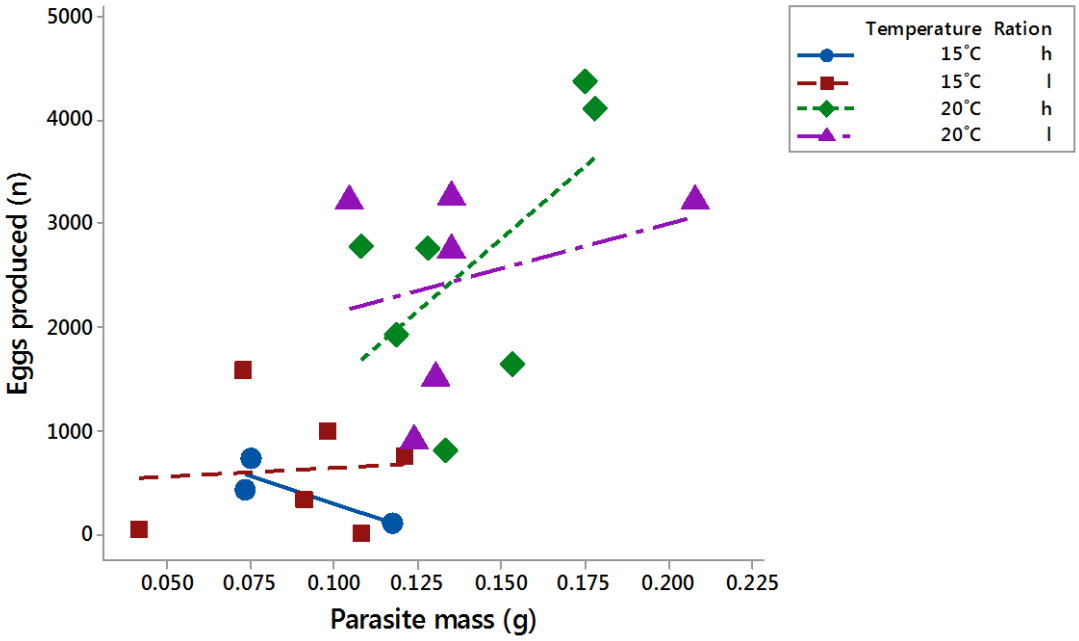

Figure 4.8 Scatterplot showing relation between the parasites mass (g) reared at two different temperatures (15 ˚C & 20˚C) and eggs produced following in vitro culture technique.

4.5 Discussion

Overall, temperature showed significant effects on the biology of host sticklebacks and *Schistocephalus solidus* parasites. The sticklebacks reared at cooler temperature grew better and were in improved body condition compared to fish reared at the higher temperature. The *Schistocephalus solidus* plerocercoids grew faster at the elevated temperature (20˚C) compared to the lower temperature (15˚C) and as a result attained a larger body size. Larger

plerocercoids of *Schistocephalus solidus* produced more eggs when cultured using a standard *in vitro* culture technique. However, host ration appeared to produce no significant additional effects on host growth or parasite fitness, over and above the effects on the mass attained by plerocercoid.

4.5.1. Effect of temperature and ration on host growth, condition and immune status

Temperature appeared to be the main factor influencing host biology. In this study, the growth (measured as SGR) of sticklebacks was increased at the lower temperature compared to a higher temperature. These results are similar to the findings of other recent studies (Franke et al., 2017, Macnab and Barber, 2012). One possible explanation of this decrease in the specific growth rate at higher temperature might probably be linked to energy conversion efficiencies which typically decrease as the temperature increases (Guderley and Leroy, 2001), and possibly the energy used to maintain metabolic rate at warmer temperature instead of being allocating to growth. Furthermore, as the body condition factor (BCF) results showed, fish reared at cooler temperatures were in a better body condition, compared to those reared at warmer temperatures. This probably due to the diversion of energy reserves to maintain higher metabolic rates at elevated temperatures, thus favouring the development of higher body condition at lower temperature. The host ration showed no effect on fish growth and condition.

In a previous study (Macnab and Barber, 2012), the food ration given to fish experiencing the two different temperature treatments was in excess. It is possible that the provision of an excess amount of food favours the enhanced growth of parasites at elevated temperatures, yet this may not be ecologically realistic. Endoparasite rely on host-derived nutrients for growth (Barber et al., 2008). To understand the role of host food ration on host and parasite growth, two different host rations 8% and 16% per day were used to feed the fish. However, the food ration failed to produce any significant differences, not only in parasite growth but in fish growth too. Perhaps, the 8% ration per day was enough to maintain the growth of fish irrespective of infection status. This shows that the effects of temperature on parasite growth are very strong even at lower host ration.

Infection status and ration had no significant effects on the specific growth rate and body condition of sticklebacks. The plerocercoids of *S. solidus* divert host energy reserves to fulfil the requirements of growing parasites, as they rely on the host to survive (Barber et al., 2008). However, in this study infection status failed to affect host growth. One possible explanation could be that the energy taken in the fixed ration was diverted to fulfil the metabolic needs at higher temperature.

The relative spleen size (splenosomatic index) is frequently used as an indicator of immune activation in teleost fish (Zapata et al., 2006, Handy et al., 2002). Infection status also had a significant impact on the splenosomatic index of sticklebacks. In this study infected sticklebacks showed a bigger spleen, which agrees with a study (Kalbe and Kurtz, 2006). Host ration, on the other hand, showed no effect on immune status of fish in this study. One likely reason that infection significantly influence immune activation is due to stress.

The relative size of the liver, expressed as the hepatosomatic index (HSI) indicates medium-term energy reserves in fish (Chellappa et al., 1995). In the present study, rearing temperature showed no significant effect on hepatosomatic index. These results are not similar to another study where sticklebacks reared at cooler temperature showed relatively bigger livers (Macnab and Barber, 2012). One likely reason of these results in the present study that there was no effect of temperature found on hepatosomatic index could be due to use of energy resources at higher temperature to maintain metabolic rate. Furthermore, the results show that infection had no effect on the energy reserves of the sticklebacks in this study. These results contrast to a previous study where sticklebacks with infection showed a lower hepatosomatic index (Tierney et al., 1996). Host ration failed to produce any significant effect on the hepatosomatic index of sticklebacks.

4.5.2 Effect of temperature and host food ration on parasite biology

Overall, rearing temperature had a significant effect on the growth of *S. solidus* plerocercoids, with those recovered from fish reared at 20˚C being considerably heavier than those recovered from host sticklebacks reared at 15˚C. The mass of parasite significantly predicts both plerocercoid infectivity (Tierney et al., 1996)

and egg production of adult worms in the avian definitive host (Dorucu et al., 2007). In this study, warmer temperatures appeared to be more favourable for parasites, perhaps because they were capable of exploiting the energetic reserves of sticklebacks more efficiently at warmer temperatures. Furthermore, sticklebacks also showed relatively poor growth and condition at warmer temperatures. These results are similar to finding of another study (Macnab and Barber, 2012). In this study, the food provided to fish was in excess. Fish were given either 16% or 8% ration per day to single out the effect of host ration on parasite development at elevated temperature.

However, the result of this study showed that there was no significant additional effect of host ration on the parasite growth and development at any temperature. Therefore, the 8% body weight ration per day was enough to sustain the growth of fish and parasite. In a study the effect of host ration 8% and 16% on growth of *S. solidus* plerocercoids was non-significant, which is similar to findings of study (Simmonds, 2015), where no other factors were considered. The results of this study show that, temperature is the main factor that influence the host-parasite interaction.

The already published data showed that a strong effect of plerocercoid mass on the parasite fecundity (Dorucu et al., 2007). Similarly, the result of this present study showed that the plerocercoids recovered from higher temperature were heavy and big in size and larger parasite mass significantly produced more eggs. The host ration showed no effects on parasite egg output. These results clearly show that temperature directly affects plerocercoid mass, which then affects egg output with few exceptions (figure 4.8). Whereas, there is no additional effect on egg output other than the effect of temperature on plerocercoid mass.

Furthermore, for future work targeted experiments by rearing fish harbouring parasite for at 15°C for longer duration (probably 12 to 16 weeks) may produce parasite with bigger mass. The eggs produced from these bigger size parasites may give insight to differentiate the effects of parasite mass and temperature on the egg output.

4.6 Conclusion

These findings show that temperature rise due to anthropogenic activities affects the parasite growth significantly and is an important factor to influence host parasite interaction, irrespective of the amount of food available to host. The effects of temperature are unlikely to diminish, even when the amount of food available to host is altered

Chapter 5.

Effect of *Schistocephalus solidus* infection and temperature on neurotransmitters in sticklebacks

5.1 Abstract

The effects of anthropogenic global warming on host-parasite interaction can have considerable ecological implications. Changing temperature has the potential to affect the severity and extent of parasite infection. Neurotransmitter play important role in controlling various behaviors in animals. Parasite are known to affect their host behavior and underling neurophysiological changes which can affect behavior. However, under temperature change which can affect severity of parasite infection, the consequences of developing parasite infection for the underlying neurophysiological changes is poorly understood. In this chapter, the results of an experimental study are reported. Three-spined sticklebacks were reared either at 15°C or 20°C and were either exposed to experimental infections with the cestode *Schistocephalus solidus,* or were sham-exposed, and held for eight weeks. The brain sample were collected at the end of experiment and by using high precision liquid chromatography technique (HPLC), the concentration of five different neurotransmitters were measured. The results showed that temperature change significantly affect the levels of dopamine neurotransmitter and its metabolite. Whereas, no significant effects of infections status found on the neurotransmitter levels.

5.2 Introduction

Animals are exposed to a variety of abiotic and biotic stressors in their natural environments. In response to these stressors, various physiological pathways are activated in their body. The physiological pathway that activates under stress is the hypothalamic-pituitary-adrenal (HPA) system and monoamine neurotransmitters (Winberg et al., 1997). For example, the removal of cage mates in male Wistar rats, and the subsequent change in their social environment, showed significant effects on the activation of the HPA axis, with elevated levels of corticosterone in the plasma of both control and chronically stressed animals (Ferland and Schrader, 2011). In addition, environmental stress in the form of elevated temperature result in higher levels of monoamine metabolites in juvenile common carp, *Cyprinus capio* (Boeck et al., 1996).

The monoamine neurotransmitters are group of chemicals, including dopamine (DA), serotonin (5-HT) and norepinephrine (NE), produced in body to control various physiological functions and pathways. For example, various aspects of animal behavior including locomotion, social and feeding behavior are influenced by monoamines (Winberg et al., 1993, de Pedro et al., 1998, Øverli et al., 2002, Øverli et al., 2004). The neurotransmitter levels are sensitive to a range of biological and environmental factors including immune status, nutritional status, the levels of circulating hormones, temperature and osmoregulatory stress (Boeck et al., 1996, Levin and Routh, 1996, Orosco et al., 1996, Lacosta et al., 1998, Leibowitz and Alexander, 1998).

In the wild, animals are exposed to multiple stressors at the same time unlike under laboratory controlled conditions, and it is increasingly recognized that these multiple stressors can have far more serious effects compared to a single stressor (Sih et al., 2004c). The interaction between multiple stressors can also influence various physiological pathways in animals. For example, two environmental factors i.e. temperature and a mixture of chemicals influence estrogenic responses in flathead minnows, *Pimephales promelas* (Brian et al., 2008). In three spined sticklebacks, *Gasterosteus aculeatus*, exposure to ecologically relevant challenges i.e. predator and fighting with unfamiliar conspecifics produce similarly elevated hypothalamic pituitary interrenal (HPI)

response but different monoamine content (Bell et al., 2007). Although there is a growing knowledge about how individual stressors and the interaction between multiple (similar) stressors can influence the physiological responses of animals, less is understood about how the interaction between parasite infection and environmental factor can affect physiological responses.

One of the main biological factors that have the potential to influence physiological pathways in animals is parasitism. For example, in roach, *Rutilus rutilus,* infections with the parasitic cestode *Ligula intestinalis* can inhibit the expression of gonadotrophin (LH) up to 50%, which may ultimately influence gametogenesis (Carter et al., 2005). In addition, the parasitic wasp *Cotesia congregata* significantly influences the octopamine content of the central nervous system of its host, the tobacco horn worm *Manduca sexta* (Adamo and Shoemaker, 2000). Furthermore, parasites are likely to influence the behavior of their intermediate hosts (Moore, 2013). These behavioral changes frequently increase the likelihood of parasites reaching the definitive host and completing their life cycles. The behavioral changes could be the side effects of infections (Poulin, 1995) or due to interference with host's neuroendocrine system (Adamo and Shoemaker, 2000, Adamo, 2013). For example, in Californian killifish *(Fundulus parvipinnis)* infections of the brain encysting trematode parasite *Euhaplorchis californiensis* showed no effect of parasite infection on host growth and reproduction. However, the locomotory behaviors of host were significantly affected by infection status and parasite density dependent differences were found in the levels of neurotransmitters in the brain of infected fish, which showed increased dopamine activity in the hypothalamus and decreased serotonin activity in the hippocampus compared to non-infected conspecifics (Shaw et al., 2009). Furthermore, in three spined sticklebacks *Gasterosteus aculeatus*, the infections with the cestode *Schistocephalus solidus* were significantly associated with variation in the levels of monoamines including serotonin (5-HT), norepinephrine (NE) and ratios between 5-HIAA:5HT (5-hydroxyindoleacetic acid: serotonin) (Øverli et al., 2001). Since the severity and extent of parasite infections can be affected by environmental change, including elevated environmental temperatures, it is therefore important to understand how such

environmental changes might interact with infection status to generate observed pattern of neurotransmitters in infected fish.

Temperature is one environmental factor that has been shown to influence host-parasite interactions, by altering the susceptibility of host animals to infection, growth, development rates of parasites, behaviour and physiology of infected animals (Paull and Johnson, 2011, Macnab and Barber, 2012, Garbutt et al., 2014, Paull and Johnson, 2014). Change in temperature can act as a stress and have the potential to influence immune status (Cairns et al., 2005). For example, in rainbow trout (*Oncorhynchus mykiss*), increased leukocyte activation was found when fish were exposed to an elevated temperature higher than 15˚C following exposure to *Aeromonas salmoncida* (Köllner and Kotterba, 2002).

To investigate the effect of *Schistocephalus solidus* infections and temperature change on the monoamine levels of sticklebacks, samples of the diencephalon were collected from fish that had been reared under different temperature regimes and differentially exposed to *S. solidus* infection (see details in Chapter 4). The questions addressed in this chapter are: (1) Does infection affect neurotransmitter levels in sticklebacks? (2) Does the temperature at which fish are reared affect the levels of neurotransmitters? (3) Does the interaction between rearing temperature and infection with parasites affect the levels of neurotransmitters in sticklebacks?

5.3 Methods

The brain samples of sticklebacks collected from fish used in the experimental study described in chapter 4. Sticklebacks that had been either exposed to infective *S. solidus* parasites, or sham-exposed (controls), were reared in the laboratory at either 15˚C or 20˚C for a period of eight weeks. During the eight weeks post exposure / sham exposure period, fish were fed a daily ration of either 8% or 16% body weight (for further details of the experimental design see Chapter 4, section 4.2). At the end of study, the fish were euthanized and brain samples were collected.

5.3.1 Brain sample collection

Following incision on the lateral sides of skull, sharp micro-dissection scissors were used to cut the anterior and posterior ends where the brain was joined to the skull and the brain was transferred gently into a clean Petri dish. The brain was cut into four sections: forebrain (cerebral hemisphere (labelled as 2 in fig. 5.1)), optic tectum (labelled as 3 in fig.5.1), and diencephalon (hypothalamus and thalamus (shaded area in ventral view of fig.5.1) and hindbrain (cerebrum and medulla oblongata (labelled as 4 in fig.5.1). Each section of the brain was transferred into a pre-weighed Eppendorf tube, and the weight of the brain tissue was recorded. Each tube containing brain tissue was labelled and immediately snap-frozen in liquid nitrogen, and stored at -80˚C prior to HPLC analysis. The diencephalon region of stickleback brain consists of epithalamus, hypothalamus, and thalamus (Wootton, 1976). In fish these parts are mainly involved in managing stress and levels of circulating hormones. Therefore, this part was used in further analysis.

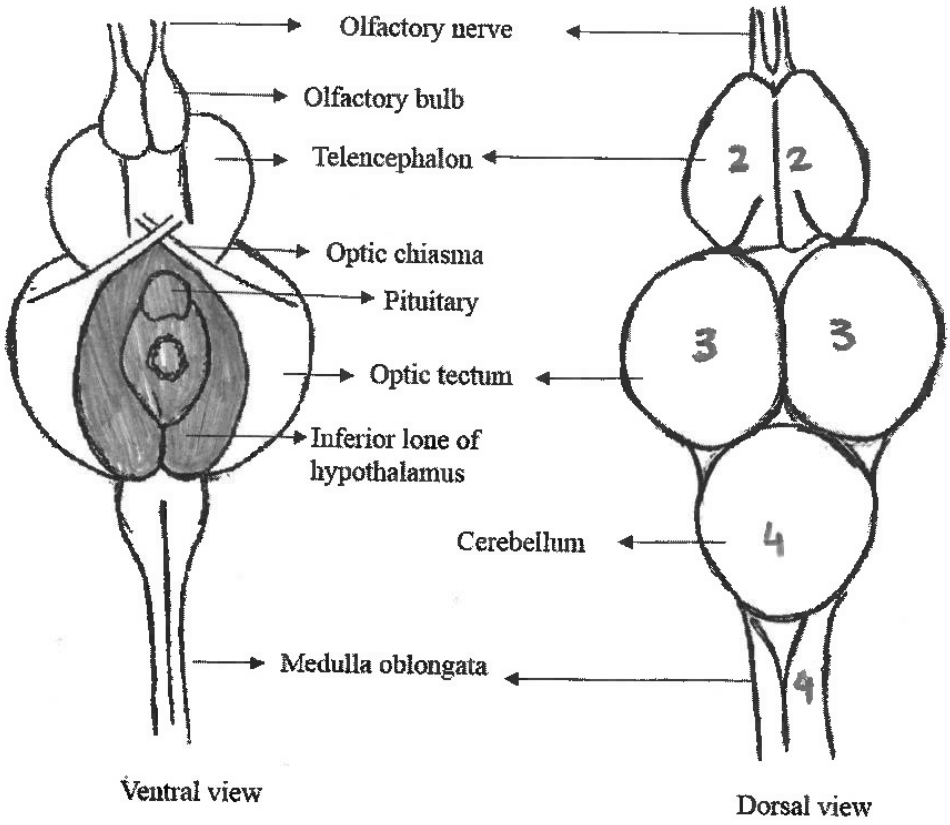

Figure 5.1 The brain of *Gasterosteus aculeate* (left ; ventral view: right; dorsal view) redrawn from (Wootton, 1976). The shaded area in ventral view shows the diencephalon. The numbers represent different regions of brain 2; forebrain, 3; optic tectum and 4; hindbrain in the dorsal view of stickleback brain.

5.3.2 HPLC analysis

5.3.2.1 Homogenisation of brain tissue

Brain samples of the diencephalon region of experimentally-infected (n = 18) and sham-exposed control fish (n = 20) were removed from -80C and put onto dry ice to be homogenised. The method 100 µl of Percholoric acid 0.1N (PCA) was added into the tube containing the brain tissue. The brain was homogenised thoroughly for one minute with a plastic pestle until no tissue lumps were noticeable. The homogenised brain tissue was placed onto ice and centrifuged at 4˚C for 15 minutes. Following centrifugation, the supernatant was collected carefully and placed into a new tube before being stored at -80˚C for further analysis.

5.3.2.2 HPLC analysis

The homogenised samples stored at -80˚C were defrosted by placing them into ice. Each sample (40 µl) was loaded into a 250 µl polypropylene tube and placed into HPLC machine in replicates of two. A 10 nM standard solution was prepared from a 1 µM concentrated standard solution (containing dopamine (DA), 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroytryptamine (5HT), 5-hydroxyindoleacetic acid (5HIAA)) by dissolving 3 µl solution in 297 µl of H_3PO_4. In each run of HPLC, three samples of standards solution and 8-10 samples of brain tissues were processed (each sample in duplicate). Eight different HPLC experiments were run for the two brain areas (four for each brain section) and the data from HPLC (acquisition sheets) were stored automatically in the computer for further analysis.

High performance liquid chromatography (HPLC), is a form of column chromatography that works on the principle of pumping pressurized liquid – the mobile phase (a solvent containing sample mixture) through a column of solid adsorbent material – the stationery phase. Each component in the solvent mixture interacts with adsorbent material differently from one another, and components flow out of the column separately due to different flow rates.

5.3.2.3 Data collection from HPLC acquisition sheets

The retention times and area covered by five different peaks (DA, DOPAC, HVA, 5-HT, 5HIAA) from the standard run was recorded. This was used to read the peaks with same or a similar retention time as the actual brain samples and to calculate the area under the peaks. All the data collected were transferred into an Excel sheet and were calibrated using the brain area weight. The pmol/mg concentration of each neurotransmitter obtained was then analysed statistically.

5.3.2.4 Statistical analysis

The data obtained had the following sample sizes: infected (n = 16), control (n = 14), 15˚C (n = 14) and 20˚C (n = 16). A two-way ANOVA test was used with infection status (control and infected) and temperature treatment (15˚C, 20˚C) as factors and the concentration of neurotransmitter as a response variable. The data that failed the assumption of a parametric test was log transformed. The

effects of host ration (8% or 16%) were not included in the result sections as the host ration showed no effects on the host and parasite biology (chapter 4) and on host neurotransmitter levels.

5.4 Results

5.4.1 Effect of temperature and infection on neurotransmitters

5.4.1.1 Dopamine (DA)

There was a significant effect of temperature on dopamine levels ($F_{(1, 26)} = 10.19$, $p = 0.004$; Figure 5.2.a), with fish reared at the higher temperature exhibiting higher dopamine concentrations. There was no effect of infection status ($F_{(1, 26)} = 0.003$, $p = 0.957$; Figure 5.2.b), and there was no interaction between temperature and infection status ($F_{(1, 26)} = 0.560$, $p = 0.46$).

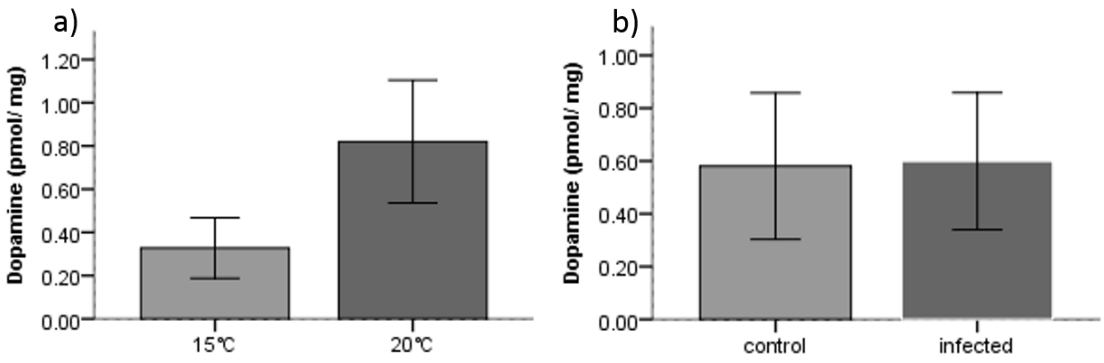

Figure 5.2 Bar charts (±2) SE showing the effect of (a) temperature (15°C: light grey bars; 20°C: black bars) and (b) infection status (infected and control) on the concentration (pmol/mg) of dopamine (DA) in the diencephalon.

5.4.1.2 3-4-dihydroxyphenylacetic Acid (DOPAC)

There was no significant effect of temperature on the levels of DOPAC ($F_{(1, 26)} = 1.919$, $p = 0.178$; Figure 5.3.a), nor was there an effect of infection status ($F_{(1, 26)}$

= 0.015, p = 0.902; Figure 5.3.b) and there was no interaction between the two factors ($F_{(1, 26)} = 0.814$, p = 0.375).

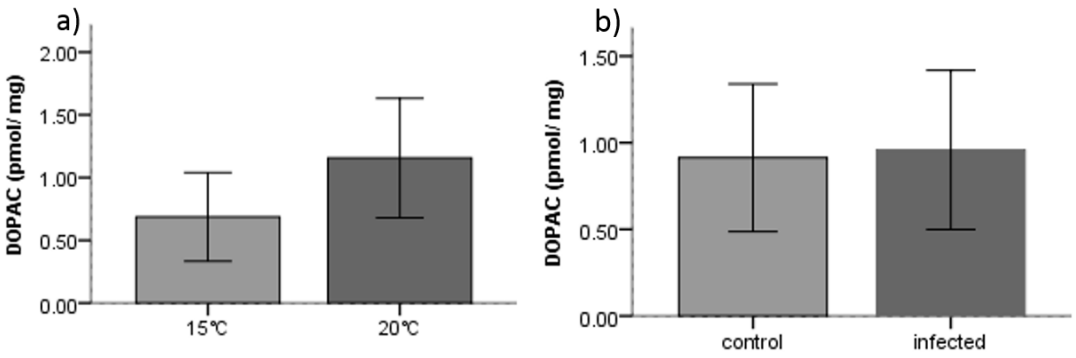

Figure 5.3 Bar charts (±2) SE showing the effect of temperature (15°C: light grey bars; 20°C: black bars) and infection status (infected and control) on the concentration (pmol/mg) of DOPAC in the diencephalon.

5.4.1.3 Homovanillic acid (HVA)

There was significant effect of temperature on HVA levels ($F_{(1, 26)} = 7.369$, p = 0.012; Figure 5.4.a), with fish reared at the higher temperature exhibiting higher DOPAC concentrations. There was no effect of infection status ($F_{(1, 26)} = 0.008$, p = 0.93; Figure 5.4.b) and there was no significant interaction between temperature and infection status ($F_{(1, 26)} = 0.588$, p = 0.450).

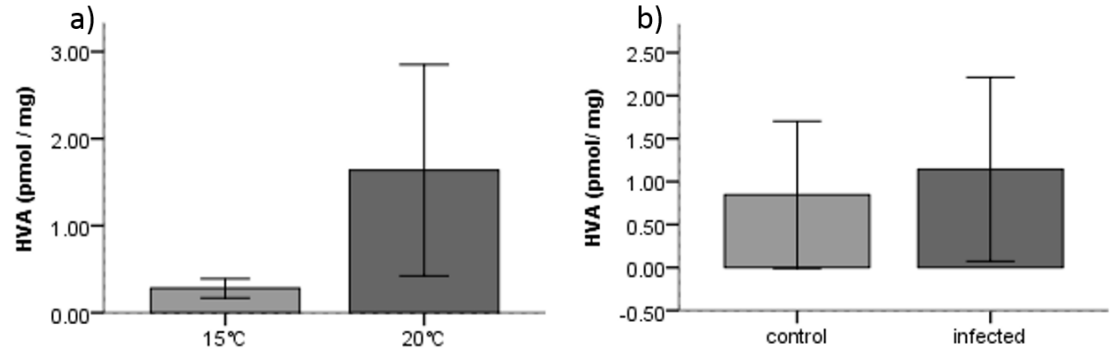

Figure 5.4 Bar charts (±2) SE showing the effect of temperature (15°C: light grey bars; 20°C: black bars) and infection status (infected and control) on the concentration (pmol/mg) of homovanillic acid (HVA) in the diencephalon of stickleback's brain.

5.4.1.4 Serotonin (5-hydroxytryptamine, 5-HT)

There was no effect of temperature ($F_{(1, 26)} = 2.263$, $p = 0.145$; Figure 5.5.a) or infection status ($F_{(1, 26)} = 0.015$, $p = 0.905$; Figure 5.5.b) on 5-HT levels, and there was interaction between the two factors ($F_{(1, 26)} = 0.008$, $p = 0.929$).

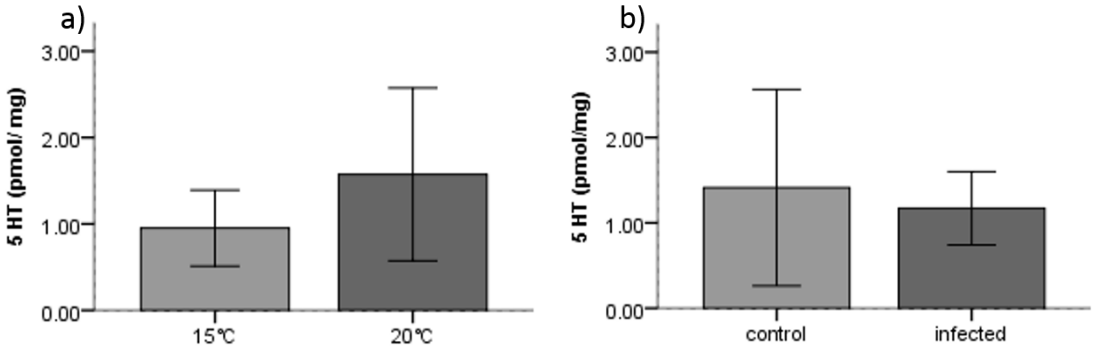

Figure 5.5 Bar charts (±2) SE showing the effect of temperature (15°C: light grey bars; 20°C: black bars) and infection status (infected and control) on the concentration (pmol/mg) of 5-HT in the diencephalon.

5.4.1.5 5-hydroxyindoleacetic Acid (5-HIAA)

There was no effect on rearing temperature ($F_{(1, 26)} = 1.741$, $p = 0.198$; Figure 5.6.a), or infection status ($F_{(1, 26)} = 0.086$, $p = 0.772$; Figure 5.6.b) on 5-HIAA levels and no significant interaction between the two factors ($F_{(1, 26)} = 0.680$, $p = 0.417$).

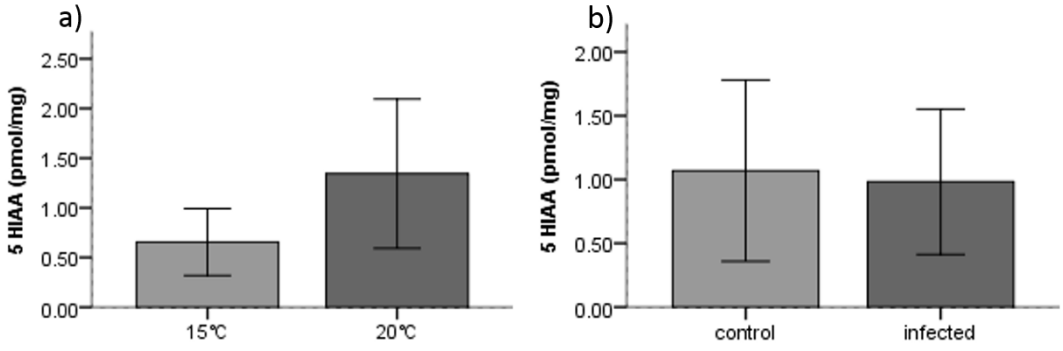

Figure 5. 6 Bar charts (±2) SE showing the effect of temperature (15°C: light grey bars; 20°C: black bars) and infection status (infected and control) on the concentration (pmol/mg) of serotonin (5HIAA) in the diencephalon.

5.5 Discussion

The overall results show that elevated rearing temperature had a significant effect on the levels of dopamine (DA), and of its metabolite homovanillic acid (HVA) in the diencephalon of stickleback brains. The concentration of dopamine and HVA is higher in the fish brain area reared at higher temperature i.e. 20˚C compared to lower temperature i.e. 15˚C. In addition, there is a clear trend that the concentration of other three neurotransmitter DOPAC, 5-HIAA and 5-HT were higher at 20˚C compared to 15˚C, but these differences were statistically non-significant.

Furthermore, experimentally-induced infections with the parasite S. *solidus* showed no significant effects on the levels of neurotransmitters recorded in this study, and there were no significant interaction effects.

5.5.1 Effect of temperature on neurotransmitters

Rearing temperature was significantly associated with differences in the concentration of dopamine (DA) and homovanillic acid (HVA), with higher levels recovered from the brains of sticklebacks reared at 20°C compared to 15°C. The levels of dopamine (DA) and its metabolite were significantly higher at the higher temperature. Serotonin (5-HT) and its metabolites 3-4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) showed a trend of higher concentration at higher temperature, however for these two neurotransmitters the effect of temperature was statistically non-significant. Dopamine is involved in thermoregulation in animals, for example, hypothalamic dopamine involved in the heat loss system in endotherms (Lee et al., 1985). Endogenous dopamine plays important role in temperature regulation in rats (Cox and Lee, 1980). In juvenile common carp, *Cyprinus capio*, fish showed higher levels of monoamine metabolites i.e. enhanced dopamine activity and serotonin metabolism at elevated temperature, with increased levels of dopamine and serotonin at increased salinity (Boeck et al., 1996). Thermal acclamation can alter serotonin (5-HT) and nor-epinephrine (NE) in the fish (Tsai and Wang, 1997). One likely reason for the results of this present study could be the stress induced by higher temperature, which led to higher levels of dopamine in sticklebacks. In fish, the

hypothalamus manages stress in response to stress corticotrophin releasing factor (CRF), arginine vasotocin (AVT), melanin-concentrating hormone (MCH) and ventral diencephalic dopamine (DA) releases, which determine adrenocorticotropic hormone (ACTH) and adrenal (interrenal) cortisol output. Cortisol is key factor which distributes energy to organs in response to environmental challenges (Gorissen and Flik, 2016).

Furthermore, the increased levels of dopamine (DA) at higher temperature may also possible due to indirect effect of depleted oxygen which can be influenced by higher temperature. Dopamine is related to movement/locomotion in organisms. Under depleted oxygen it may possible that fish were coming to surface to get more oxygen. As a result, fish were under stress of depleted oxygen and higher metabolic rate which may led to higher levels of dopamine.

5.5.2 Lack of an infection effect on neurotransmitter levels

There was no significant effect of *S. solidus* infection on dopamine, DOPAC, 5HIAA, 5-HT and HVA. These results are not consistent with the findings of other studies. For example, three spined sticklebacks infected with *S. solidus* showed elevated 5-HIAA:5-HT ratios in the brainstem and hypothalamus (Øverli et al., 2001). In response to stress, serotoninergic activity typically increases in animals (Winberg and Nilsson, 1993). In the present study, the sticklebacks were exposed to *S. solidus* for eight weeks, meaning that it was expected that fish will have higher levels of 5-HT due to chronic stress. There are two possible explanations that infections can change levels of neurotransmitters; firstly, due to simple host response to chronic stress, and secondly, due to adaptive manipulation of parasites to their hosts. If parasites attain infectivity (i.e. grow to ≥ 50 mg in size) in the second intermediate host and are therefore ready to be transmitted to the definitive host they will influence monoamine levels, which will change host's behavior in favor of the parasite to reach definitive host. According to this explanation, higher monoamine levels should have been detected among infected fish, because the parasites recovered from fish in this present study were almost all bigger than 50 mg in size. However, the results of this study showed no effect of infection on monoamine levels. One possible explanation of these results could be the infection levels. 54% of infected fish in total sample had

double infection (two plerocercoids), whereas in case of *S. solidus*, at a given parasite mass the single infections are far more debilitating for its host compared to multiple infections (Nordeide and Matos, 2016).

5.6 Conclusion

This experimental study showed that temperature change significantly affects the levels of dopamine and homovanillic acid in sticklebacks. However, infection status had no effect on monoamines levels. It may possible that the under changing temperature the effects of parasite infections are less pronounced and temperature appeared to be the main factor that cause stress and influence monoamine levels in sticklebacks.

Chapter 6.

How do interactions between multiple stressors affect parasite fitness?

6.1 Abstract

The effects of environmental factors can have considerable impacts on host-parasite interactions. However, the consequences of multiple stressors, such as are encountered simultaneously in degraded ecosystems, on the fitness of multiple-host parasite is poorly understood. In this chapter, an experimental study was undertaken to examine the effects of pre-exposure to an important aquatic pollutant, the heavy metal, copper (Cu), and elevated temperature on the fitness of *Schistocephalus solidus* as it progresses through its life cycle. In the study, the parasite was first exposed to either copper or a control (distilled water) in its first-intermediate host, the copepod *Cyclops strenuus*. Once the parasites had attained infectivity to the second intermediate host, they were fed to sticklebacks in controlled experimental infections. Parasite-exposed fish were then held at either 15˚C or 20˚C for six weeks. After six weeks, the exposed fish were euthanized and data from both recovered parasites and sticklebacks was recorded. The results shows that the number of plerocercoids developed in fish was very low. However, the number of parasites developed at higher temperature was twice the number of parasites developed at lower temperature. More experimental studies clearly required to investigate the effects of changing temperature on susceptibility of developing infections.

6.2 Introduction

Animals are potentially exposed to a wide range of abiotic and biotic stressors in their natural environments. These stressors include anthropogenic stressors, such as altered thermal regimes, pollutants, and natural environmental stressors, including parasites, competitors and the threat of predation (Christensen et al., 2006, Coors and De Meester, 2008). Chemical and natural stressors interact and affect living species in different ways (Holmstrup et al., 2010, Laskowski et al., 2010) and exposure to multiple stressors can be more harmful than single stressors (Sih et al., 2004c).

Parasites themselves are sensitive to environmental change and can be used to monitor environmental pollution as caused by anthropogenic activities, including industrialization (Sures, 2004). Parasites may respond to pollution in a number of ways, acting as pollutant sinks, indicators of ecosystem health, as bio indicators (Sures et al., 2017b) and they may act as driving agents for key ecological processes (Sures et al., 2017a). For example, the acanthocephalan parasite *Pomphorhynchus laevis* accumulated higher levels of cadmium (Cd) and lead (Pb) than the host fish (Sures and Taraschewski, 1995). Much research has focused on host-parasite interactions under altered environment conditions (Lafferty and Kuris, 1999, Sures, 2003, Sures, 2006, Gerard et al., 2008). The effects of multiple stressors on the health of living organisms are often unpredictable (Marcogliese et al., 2010). In some cases, the interaction between multiple stressors lead to synergistic, negative, additive and or antagonistic effects (Aufauvre et al., 2012, Holmstrup et al., 2010, Brian et al., 2008). However, less is known about the effect of interaction between multiple environmental stressors on parasite fitness, and the effects of multiple stressors on the fitness of parasitic organisms remains poorly understood.

Anthropogenic activities mainly industrialization produce a number a heavy metals which are becoming part of aquatic ecosystem. Copper (Cu) is one such element which also exists naturally. It is an essential micronutrient that can lead to harmful effects if available in excess. In crustaceans, Cu uptake is either by food intake or through permeable body surfaces and is then transported in the haemolymph within the body upon binding to metabolites (Marsden and

Rainbow, 2004). For example, during developmental stages of the freshwater copepod *Mesocyclops pehpeiensis*, exposure to heavy metals reduces the development of nauplius larvae compared to control in both acute and chronic toxicity tests (Wong and Pak, 2004). In another study, exposure to a higher concentration of cadmium increased the mortality of the copepod *Diaptomus forbesi* (Ghosal and Kaviraj, 2002). Despite this, and the importance of copepods as intermediate hosts of a wide range of fish parasites, studies of disease progression in copepod hosts under altered heavy metal conditions are limited. In *Cyclops strenuus* infected with procercoids of *Bothriocephalus acheilognathi*, tapeworms exposed to 100 µg/L Cd showed decreased copepod survival (Khalil et al., 2014) suggesting a detrimental effect of heavy metal exposure and parasitism on copepod mortality.

An experimental study was therefore designed to investigate the effect of two important stressors of aquatic ecosystems – elevated environmental temperature and heavy metal pollution – on aspects of the fitness of the parasite *Schistocephalus solidus.* The first stage larvae of *S. solidus* grow and develop in the haemocoel of copepods, the first intermediate hosts of the parasite (Smyth, 1969). One of the study have reported copper concentration in the UK rivers range between 0.02µg/L to 133µg/L (median concentration of 4.7µg/L) (Donnachie et al., 2014). In another study, the concentration of copper measured in treated and untreated wastewater from biological wastewater treatment plant of Riberirao Preto (RP-BWTP), Brazil, showed 2.13-19.87 µg/L (mean 9.66 SD 4.86) and 10.66- 28.50 µg/L (mean 17.31 SD 4.93) (da Silva Oliveira et al., 2007). *Schistocephalus* infections in copepods were exposed to 10 µg/L Cu until they had attained infectivity, and then were fed to three-spined stickleback fish (the second intermediate hosts). Fish consume copepods as food and the parasite sheds its outer layer in the host's digestive tract and resides in the body cavity as it develops further (Hammerschmidt and Kurtz, 2007). Sticklebacks exposed to *S. solidus* were then maintained at two different temperatures (15˚C and 20˚C) for a period of six weeks. Water temperature has already been shown to have a significant effect on the growth of parasites in fish hosts (Franke et al., 2017, Macnab and Barber, 2012). The study therefore addressed the following questions: does pre-exposure of early developmental stages of the parasite to a

pollutant (copper) affect (1) the probability of establishment of infection, and / or (2) parasite fitness, in stickleback hosts held under the two temperature conditions?

6.3 Method

6.3.1 Preparation of stock solution

A stock solution of 100 mg/L Cu was prepared by dissolving 0.393 g of $CuSO_4.5H_2O$ in 1000 ml of distilled water. To prepare 10 µg/L Cu solution, 50 µl stock solution was added to 499.95 ml of distilled water in a 500 ml bottle. The salt was measured by using an electrical balance and water with a graduated measuring cylinder. The stock solution was prepared at the beginning of the experiment and the 10 µg/L Cu solution was prepared fresh on every third day to replace the old water.

6.3.2 Copepod exposure to treatment and *S. solidus* infections

Copepods were sieved from the stock flask using a 200 µm sieve and separated for batch and individual exposure to *S. solidus*. The sieved copepods were either exposed to 10 µg/L Cu solution or distilled water (as control) for 24 hours to acclimate to the new water conditions. The copepods were either kept as a batch with a group of 30 copepods in 250 ml conical flask with water volume of 180 ml, or individually in 24-well plates (individual exposures). For individual exposures, three replicates were used and batch exposure was carried out as back up in case of failure of development of infection in individual exposure. The eggs of *S. solidus* obtained by *in vitro* culturing of plerocercoids, obtained from lab bred and infected sticklebacks (Smyth, 1946). Following harvesting, the eggs were kept at 20˚C in the dark to develop. They were then exposed to light after 13 weeks. On the following day, any hatched, free swimming coracidia were transferred into new Petri dishes by using a glass pipette under a dissecting microscope. The acclimated copepods (10µg/L Cu solution and/or distilled water) were either batch exposed in a 250 ml conical flask with a 180 ml water column or individually exposed in a 24 well-plate with 3-4 coracidia per copepod. Following exposure to *S. solidus*, the copepods were kept at 20˚C under a 12L: 12D photoperiod and were fed with alfalfa infusion on the next day. On every third day, the water was

replaced either with 10 µg/L Cu solution and/or distilled water and copepods were fed with the alfalfa infusion.

6.3.3 Copepod screening and stickleback infections

Copepods were screened under a microscope to identify the presence or absence of the cercomer (a hooked developmental structure that indicates parasite infectivity). Copepods containing a single infective procercoid were used for subsequent experimental infections of sticklebacks. Adult, lab-bred three spined sticklebacks of River Welland origin (n = 45) were selected at random from stock tanks and fed a *S. solidus* infected copepod that had been reared in the 10µg/L Cu solution or in distilled water, to examine the impact of copepod rearing treatment on parasite development in the second intermediate host, at two different temperatures (see Figure 6.1). Following exposure, fish were fed with frozen bloodworms at a ration of 10% body weight each day, and were weighed at week 0, 3, and 6 to allow the 10% body ration to be recalculated. Water temperature was maintained using thermostatic control, at a mean (± SD) of 15.66 ± 0.44 for 15˚C and 19.67 ± 0.34 for 20˚C. Further details of the husbandry setup in which the fish were maintained during the experiment are found in section 4.1.1.

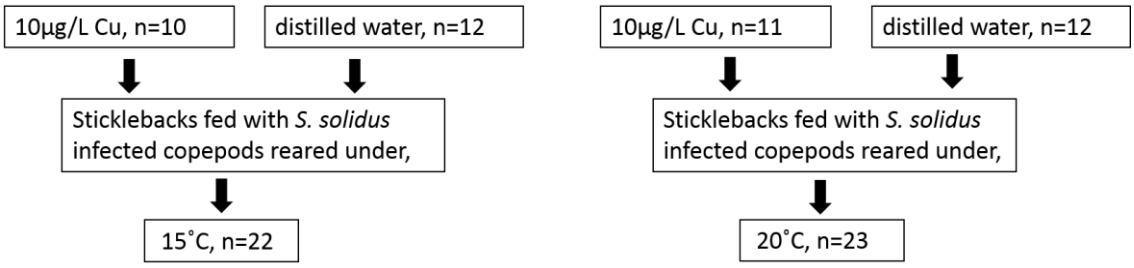

Figure 6.1 Pictogram showing detail of the treatment and number of sticklebacks exposed to *S. solidus* reared in copepods at two different treatments for 6 weeks.

6.3.4 Fish dissection and parasite collection

At the end of post-exposure week six, fish were euthanized by using a Home Office approved Schedule 1 methods (overdose of Benzocaine anaesthetic). The fish final mass (M, to 0.001g) and standard length (SL, to 0.01mm) were recorded

using an analytical balance and dial calliper respectively. Any plerocercoids recovered from infected fish were weighed individually and transferred immediately into RPMI-cell culture media in a Petri dish, to allow them to be cultured *in vitro* for subsequent quantification of egg output (for further details, see Chapter 4). One fish that had been fed an infected copepod reared under 10 µg/L Cu and held at 20˚C, and three that had been fed infected copepods reared under distilled water treated *S. solidus* (two held at 20 ˚C and one held at 15 ˚C) died during the experiment. No fish were reared under heavy metal treatment in the experiment. One fish was found to contain two plerocercoids at the time of dissection and so the data collected from it was not included in the parasite fitness analysis.

6.2.5 Statistical analysis

To test the effect of water treatment (Cu vs distilled water) on the probability of developing infections after being fed infective parasites, a Chi-square test used. Very few plerocercoids developed in fish held at 15˚C (1/10 fish fed copepods reared at 10µg/L Cu; 2/12 fish fed copepods reared in distilled water), so only plerocercoids recovered from fish held at 20˚C were used for further statistical analysis. A Mann-Whitney U test was used to test the effect of pre-treatment (10µg/L Cu and distilled water) on the parasite index and parasite mass of *S. solidus* plerocercoids recovered from fish reared at 20˚C.

All the analysis was carried out in Graph Pad Prism 7 and SPSS IBM Statistics for Windows.

6.4 Results

6.4.1 Effect of pre-exposure to copper in first intermediate host on susceptibility of developing infection in second intermediate host

Exposure of infected copepods to copper had no significant effect on the likelihood of *S. solidus i*nfections establishing in exposed sticklebacks (χ^2) = 0.408, DF =1, P = 0.523, Figure 6.2.b. Furthermore, fish rearing temperature also had no significant effect on the probability of sticklebacks developing an infection following parasite exposure (χ^2 = 2.383, DF =1, P = 0.123, Figure 6.2.a.

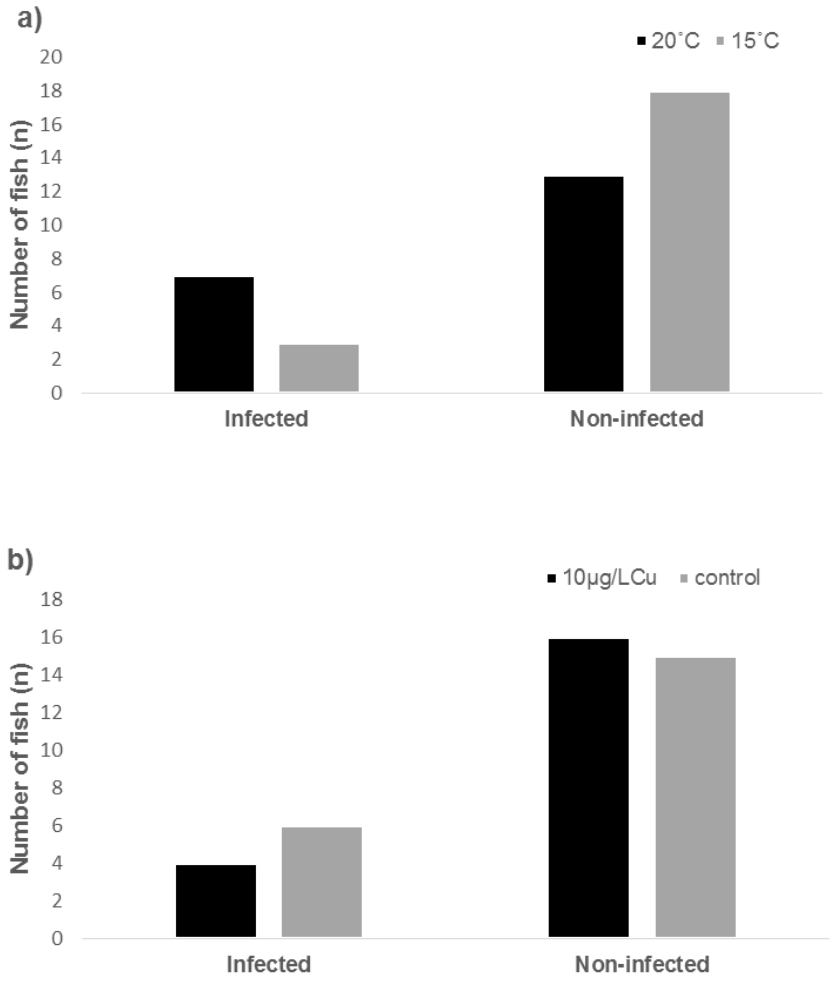

Figure 6.2 Bar charts showing the effect of (a) temperature (15˚C and 20˚C) and (b) pre-exposure to 10 µg/L Cu and distilled water-treated *S. solidus* procercoid and (b) on susceptibility of developing infections of S. solidus in three spined sticklebacks.

6.4.2 Parasite fitness

The results show that there was no significant effect of copper pre-treatment (10µg/L Cu vs distilled water) on either the absolute mass of plerocercoids recovered from fish reared at 20˚C, (U = 2.0. P = 0.229, r = -0.53; Figure 6.3) or the parasite index (U = 2.0, P = 0.229, r = -0.53; Figure 6.4).

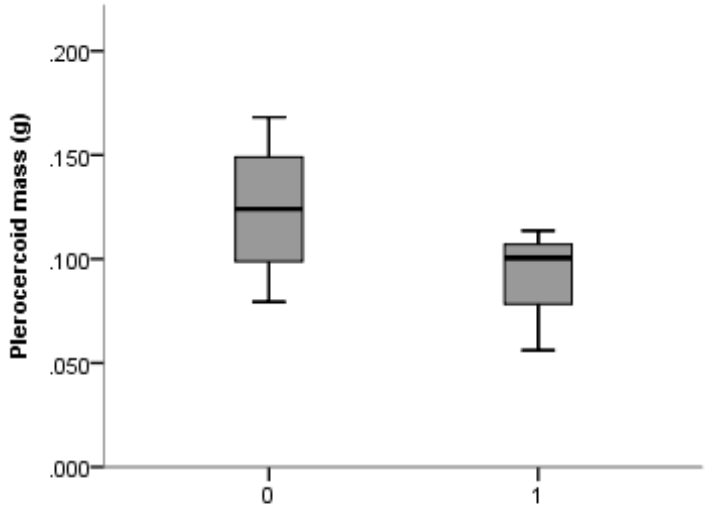

Figure 6.3 Box plots showing effect of pre-treatment (distilled water and 10µg/L Cu) in the copepod host on the parasite mass of *S. solidus* recovered from sticklebacks reared at 20˚C for six weeks. The zero (0) represents distilled water (n= 4) and 1 represents 10µg/L Cu (n=3) treatment.

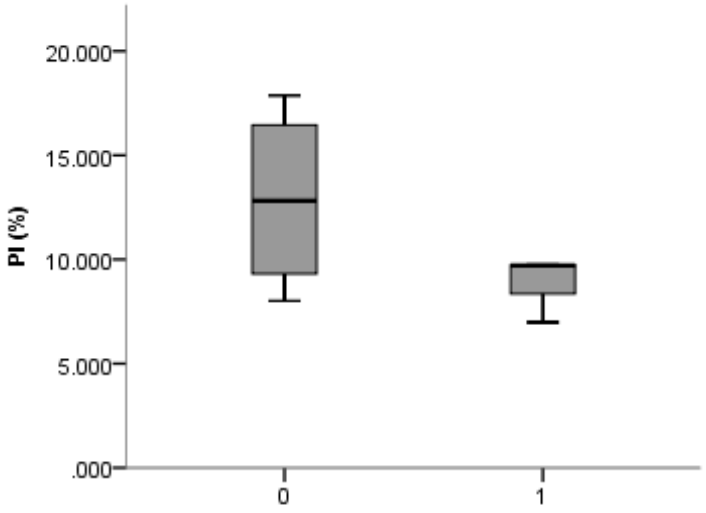

Figure 6.4 Box plots showing effect of pre-treatment (distilled water and 10µg/L Cu) in the copepod host on the parasite index of *S. solidus* recovered from sticklebacks reared at 20˚C for six weeks. The zero (0) represents distilled water (n= 4) and 1 represents 10µg/L Cu (n=3) treatment

6.5 Discussion

The results shows that there was no significant effects of pre-exposure to copper treatment (copepod stage) and rearing temperature(fish stage) on probability of developing infections of *S. solidus* in sticklebacks. Although parasite infections were more than twice as likely to occur in fish reared at 20˚C than at 15˚C, due to the small sample sizes, this effect was non-significant.

6.5.1 Effect treatment on parasite fitness

Overall, the parasites recovered from fish reared at 15˚C were < 50 mg in mass, whereas at 20˚C ≥ 50 mg. The number of plerocercoids developed at 15˚C were lower therefore not included in the result section. The plerocercoids that were recovered from fish held at 20˚C, which had developed from copepods exposed to the 10µg/L Cu treatment, weighed 0.090 ± 0.03 g (mean ± SD) while those developing from control copepods held in distilled water were 0.138 ± 0.026 g. There was no significant effect of pre-treatment of 10µg/L concentration of copper found on the parasite fitness parameters including, plerocercoids mass, and parasite index of *S. solidus* plerocercoids recovered from sticklebacks held at 20˚C for six weeks. To date, and according to my knowledge, the literature on toxic effects of heavy metals on the growth and development of cestode parasites *in vivo* is not available. However, some studies have previously reported the toxic effect of different heavy metal on the different free swimming infective stages stages of aquatic parasites. For example, copper toxicity showed reduced longevity and infectivity of *Echinoparyphium recurvatum* cercaria when exposed to different concentration in both soft and hard water (Evans, 1982). In *Diplostomum spathaceum*, exposure to heavy metals i.e. cadmium and zinc, reduces survival as concentration increases (Morley et al., 2001). Direct exposure to heavy metals, e.g. cadmium, decreases the longevity of free living *Diplostomum spathaceum* cercaria (Pietrock et al., 2002). Furthermore the effect of temperature on chronic metal toxicity of Copper (Cu), Nickle (Ni) and Zinc (Zn) to *Daphnia magna* decreases as temperature increase (Pereira et al., 2017).

The infections were more than twice more likely to establish in fish at 20˚C than 15˚C. The rearing temperature showed effects on the establishment and

development of *S. solidus* with bigger mass and two fold establishment of infections at 20˚C. These results are interesting, as they show the possible effects of temperature change on parasites susceptibility to establish and develop infections in fish host. The infections developed in the second intermediate host is very low in number, it is difficult to explain the underlying reason of these results. However, for future studies with larger sample size will give more insight that, how changing thermal regimes can mediate ecological interaction between species, and potentially impact host-parasite interactions.

Chapter 7.

Preliminary laboratory work for developing *Ligula intestinalis* and *Pimephales promelas* as a model parasite-host system

Abstract

Parasite infection can have wide cological and economic importance due to their castration effects on fish host, hence cause serious effects on subsistence or commercial fisheries. A wide range of tropical and temperate fish are suitable host of *Liguls intestinalis* – cestode parasite. Most of the data avialable on the effects of *Ligula* on fish host is from naturally infected fish hosts, whereas the experimantal model is not well developed. One of the main aim of this chapter was to develop an experimental host-parasite model consisting of *Ligula intestinalis* and *Pimephales promelas* to answer important questions of experimental biology and behavioural ecology in laboratory controlled conditions. The results show *Pimephales promelas* can be used as model to study behavioural ecology. Whereas, developing life cycle of *Ligula* in laboratory requires further work by use of exposing copepods from wild to *Ligula* coracidia (freee swimming stage) in laboratory controlled conditions.

7.1 Preliminary laboratory work for developing *Ligula intestinalis* as model parasite

7.1.1 Introduction

Ligula intestinalis is a diphyllobothriidean cestode that is considered to be an important parasite in various fields of biology, including ecology, parasitology and genetics. The parasite is cosmopolitan in its distribution and has attained considerable attention over the past few decades due to its detrimental effects on variety of cyprinid fish hosts (Bouzid et al., 2008, Morgan, 2003, Olson et al., 2002, Hoole and Arme, 1983, Hoole et al., 2010). One of the main effects of *Ligula intestinalis* infection on its fish hosts is to affect reproductive development by influencing the gonado-pituitary axis of host fish (Trubiroha et al., 2010, Trubiroha et al., 2011). As a result, infections may impact spawning success and recruitment, with consequences for population survival in natural populations and subsistence fisheries (Bean and Winfield, 1989, Kennedy et al., 2001).

The parasite has a complex life cycle (Figure 7.1) consisting of one free-living stage (the coracidium), two intermediate hosts (IH1: a copepod; IH2: a cyprinid fish) and a definitive host (DH: a piscivorous bird) (Smyth and McManus, 1989). The effects of parasite are more severe on the second intermediate host (cyprinid fish) compared to first intermediate and definitive host.

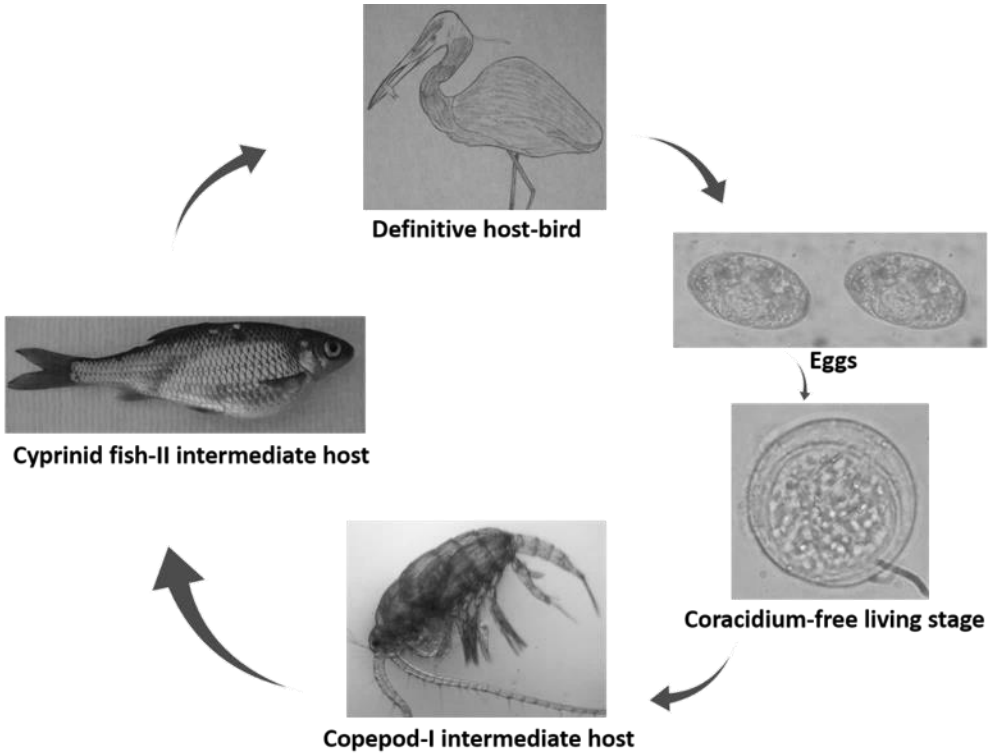

Figure 7.1 Life cycle of *Ligula intestinalis* (Images by Yaqub. S)

Ligula-infected cyprinid fish show abnormal swelling in the abdominal cavity due to the large size attained by individual plerocercoids. In heavy infections, the combined mass of plerocercoids can reach 5g which leads to complete deformation and dislocation of the pectoral fins (Loot et al., 2002a, Loot et al., 2002b). In a recent study the mass of a single plerocercoid was reported up to 16g in predator carp, *Chandodichthys erythropterus* (Sohn et al., 2016).

In addition, deformation of the body may maximise the chances that infected fish are targeted and caught by piscivorous birds (Palikova et al., 2014, Museth, 2001). Furthermore, the infected fish tend to have altered schooling behaviour from their non-infected shoal mates, which is likely due to manipulation of fish behaviour by the parasite in order to reach its definitive host by increasing the chances of predation (Barber and Huntingford, 1996).

Ligula act as a natural stressor of fish and it is also likely to affect the levels of biomarkers, for example, heat shock protein (HSP70) and hepatic glutathione-S-transferase (GST) in their second intermediate hosts (Frank et al., 2013, Frank

et al., 2011). *Ligula* plerocercoids can also cause castration and slow down the reproductive efficiency of host fish (Morgan, 2003) by triggering immature gonad development (Dejen et al., 2006). Effects of 'ligulosis' (the name given to the fish disease associated with infection by *Ligula* plerocercoids) on sexual development are very obvious, with more pronounced effects in females compared to males, with the diversion of energy for plerocercoid development resulting in a larger drop in GSI (gonado somatic index) among female than male fish, because perivisceral fat reserves in females normally nourish the growth of *Ligula* plerocercoids (Vanacker et al., 2012). *Ligula* obstructs the host's reproductive development through endocrine disruption (Trubiroha et al., 2011) and inhibits gonadotropin expression in the pituitary through the brain-pituitary-gonadal axis of the fish hosts, which distorts the normal growth of gonads (Carter et al., 2005, Arme, 1997).

The majority of knowledge regarding *Ligula* infections in the studies mentioned above and elsewhere has been gathered from studies carried out on naturally infected fish, and these studies clearly suggest detrimental effects of the parasites on fish host biology. However, to date there has been no study that has been carried out on experimentally infected fish. An original aim of the present PhD thesis was to develop the fathead minnow *Pimephales promelas* as an experimental host for investigating the impact of experimentally-induced *Ligula intestinalis* infections on host behaviour and personality. Due to limited success in developing the working model, the focus of the thesis subsequently shifted and an alternative model was used to test these key questions. This chapter therefore documents the progress that was made towards developing the fathead minnow-*Ligula intestinalis* system for experimental parasitology. The aim of this first section of the chapter (7.1) is to establish *Ligula intestinalis* in the laboratory under controlled conditions to shed more light onto different aspects of the host-parasite interactions. Work undertaken to develop the fathead minnow as an experimental host suitable for behavioural study is described in the second part of the chapter (7.2).

7.1.2 Methods

7.1.2.1. Fish collection, husbandry and parasite collection

European roach, *Rutilus rutilus*, naturally infected with the plerocercoids of *Ligula intestinalis* were brought into aquariums in University of Leicester in November 2014 after being collected, by the U.K. Environmental Agency under licence, from a non-identified site with endemic *Ligula* infection in eastern England. Infected roach were held in a large holding aquarium (182.8 × 182.8 × 152.4 cm) on a recirculating system, under a natural photoperiod and were fed *ad libitum* with frozen bloodworms (*Chironomus* sp. larvae) and Discus pellets.

Fish were euthanized by using a Home Office approved Schedule 1 method (overdose of Benzocaine anaesthetic). Plerocercoids of *L. intestinalis* were recovered from three visibly infected fish. Individual plerocercoids were blotted dry, photographed, weighed and immediately transferred into 9cm diameter Petri dish containing RPMI-1640 Cell Culture media (Sigma, U.K.) in preparation for *in vitro* culture.

7.1.2.2. *In vitro* culture of *Ligula intestinalis* plerocercoids

Six plerocercoids recovered from three infected fish were cultured *in vitro*, using one of two different methods: a previously published protocol for *L. intestinalis* (Smyth, 1990) and a method adapted from (Dorucu et al., 2007) previously used for the *in vitro* culture of *Schistocephalus solidus*. Two different methods were used in order to get more and viable eggs. In the first method, a 20 cm loop of 8 mm dialysis tubing (Medicell Membranes Ltd., London U.K.) was suspended in 90 ml of medium consisting of 50% heat inactivated horse serum (Sigma Life Science, U.K.) and 50% RPMI-1640 cell culture medium (Sigma U.K.) within a sterilized 100-ml capacity Pyrex boiling tube. A rubber bung with two glass tubes passing through it was used to cover the top of tube and the ends of dialysis tubing were connected to the glass tubes. White cotton plugs were used cover the top of glass tube (Figure 7.2.a.).

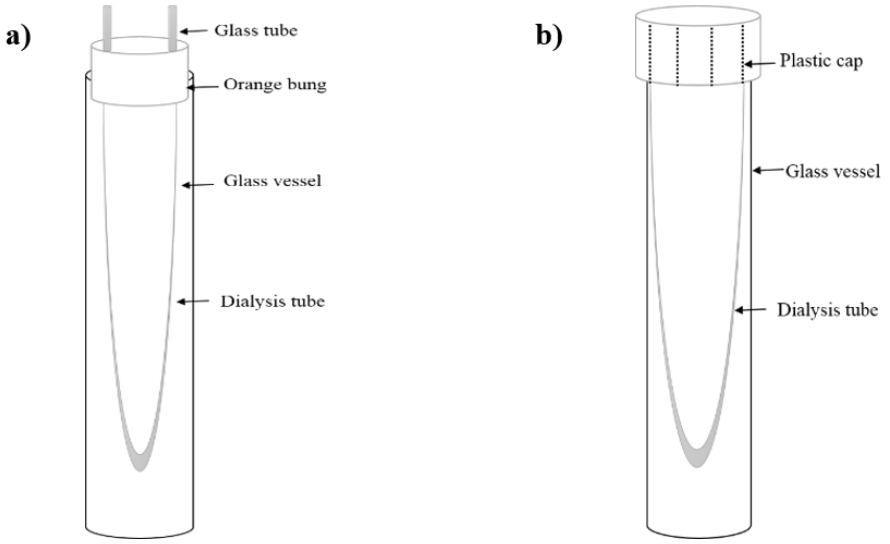

Figure 7.2 The culture tubes used for in vitro culture of *Ligula intestinalis* (a) first method (b) second method.

In the second method (Figure 7.2.b) an autoclaved 70 ml screw-top Pyrex culture tube (Fisher, U.K.) with a 15cm loop of 8 mm diameter dialysis tube (Medicell Membranes Ltd., London U.K.) was filled with 50% RPMI-1640 cell culture medium (Sigma UK), 50% heat-inactivated horse serum (Sigma Life Science, UK). In both culture methods, the plerocercoids were cut into equal segments of 2-3 cm length using sterilised dissecting scissors. Segments of plerocercoids were cultured as a single or paired segment. In case of paired culture, each segment was taken from different single worm were placed together in dialysis tubing. In addition, 0.05 ml of the antibiotic Penicillin from stock was added to the culture tube in order keep the culturing media sterile.

Culture tubes were then transferred to a water bath set to a temperature of 40˚C (to mimic the body temperature of the natural final host, a piscivorous bird), shaking at 122 rpm/minute. The culture media was replaced every 72 hours until the egg deposition was noticeable (Figure 7.3).

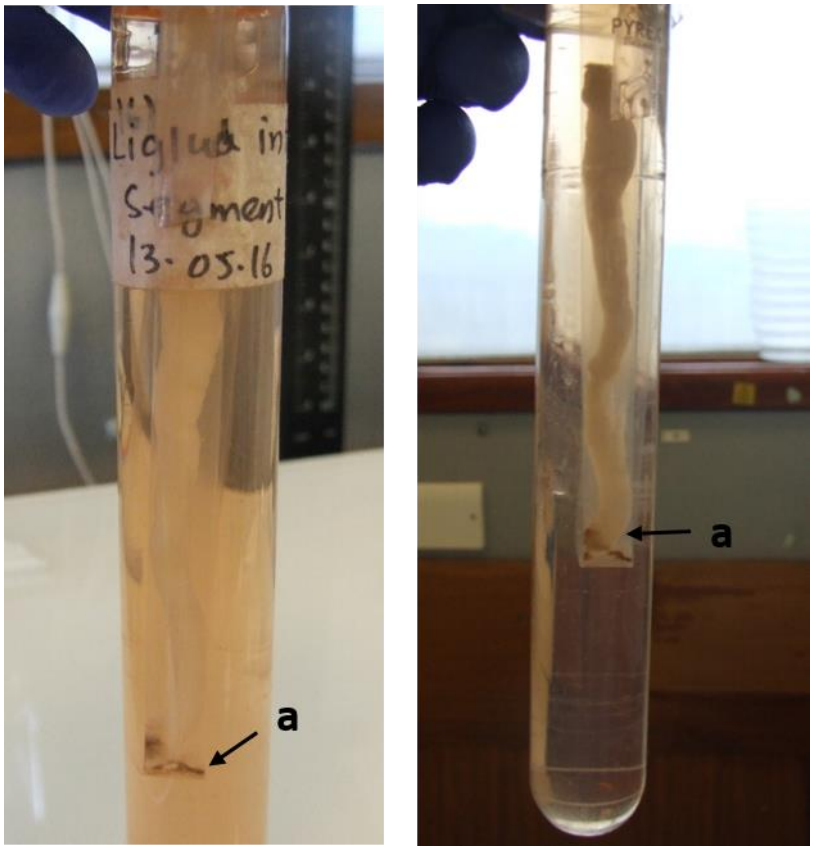

Figure 7.3 In vitro culture of plerocercoids of *Ligula intestinalis* showing deposition of eggs (a).

7.1.2.3. Egg collection

Eggs were collected from the tubes on the tenth day of culture into a 9cm diameter Petri dish. Harvested eggs were washed thoroughly with double distilled water (ddH_20) to remove any culture media, and waste produced by parasite was removed under a dissection microscope with the help of a glass pipette. After washing, eggs were split into three to four Petri dishes, depending on the total volume of eggs. ddH_20 was added and the Petri dishes were sealed by using Parafilm to minimise the chances of evaporation of water during the development period of 21 days at 20˚C.

7.1.2.4. Egg hatching

After 21d, eggs were taken from the 20°C incubator, unwrapped and placed in the sunlight to initiate the hatching process (Dubinina, 1980). After first exposure to light, the eggs were placed back in dark and exposed to light second time the

following day. After 24 hours, eggs were viewed under a microscope and free swimming coracidia were observed (Figure 7.4.)

7.1.2.5. Exposure to the first intermediate host, the copepod *Cyclops strenuus*

A range of freshwater cyclopoid and calanoid copepods, including *Cyclops strennus, C. vicinus, C. furcifer* and *Eudiaptomus gracialus* have previously been recorded as the first intermediate hosts of *Ligula intestinalis* (Glazunova and Polunina, 2009). A lab culture of *Cyclops strennus abyssorum* (Sciento, Manchester, UK) was maintained and copepodite stages were sieved from the main stock for use in the experimental infection studies.

The copepods were sieved into different size classes (nauplii, copepodites and adults) by using sieves with increasing mesh size of 45µm, 150 µm and 250 µm respectively. Three different techniques to expose first intermediate host-copepodites to *Ligula intestinalis* coracidia and/or hatched eggs. Three different techniques were used to maximise the chances higher rates of infections in copepods. In the first method, the sieved copepodites were transferred into 250 and 500 ml conical flasks with a water volume of 50 and 100 ml. The Petri dish containing coracidia and/or hatched eggs was poured gently into the conical flask containing copepodites. In the second method, two 24 well-plates were used and one copepodite was placed in each well with hatched eggs and/or coracidia. In the last method sieved copepodites were transferred into the Petri dish containing hatched eggs and/or coracidia.

After being exposed to *Ligula* hatched eggs and/or coracidia, copepodites were transferred to aquaria and kept at a temperature ranging from 10-18˚C for 18-21 days to further develop to the procercoid stage in the first intermediate host. After three days, the copepodites exposed to coracidia were fed with an infusion of alfalfa and this was repeated once per week for three weeks.

After 18-21 days, the copepods exposed to *Ligula* coracidia were screened for the development of *Ligula* procercoids under a compound microscope. The exposed copepods were caught with a glass pipette and placed on the slide with one drop of water. After these three to four drops of carbonated water were added

to the slide with the exposed copepods, which lightly anaesthetises the copepod to restrict its movement for 60-90 seconds. During this time, each copepod was observed carefully under a microscope (4×/0.1 and 10×/0.25 magnification) to look for procercoids (infective stage of *Ligula*).

7.1.3. Results

The *in vitro* culture technique to produce fertile *Ligula* eggs was successfully undertaken in the lab, and free swimming coracidia were hatched from the embryonated eggs (Figure 7.4.). However, multiple attempts to infect copepods were unsuccessful, even though seven exposure experiments (i.e. exposure of copepods to infective stages of parasite) were undertaken.

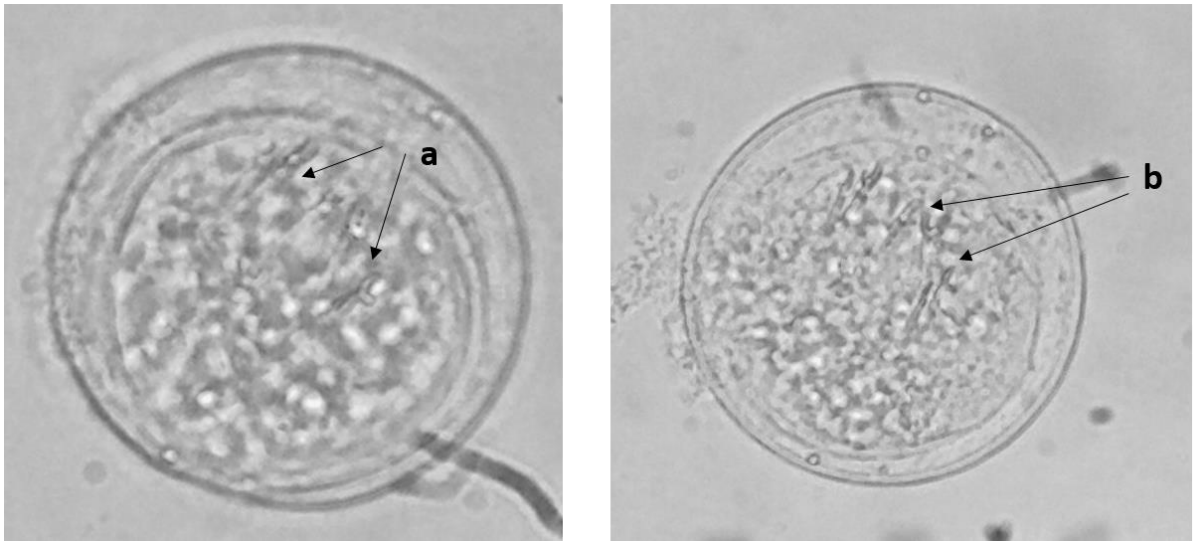

Figure 7.4 Coracidia (free swimming larval stage) of *Ligula intestinalis* (a) four larval hooks (b) larval hooks.

Table 7-1 Table showing the detail of copepod cultures set up with *Ligula intestinalis.*

Culture	Temperature (Copepods kept)	Copepods exposed	Copepods screened	Copepods infected
1	10˚C	60	38	0
2	10˚C	50	41	0
3	11˚C	50	38	0
4	15˚C	50	33	0
5	18˚C	50	26	0
6	18˚C	30	18	0
7	18˚C	40	26	0

7.1.4. Discussion and future work

The results of this preliminary work have shown the successful development of plerocercoids into sexually mature adult worms using *in vitro* culture techniques, the production of viable eggs and the hatching of free swimming coracidia of *Ligula intestinalis* under controlled laboratory conditions. However, the next stage required to develop the complete life cycle – i.e. experimental infection of lab-bred copepods – was unfortunately unsuccessful. One possible reason could be that the conditions in the lab, especially the temperature at which the eggs were being kept for 21 days, might not be suitable to promote further development. There is no literature available regarding conditions for maintain life cycle of *Ligula intestinalis* in the lab. So far, the same temperature and conditions that have been successfully used for another cestode parasite *Schistocephalus solidus.* In future, it would be worth trying different temperatures to incubate the eggs. Secondly, from the available literature it appears that calanoid copepods are most frequently used as the first intermediate host for *Ligula intestinali*s (Glazunova and Polunina, 2009) compared to cyclopoid *Cyclops strenuus.* In an attempt to increase success rates, a mixed culture of calanoid copepods (*Diaptomus* spp.) was collected from the wild from Eyebrook Reservoir (Leicestershire, UK) and their culture was attempted under standard aquarium conditions. However, the wild-caught culture of calanoid copepods showed poor survival and was challenging to maintain under laboratory conditions, most likely due to their specialised dietary requirements which are mainly phytoplankton,

unlike cyclopoid copepods, which are omnivorous. In future, the use of calanoid copepods from the wild might help in developing *Ligula* life cycle in lab.

7.2 Experimental work for developing the fathead minnow (*Pimephales promelas*) as an experimental model host for experimental parasitology and behavioural study

7.2.1 Introduction

7.2.1.1 The fathead minnow, *Pimephales promelas,* as an experimental model

The fathead minnow *Pimephales promelas* is a common species of cyprinid fish found in freshwaters of North America (McCarraher and Thomas 1968, Grant and Tonn, 2002, Danylchuk and Tonn, 2006). This species is tolerant to a variety of basic water quality characteristics such as temperature and pH (McCarraher and Thomas 1968). Adult fathead minnows range from 5-9 cm in size, with 42-48 lateral scales, an incomplete lateral line and a fleshy and fat body (Maitland, 2000). Male fathead minnows usually grow larger in size and reach the length of 90-101 mm (McCarraher and Thomas 1968). Among secondary sexual characteristics, nuptial tubercles are mostly absent in females, but are noticeable in males, arranged in a bilaterally symmetric pattern, and their number reach to 8 between nares and around the eyes. In some males the number of tubercles recorded up to 30, which are arranged in two parallel lines above mouth and below the nares and are often shaped as asterisk (Jensen et al., 2001).

The natural breeding season of fathead minnows is from June-August, when sexually mature males possess a fleshy pad behind their head. Sexually mature males begin to occupy stones and under sides of lily leaves as a nest and begin to spawn with one or more females (Maitland, 2000). Males exhibit conspicuous courting behaviour such as approaching towards females and display in which females are led to the appropriate spawning substrate (Cole and Smith, 1987). Females usually lay eggs on the stem of submerged plants when the surface water temperatures usually ranges from 14-18.5˚C in the start of spring season (McCarraher and Thomas 1968). In the breeding season, males release chemicals from their bodies into the water, which acts as a stimulant to attract females for courtship. Reproductively active females can distinguish between the chemical stimuli released by conspecific females or immature males (Cole and

Smith, 1992). Eggs fertilised by socially dominant males hatch and grow quickly into large size individuals at the end of the growing season as compared to those laid by socially suppressed males, most likely due to competition for food (Danylchuk and Tonn, 2001). The timing of reproduction is crucial in determining recruitment dynamics, with fry that hatch early in the season usually attaining a larger size, whereas delayed spawning restricts the growth and maturation of offspring (Divino and Tonn, 2007). After spawning, males continue to guard the nest and the eggs take 5 days to hatch at a temperature of 25˚C. When these new offspring reach a body size of 5-7 cm they begin to spawn and continue to live for 2-3 years (Maitland, 2000). Social status regulates reproductive activity during the breeding season (Danylchuk and Tonn, 2001).

7.2.1.2 Fathead minnows as a model for behavioural and eco-toxicological studies

Fathead minnows have been extensively used as model animal in behavioural studies. This species show prominent social, reproductive and nesting behaviour (Danylchuk and Tonn, 2001, Divino and Tonn, 2008, Jung and Tonn, 2011), predatory behaviours and its dynamics under normal and altered environmental conditions (Jones and Paszkowski, 1997, Godard et al., 2013, Chiu and Abrahams, 2010, Pollock et al., 2006, Jung and Tonn, 2011, Abrahams and Sloan, 2012), avoidance behaviour from parasites (Stumbo et al., 2012b) and territorial behaviour in males (Pyron and Beitinger, 1989, Danylchuk and Tonn, 2001, Divino and Tonn, 2008).

Fathead minnows are regarded as a prime experimental model fish species for eco-toxicological studies, largely because of various physiological chemicals produced by the fish that can act as a biomarker of sexual and other developmental processes. This enables them to indicate the adverse effects of various environmental toxins. For example, oestrogens in wastewater produced by anthropogenic activities causes physiological changes in male fathead minnow by increasing the levels of plasma vitellogenin (Hemming et al., 2004). Recent studies have shown that the fathead minnow FET (fish embryo toxicity) test can be a suitable alternative for chemical toxicity tests (Jeffries et al., 2014). Short-term reproduction assays with fathead minnow, testing both oestrogenic

and androgenic chemicals, revealed that alterations in the endocrine system resulting from reproductive toxicity can be under both androgenic and oestrogenic control (Ankley et al., 2001).

An early-life stage toxicity test to study the effect of chronic waste and surface water toxicants has been carried out on fathead minnows as an animal model (Stoddard and Huggett, 2014). Recent studies have used fathead minnows as a biological indicator in water bodies to detect the effects of environmental gestagens (Ellestad et al., 2014). Toxins like environmental oestrogens are likely to be responsible for comparatively poor reproduction within large wild populations of fathead minnows and ultimately resulted in irregular and adverse patterns of gene flow within populations (Martinovic et al., 2007). Fathead minnows inhabiting a natural pond system, which contained relatively higher concentrations of naphthenic acids (≥ 25mg/l) due to local oil sand processing, exhibited altered reproductive and body growth with lower levels of plasma 11-ketotestosterone, a lower splenosomatic index (SSI) and abnormal growth of the operculum (Kavanagh et al., 2013). Exposure to chemical pollutants decreased the level of the sex- steroid biomarkers17β-estradiol (E2) and testosterone, by affecting the hypothalamic-pituitary-gonadal (HPG) axis either directly or indirectly which ultimately resulted in lower fecundity in fathead minnows, with decreased production of vitellogenin by females (Ankley et al., 2008). Reproductive toxicants, such as methoxychlor (an oestrogenic compound) and methyltestosterone (an androgenic chemical) decreased the reproductive rate, and the latter one caused masculinization in females and affected gonad development (Ankley et al., 2001). Environmental pollutants like Cadmium (Cd) pose potentially serious implications for the reproductive success of fathead minnows and alter reproductive behaviours, which in turn lowered egg-laying frequency and fertility (Sellin and Kolok, 2006). Furthermore, selenium as a toxicant in the aquatic environment possess serious threats to fathead minnow especially in the early life stages survival, swimming performance, aerobic capacity and energy balance (McPhee and Janz, 2014).

7.2.1.3 The parasite fauna of fathead minnows

Pimephales promelas acts as host for different parasites such as brain-encysting trematode *Ornithodiplostomum ptychocheilus* (James et al., 2008, Shirakashi and Goater, 2005, Wisenden et al., 2012). Metacercaria of *Ornithodiplostomum* redirects the fathead minnow's metabolic energy towards its own development (James et al., 2008) alters behaviour because of pathological changes linked with the development of the parasite larvae and causes a reduction in motor execution, the optomotor response (OMR) (Shirakashi and Goater, 2005). Furthermore, this species learn to show avoidance behaviour after one exposure to cercaria of *Ornithodiplostomum* (James et al., 2008). Metacercaria of *Ornithodiplostomum* species develop and consist of different phases like, obligate migration, growth, encystment and consolidation (Matisz and Goater, 2010) and causes oxidative stress due to the development of growing larvae and compromised immune response by fathead minnows (Stumbo et al., 2012a).

Crassiphiala bulboglossa (black spot) has been reported to be parasite of fathead minnows by (Wisenden et al., 2012, Kavanagh et al., 2013) with a prevalence of 60% and a median intensity of 12 and 20 metacercaria per minnow, however, such a parasite load has no effect on shoaling affinity and anti-predator response to a mechanical model (Wisenden et al., 2012). Furthermore, fathead minnows inhabiting polluted water containing a high concentration of naphthenic acid had damaged opercula and gills as well as many parasites residing in the gills belonging to *Trichodina* spp. (Kavanagh et al, 2013). *Ornithodiplostomum ptychocheilus* and *Posthodiplostomum minimum* have metaceraia as developing stage which affect the overall activity, growth and survival of infected minnows (Stumbo et al., 2012b).

7.2.1.4 *Pimephales promelas* and *Ligula intestinalis*

Pimephales promelas is the natural host of the cestode parasite *Ligula intestinalis,* which is found in the coelom cavity of host (McCarraher and Thomas 1968, Kavanagh et al., 2013). In natural environments fathead minnows infected by *Ligula intestinalis* show poor reproductive potential because the parasite greatly restricts egg production and infected fish produces 42% fewer eggs than the fish with no infection. Furthermore this parasite also causes multiple infestations in

fathead minnow and the number of plerocercoids in each infected minnow ranges from 1-22 (McCarraher and Thomas 1968).

The present study aimed to develop this species as a model organism to set up preliminary behavioural work investigating the interactions between host personality and parasite infections in fish. Work to develop the experimental *Ligula intestinalis* parasite model was undertaken alongside (see 7.1, above) with the aim of developing this host-parasite model system for future studies.

In this part of the chapter, the suitability of fathead minnows as subjects for personality studies was investigated. The consistency of individual differences in two personality traits – activity in a novel environment and exploration of a novel object – was tested under both social and asocial contexts (for justification and details see Chapter 2, introduction).

7.2.2 Methods

7.2.2.1 Fish supply and husbandry

Juvenile fathead minnows were generated by inducing natural spawning (Uguz, 2008) of sexually mature adult *Pimephales promelas* in laboratory aquaria. The adults used for natural spawning were originally sourced from excess laboratory stock from Brunel University, U.K. The lab-bred juvenile fish were reared under controlled conditions in the aquarium facility at the University of Leicester. Twenty adult laboratory-bred fathead minnows were randomly selected from stock tanks and each fish was transferred into individual compartments in the holding aquarium one week before the start of behavioural assay to acclimatise. Two holding aquaria (60 × 40 × 40 cm each) were set up with gravel, enriched with plastic plants and supplied with power filters for filtration and an air stone for air supply. Furthermore 10 plastic (2 L) drink bottles (9 cm radius) with 20 holes (2 - 3 mm diameter) for continuous water transfer in and out were used to keep fish individually in each holding aquarium. These tanks were set up one week before the start of acclimatisation and were kept at 12L: 12D photoperiod with temperature ± 23˚C. Fish were fed fish pellets (Tetra Prima) daily and water was changed once a week.

7.2.2.2 Experimental arena

The experimental arena was set up to carry out behavioural tests for activity in a novel environment and exploration of a novel object in two different contexts – a social context and asocial context. Two experimental arenas were used for testing activity in a novel environment and exploration of a novel object. For activity in a novel environment, a rectangular tank (32.2 × 17.2 × 18 cm), with a grid consisting of 40 squares (32 square – activity zone, and 8 square – acclimation zone) used to quantify activity. For exploration of the novel object, a circular ring (20 cm diameter) was used in which the novel object was dropped gently in the middle at the start of the trial. In addition, while testing activity and exploration for a novel object in a social context, a conspecific shoal was placed in a square tank (50W × 50L × 20H cm) in the middle of the experimental arena.

7.2.2.3 Behavioural assays

Individual fish were caught using a net, and transferred gently to a static tank. After a few minutes, they were transferred by net into the acclimation zone (AZ) in the experimental arena to measure activity in a novel environment. The door was closed for 300 seconds to reduce any stress caused by handling and transfer. After 300 seconds the door of the acclimation zone (AZ) was gently and remotely opened and the behaviour (activity in a novel environment, NE) was recorded from above, using an HD Camcorder (Sanyo 16x advance HD) focussed on a 45º reflected mirror image, set up on the top of the experimental arena, for 300 seconds, for subsequent analysis.

For exploration of the novel object, fish were gently transferred into the circular ring and allowed to settle for 300 seconds. The novel object was dropped gently into the arena from above, and video was recorded for the next 300 seconds. The behavioural trials in the social context were started with first behaviour (activity in a novel environment) one week after acclimation. The test for the second behaviour (exploration of a novel object) was carried out the following day. The second trial for each behaviour was carried out after four days. The same schedule was followed for each trial in the asocial context on completion of three trials in the social context.

7.2.2.4 Statistical analysis

For activity in a novel environment, three variables were scored from recorded films by replaying using Windows Media player on a Windows desktop computer: latency to leave the acclimation zone (LE-AZ), the number of squares fish moved in the activity zone (N sq.-NE) and the number of squares crossed in the activity zone (F sq. - NE). For exploration of the novel object, the time spent in the more exploratory zone (MEZ) was recorded. A Spearman rank correlation analysis was used to test the temporal repeatability of each variable in each context, i.e. the social context and the asocial context. Minitab 17 Statistical Software for Windows used to analyze data, and graphs were plotted using Microsoft Excel 2013

7.2.3 Results

7.2.3.1 Activity in a novel environment

7.2.3.1.1 Activity in a social context

In the presence of conspecifics, there was no temporal consistency found in the latency to leave the acclimation zone, the number of squares crossed or the number of times that individual squares were crossed in the activity zone across three trials for activity in novel environment (Table 7.2).

Table 7-2 Table showing the results of Spearman's correlation analysis across three trials for each single variable for activity in novel environment in social context.

Trial no	Latency to leave acclimation zone		No of squares crossed in activity zone		No of times Squares crossed in activity zone	
	Spearman rho	p-value	Spearman rho	p-value	Spearman rho	p-value
Trial I& II	-0.213	0.395	-0.017	0.948	0.263	0.292
Trial II & III	-0.364	0.137	-0.126	0.619	0.002	0.994
Trial I & III	0.198	0.430	-0.101	0.689	-0.100	0.692

7.2.3.1.2 Activity in the asocial context

In the absence of conspecifics, statistically significant temporal consistency was found in the latency to leave the acclimation zone (r_s = 0.599, p = 0.009; Figure 7.5 and Table 7.3.) between trial II and III, and the number of squares crossed in the activity zone (r_s = 0.485, r = 0.041; Figure 7.5 and Table 7.3.) between trial I and III.

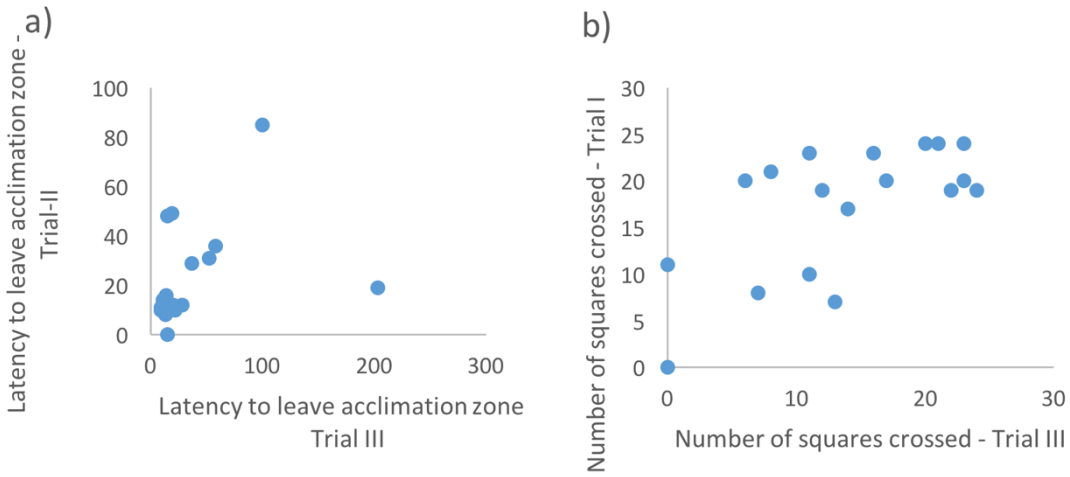

Figure 7.5 Scatterplots showing relationship (a) latency to leave acclimation zone between trial II and III, (b) number of squares crossed between trial I and III, for activity of *Pimephales promelas* in a novel environment in the presence of conspecifics.

Table 7-3 Table showing the results of Spearman's correlation analysis across three trials for each single variable for activity in novel environment in asocial context.

	Activity in Asocial context (n=18)					
Trial no	*Latency to leave acclimation zone*		*No of squares crossed in activity zone*		*No of times squares crossed in activity zone*	
	Spearman rho	p-value	Spearman rho	p-value	Spearman rho	p-value
Trial I & II	0.065	0.796	0.191	0.449	0.356	0.148
Trial II & III	***0.599***	***0.009***	0.171	0.498	-0.030	0.904
Trial I & III	0.173	0.494	***0.485***	***0.041***	0.349	0.155

7.2.3.2 Exploration of Novel Object

7.2.3.2.1 Exploration of novel object in social context

In the presence of conspecifics, the amount of time spent in the more exploratory zone was significantly correlated between trial I and II (r_s = 0.674, p = 0.001). However, there was no significant correlation in the amount of time spent in the more exploratory zone in trials II and III (r_s = 0.365, p = 0.114) or between trials I and III (r_s = 0.311, p = 0.182).

7.2.3.2.2 Exploration of a novel object in an asocial context

In the absence of conspecifics, the amount of time spent in the more exploratory zone was significantly correlated between trials II and III (r_s = 0.619, p = 0.004). Whereas there was no significant correlation found in the amount of time spent in the more exploratory zone in trials I and II (r_s = 0.235, p = 0.319) and trial I and III (r_s = 0.292, p = 0.211).

7.2.4 Discussion

7.2.4.1 Activity in a novel environment (social and asocial context)

This preliminary study on consistent inter-individual differences of fathead minnows tested across two different behavioural contexts, measured their activity in a novel environment and exploration of a novel object. Activity is a very basic behavioural trait, which has been shown to exhibit significant individual variation between individuals within populations (Reale et al., 2007, Sih et al., 2004a). Fathead minnows tend to show a strong social structure (Danylchuk and Tonn, 2001), the main aim of the study to test the effects of social context on their personality. However, the results of this study showed an absence of temporal consistency for activity in a novel environment in a social context.

Social context has already been shown to have a significant effect on personality (Schuett and Dall, 2009b, van Oers et al., 2005) which contrasts with the results of this study, where individuals have shown no consistency in activity scores in the presence of conspecifics as compared to their absence. One possible reason could be that when experiments start with behavioural trials in the presence of conspecifics, fish are likely to be fearful of the new environment and tended to behave without any temporally consistent pattern. Secondly the order of testing repeatability of behaviour might have influenced the temporal consistency. Fish were tested in the social context first and then in the asocial context. In future studies, it fish should be tested in a randomised way irrespective of their context.

7.2.4.2 Exploration of novel object (social and asocial context)

For the exploration of the novel object, the results show temporal consistency in exploring the novel object in terms of time spent in more exploratory zone. In the presence of conspecifics temporal consistency was found between trials I and II, whereas in the absence of conspecifics the temporal consistency shifts between trail II and III. The lack of consistency between the final trial and the other two trials could be attributed to habituation to the environment (Reale et al., 2007). This could mean that individuals become less responsive to a repeated stimulus in the presence of a social context. However, in an attempt to mitigate this

problem, three different novel objects were used in behavioural trials for exploration.

In the social context, the response to exploring novel object was consistent over time (for first and second trial). This result is similar to the results of another study conducted on male Mozambique tilapia (*Oreochromis mossambicus*), in which presence of conspecifics facilitated exploratory behaviour (Galhardo et al., 2012). Furthermore, the novel object in these tests was dropped from above slowly which could lead them to respond in a different way (inconsistent) in the absence of conspecifics around. However, as trials progress (from II to III) fish may tend to become acclimatized to the dropping of the novel object, even in the absence of conspecifics and tend to behave more consistently.

7.3 Concluding remarks

In summary, although the attempts to develop *Ligula intestinalis* as a model parasite in the laboratory were only partially successful, it remains important to develop this parasite and a suitable host species as a model host-parasite system. *Ligula* has significant impacts on its cyprinid hosts, which are beginning to impact subsistence fisheries. Furthermore, these preliminary studies on the behaviour of fathead minnows showed that this species can be used to answer questions related to animal personality, especially the impacts of social interactions, as this species is naturally social in wild.

Chapter 8.

General Discussion

8.1 General discussion

Parasites have the potential to affect various aspects of host biology, including growth, morphology, reproduction and behaviour (Barber and Svensson, 2003, Lafferty and Kuris, 2009, Robar et al., 2010, Trubiroha et al., 2011, Moore, 2013). However, the implications of consistent variation between individuals in terms of their behaviour (i.e. personality) for parasite infections is poorly understood. Understanding how host personality can interact with parasite infections to affect subsequent host-parasite biology can also give insight into the behavioural ecology of animal personality.

Anthropogenic activities are a leading cause of climate change, including global temperature change, and rapid industrialization increases concentration of pollutants in aquatic environments. Experimental studies examining the consequences of these types of environmental change on host-parasite interactions can provide an insight that how anthropogenic activities have the potential to affect ecosystems through their effects on species interactions.

The initial focus was to develop fathead minnow-*Ligula intestinalis* host-parasite model system to study these research themes. However, due to limited success stickleback-*Schistocephalus solidus* model host-parasite system used to investigate several related research questions. In first part of this thesis, the effects of interaction between host personality and parasite infections on subsequent host-parasite biology investigated. In addition, the effects of social context and parasitism on behaviour examined. The second part of this thesis highlights studies examining how single and multiple environmental stressors can affect parasite infections in fish hosts.

8.1.1 Summary of main findings

The main aim of this thesis was to investigate the consequences of host personality and environmental change for parasite infections in freshwater fish. The effects of social context on consistent inter-individual differences in behaviour (personality) in three spined sticklebacks was investigated in chapter 2 of this thesis. In addition, the effects of parasitism on the behaviour of sticklebacks were examined. The results of this experimental study showed

significant effects of social context on personality and of parasite infections on host behaviour.

The consequences of host personality for parasite infections was investigated in the chapter 3 of this thesis. The results of this experimental study demonstrated that sticklebacks showed significant temporal consistency in their behaviour. The personality showed no effect on the voluntary food of host and parasite fitness. However, the infection status showed effect on the host voluntary food intake as the infection progresses from the 4th week onwards in the experiment.

The experimental study documented in chapter 4 investigated the effects of changing temperature and host ration on the subsequent host-parasite biology. The results showed significant effects of elevated temperature on parasite growth and parasite grew bigger in size, whereas fish were in improved body condition at lower temperature with no additional effects of host's food intake ration. Chapter 5 looked at the effects of parasite severity and infection (as a result of higher temperature) on the host neurophysiological responses in sticklebacks. The results of this study showed that being reared at higher temperatures was associated with altered levels of some monoamines, but no effects of *S. solidus* infections on neurotransmitters in stickleback's brain were detected.

To investigate the effect of multiple environmental stressors, specifically elevated environmental temperature and heavy metal pollutants, on parasite infections in fish, an experimental study was conducted and reported in chapter 6 of this thesis. Despite being limited by sample size, as a result of low levels of infection developing in the study, there was a clear trend indicating that temperature change has the potential to affect the susceptibility of developing parasite infections in fish hosts, whereas no effects of pre-exposure of copper (in copepod host) found on parasite susceptibility on developing infection in fish host.

These key results of experimental studies reported in this thesis are discussed in the following sections.

8.1.2 Social context, personality and parasitism

The social context (i.e. the presence or absence of conspecifics) and parasitism were two main factors investigated to understand their effects on animal personality. Many animals tend to live in groups in the wild, yet in experimental studies designed to investigate the consistency of inter-individual behavioural differences, subjects are typically held individually and the potential consequences of this gap between the experimental and real-life social context on animal behaviour is often underestimated. One of the main findings of this study appears to be a significant effect of social context on the expression of behaviour of focal individuals. Sticklebacks were more active in a social context in a novel environment compared to asocial context. The possible explanation of these results is that, the presence of conspecifics facilitates the expression of behaviour (Webster et al., 2007, Magnhagen and Bunnefeld, 2009).

Furthermore, there was a significant effect of parasite infection on behaviour, with experimentally infected fish being more active when compared to sham-exposed (control) fish. Parasite infections frequently affect the behaviour of host organisms (Barber et al., 2000, Moore, 2002). There can be two possible explanations of this result that infection increases the activity of fish. Firstly, this could be deliberate adaptive strategy of parasites to increase activity of fish, which may help parasites, either by increasing host predation susceptibility or increased food finding by the fish host. The other possible reason could be side effects of parasite on host behaviour which can be evolutionarily neutral and have no benefit to host or to parasites.

However, parasite infections had no effects on the shoaling behaviour of host fish. The parasite infection can affect shoaling behaviour of animals within a shoal (Tobler and Schlupp, 2008), whereas, the results of this study are different. One likely reason for the result in this present study could be the infectivity of parasite infections. For example, in sticklebacks the parasite infections of *S. solidus* increased the dorsal profile by more than 40% (Barber, 1997) and heavily infected sticklebacks are likely to appear different to focal fish. Whereas, in the infected fish in this present study had 6% parasite mass. It may possible that the parasites were not infective at this point and hence no alteration in the behaviour

were found (i.e. parasite decision). Furthermore, from another perspective-phenotype matching (i.e. joining or not joining a shoal of fish depends on focal fish host decision) and it is likely that focal fish appeared as a part of the shoal instead infected and no difference found in the shoaling behaviour of infected and sham-exposed (control) and exposed-non-infected fish.

From growth-mortality trade-off theory, the personality and energetics of animals are likely to linked (Stamps, 2007, Sih et al., 2015). Food intake is the main source that fulfils the energetic demands of animals and variation among individual food intake rates might be expected to be linked to variation in personality type. For example, bold individuals have been demonstrated to ingest more food when compared to shy individuals (Jolles et al., 2016). Since parasite infections rely on the host's energetic reserves for their own nutrition (Barber et al., 2008), host food intake rates (and hence nutrients available to growing parasites) are potentially influenced by host personality type, with subsequent consequences for host and parasite biology. The effects of interaction between personality and parasite infections on host growth, effects of personality on host food intake and parasite fitness and effects of parasite infections on host voluntary food intake and post exposure behaviour were investigated by using three spined sticklebacks – *Schistocephalus solidus* in the chapter 3 of the thesis. The sticklebacks showed significant consistent inter-individual difference over time in the novel environment. There was significant difference in the voluntary food intake over time. The host personality did not affect host voluntary food intake, whereas there was clear trend that infection significantly affected host voluntary food intake from 4th week of the study.

Recent studies have suggested that personality differences between individuals can affect their levels of food intake (Jolles et al., 2016). However, in this present study, personality type showed no effect on the voluntary food intake of individual fish. It is not clear why personality in the present study was not found to affect food intake. One possibility is that aspects of the experimental design masked personality effects. It is known that the environmental context can influence food intake; for example in three spined sticklebacks, the pair of bolder individuals with higher taking (predation risk) behaviour ate more prey items compared to shy

sticklebacks (Ioannou et al., 2008). Whereas, in this present study the food intake trials were carried out in the home compartment, which was both familiar to the fish, presented little risk and in which foraging was non-competitive. For future work, food intake trials in a competitive and/or risky environment, where individuals have the maximum chances to show their personality may lead to different results. The parasite infections showed an effect (though in interaction with week) on food intake in the present study. The infection may affect food intake of host in two ways, either by affecting the competitive ability and foraging behaviour of host or by affecting the stomach capacity and appetite of infected individuals (Barber et al., 2000). In this present study, the parasite infection significantly affected food intake in the 4th week of the study, which may possible due to growing parasites which cost nutritional drain on their host. However, for future work the food intake of heavily infected/parasitized fish in a competitive environment likely to lead different result and will give more insight.

8.1.3 Effects of environmental change on host-parasite interaction

Changing environments can have serious consequence for living organisms. Elevated environmental temperature had a significant effect on a number of aspects of both host and parasite biology (Franke et al., 2017). The effects of elevated temperature are multi-dimensional and can influence the availability of food (De Stasio et al., 1996, Woodward et al., 2010). In an experimental study, the effects of host food intake ration under changing temperature were studied on host and parasite growth and egg production of parasite. The results of the study showed that, higher temperature significantly affected host and parasite growth. The parasite were in bigger mass at higher temperature compared to lower temperature, whereas the growth and body condition of host (fish) was better at lower temperature. There was no significant effect of host food intake ration found on host or parasite growth. The one possible explanation of these result could be that, parasite exploiting the higher temperature conditions for their growth, consequently the parasite with bigger mass in adult stage through *in vitro* culture produced more eggs. Published data showed significant effects of parasite mass on the parasite fecundity (Dorucu et al., 2007). These results showed that, changing environmental temperature is the main factor that can

influence the balance between host-parasite interaction, by increased parasite growth rate which subsequently influenced the production of more eggs (by adult worm) irrespective of the food intake ration of host.

Temperature change can affect parasite severity therefore to understand the effect of parasite infection under temperature change on the neurophysiological state of host was investigated in chapter 5 of this thesis. The results of this experimental study demonstrated that, temperature change significantly affects the levels of dopamine and homovanillic acid in sticklebacks. One likely reason could be the stress induced by higher temperature which ultimately affect the levels of dopamine and its metabolite. However, the parasite infections showed no effects on monoamine levels in sticklebacks. It may be possible that, under changing temperature the effects of parasite infections are less pronounced and temperature appeared to be the main factor that cause stress and influence monoamine levels in sticklebacks.

Elevated temperature was shown to have significant effects host growth and body condition in improved state at lower temperature and improved parasite growth at higher temperature. In the wild, animals typically face multiple environmental stressors simultaneously, but this contrasts with the way that such stressors are studies in the laboratory, where typically they are studied in isolation. To test the effects of multiple environmental stressors (i.e. elevated temperature and heavy metal pollutants) on parasite fitness as it progresses from first intermediate host (copepod) to in second intermediate host (fish) in its life cycle, an experimental study conducted in chapter 6 of this thesis. The results of this study on the parasite fitness showed that temperature can influence the susceptibility of developing infections. However, the number of sample is small therefore statistically no significant effects of multiple stressors found.

8.1.4 Ideas for future work

This thesis illustrates the potential role of parasite infections for host behavior in social context. Infections with energetically demanding parasites such as *S. solidus* have the potential to generate differences in state among individuals in a population, and hence generate state-dependent personality differences among

individuals in a parasitized host population. Parasites can therefore potentially act as a selection agent by influencing host behaviour, temporal consistency, host reaction norms and behavioural syndromes (Barber and Dingemanse, 2010, Poulin, 2013). The social context appeared to affect host personality in this thesis, future experiment work highlighting effects of parasite infection on personality, it is important to consider the social context in future studies as animals in wild live in groups. Furthermore, as parasite have the potential to influence social interactions of their hosts, their effects on personality in social context can be studied in future. These studies will help understand the evolutionary ecology of animal personality under parasitism.

In this thesis, the effects of personality on susceptibility of developing infection following encounter, on infection phenotype and parasite fitness were studied. The infection showed effects on the voluntary food intake. However, the limited sample size and the benign experimental conditions hinder to get the pronounced effects of both host personality and parasite infections on the voluntary food intake. It is clear that further experimental studies are required to investigate (a) how personality differences can affect the susceptibility of developing infections following exposure/encounter with bigger sample size. It is also important to profile that detail immunophsyiological conditions of host pre-exposure to infections. This will help to understand and find out the role of immunophsyiolgical conditions of different personalities on susceptibility of developing infections. (b) The effects of developing infections on host (with different personalities) biology (i.e. growth, body conditions, detailed immune profiling) post-encounter/exposure to controlled infections. (c) The role of personality type on the voluntary food intake of their host under more competitive environment (i.e. risk taking) with bigger sample size. (d) The role of parasite infection on the voluntary food intake of their host with controlled single experimental infections to track the growth and development of both host and parasite. Such experimental studies will give insight about behavioural ecology of animal personality.

This finding of the thesis highlight the fact that environmental temperature changes due to anthropogenic activities are likely to be a major factor influencing host-parasite interaction. Elevated temperatures were found to influence the growth rate of *S. solidus* plerocercoids, resulting in increased parasite mass in

infected fish. This increased parasite mass led adult worm to produce more eggs. The small sample size hindered to single out the effects of temperature or parasite mass on the parasite egg production. Therefore, it is important to carry out targeted experiment in future to single out the effects of temperature on parasite egg production. For example, parasites can be allowed to grow at lower temperature for longer (12 to 16 weeks' post exposure) to attain the mass equal to the mass of plerocercoids (> 50mg) can attain at higher temperature (8 weeks post exposure). Furthermore, temperature change can influence the susceptibility of developing infections in the stickleback host. The sample size is small, however, for future studies with larger sample size will give more insight that, how changing thermal regimes can mediate ecological interaction between species, and potentially impact host-parasite interactions.

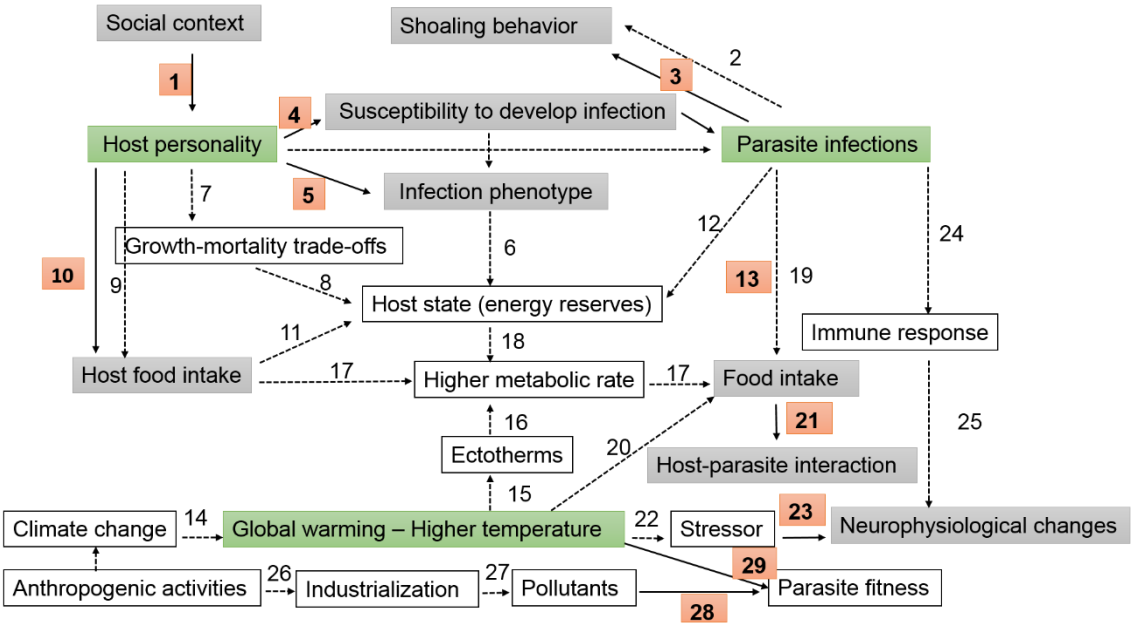

Figure 8.1 Schematic diagram outlines the summary of the experimental studies and relationship between experimental studies reported in the thesis. The dashed arrows represent the links between different factors/concepts already published in literature. The straight arrows, grey and orange boxes represent the research questions investigated and reported in this thesis. Literature reported that parasite infection can affect shoaling behaviour (2) and infection phenotype can affect host energy reserves (6). In addition, studies showed that host personality may influence the growth-mortality trade-offs (7) and growth-mortality trade-off could be linked to host energy reserves (8). Research have also explored the influence of host personality can influence host food intake (9), food intake linked to host energy reserves (11) and reporting on the effect of parasite infection on host energy reserves (12). Furthermore, temperature and climate change were shown to influence ectotherms and their metabolic rate (14, 15 & 16). More so, metabolic rate and parasite infection were shown to influence food intake and host energy reserves (17, 18 & 19), also temperature was shown to affect food intake (20) and may influence other physiological pathways in animals (22). Literature also showed that parasite infection can influence immune responses (24) which may influence neurophysiological responses (25). In additions, anthropogenic activities leads to rapid industrialization which produce heavy metal pollutant in aquatic environments (26, 27).

This thesis investigated the role of social context in influencing personality (1); the role of experimental infections on the shoaling behaviour of parasitized and non-parasitized individuals (3); the consequences of host personality type on susceptibility of developing infection following exposure (4); and how interaction between host personality and parasite infections can affect subsequent host parasite biology (5). Also, how host personality and parasite infection can affect host food intake was investigated (10 & 13). In addition, how does interaction between temperature and food intake influence host parasite interactions (21) and the effects of temperature on the neurophysiology investigated (23). Furthermore, animals are exposed to multiple stressors, how does pre-exposure to pollutant (Copper) in copepod host (28) can affect parasite fitness in fish host under global warming-higher temperature (29).

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