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DOCTORAL THESIS

Temperature effects on the *Schistocephalus solidus* life cycle

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Abstract

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Changing environments are affecting individual species and the interactions between them. This includes host-parasite interactions. Although much is known about temperature effects on individual species, much progress is still to be made in understanding how complex parasite life cycles can be affected by changing temperatures. Here, several experimental approaches are presented to help study host-parasite interactions under changing temperatures. Using these experimental data on different stages of the *Schistocephalus solidus* under a range of temperatures are collected. Combined with (re-analysed) literature data and these newly collected data are inputted into a mathematical model that is constructed to simulate the whole life cycle. These different studies show that most parts of the parasite life cycle respond in a dome-shaped fashion to changing temperatures with optimum temperature higher than current means. Overall, densities of the different life stages of *S. solidus* do not appear to be strongly affected by changes in mean temperature when including different, antagonistic effects on each life stage using a mathematical modelling approach.

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Chapter 1

Introduction

1.1 Impacts of climate change on host-parasite interactions

Host-parasite interactions play a critical role in food-webs (Dunne et al., 2013) and can play important ecological roles in general (Mouritsen & Poulin, 2002; Shields, Groves, Rombaugh, & Bellmore, 2002). These interactions are often directly and indirectly affected by environmental factors, such as temperature. It is thus important to study host-parasite interactions when trying to understand the effects of changing thermal regimes on ecosystems. In particular, parasites with ectothermic hosts should be disproportionally affected by climate changes as their hosts do not provide the same thermal buffering that endothermic hosts do (Harvell et al., 2002; Marcogliese, 2008). However, both hosts and parasites can be independently affected by temperature, so both sides of the interaction will be discussed seperately while putting the relevance of these environmental changes into the context of host-parasite interactions. However, first a brief overview of different parasite life cycles will be given to indicate how different groups of parasites could be differently affected by changes in environmental temperatures.

1.1.1 Parasite life cycles

Parasitism has evolved many times (Weinstein & Kuris, 2016) and in a range of different groups of multicellular animals and major parasite groups include species in the nematodes, plathyhelminths (including trematodes and cestodes), arthropods and nematomorphs (Goater, Goater, & Esch, 2013). Among parasite life cycles a distinction

is made between direct and complex (or indirect) life cycles. Direct life cycles can involve multiple life stages and multiple different host species, but it is possible to complete the life cycle on a single host species no intermediate hosts are required (Figure 1.1A). In contrast, parasites with complex life cycles require at least one intermediate host species during their development (Figure 1.1B). Intermediate host species typically serve for development and transmission, but sexual maturation occurs in the definitive host (Goater et al., 2013). Studying the effects of environmental change on parasites with direct life cycles is relatively simple as only the effects on the host, parasite and their interactions need to be investigated. For parasite with complex life cycles this become exponentially more difficult, with increasing numbers of intermediate host species involved all interactions need to be considered (Barber, Berkhout, & Ismail, 2016).

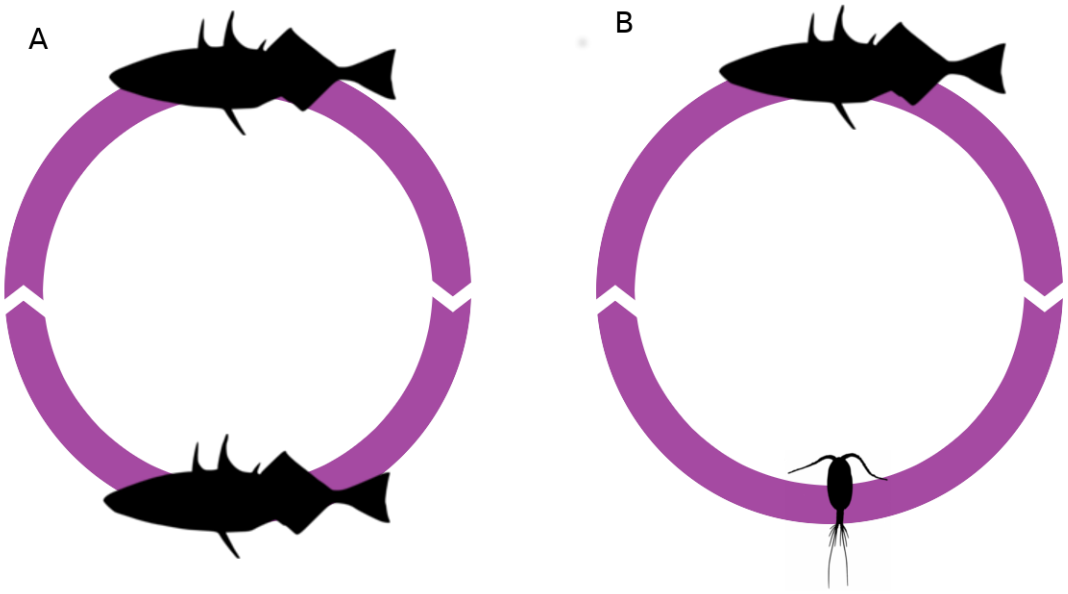


FIGURE 1.1: Schematic example of direct (A) and indirect (B) parasite life cycles. Parasites with a direct life cycle, can move between hosts, but are not obliged to do this in order to complete their life cycle (A). Parasites with complex life cycles are obliged to migrate between host species in order to complete their life cycle (B). With each additional host species, additional interactions with other species are added (e.g. predator-prey intearctions), making the study of changing environments more difficult. Adapted from (Barber et al., 2016), silhouettes obtained from `http://phylopic.org`.

1.1.2 Host responses to temperature change

1.1.2.1 Host behaviour and exposure

Warming temperatures can increase activity in ectothermic animals (Piersma & Van Gils, 2010) and, consequently, increased feeding rates to sustain this increase in activity. Therefore, the exposure of potential hosts to trophically transmitted parasites, and the transmission rate of these parasites may be increased under increasing environmental temperatures. Increased activity could also lead to increases in overall movement patterns, allowing hosts to visit a greater number of microhabitats. In these different microhabitats they could be exposed to a larger number of parasites. Another way parasite exposure may be elevated at higher temperatures are increased ventilation rates of aquatic animals (Burton, 1979) due to a combination of increased oxygen requirements and a decrease in oxygen saturation of water at warmer temperatures (Benson & Krause, 1984). This is especially true in fish, where the gills are exposed to potential parasites whilst breathing (Mikheev, Pasternak, Valtonen, & Taskinen, 2014). This could also lead to stronger chemical plumes which motile parasites could use to track the potential host (Haas, Haberl, Kalbe, & Kömer, 1995), further enhancing the infection potential. Alternatively, selection of cooler microhabitats by free-living animals is known to be effective in ameliorating some of the negative impacts of warming effects on physiology.

Furthermore, in some species infected hosts are able to select thermal conditions that suppress parasite development or aid recovery of the host from infections (Mohammed et al., 2016; Moore & Freehling, 2002). Depending on whether hosts select for cooler habitats (behavioural chills) or warmer habitats (behavioural fevers), this might be easier or more difficult under future thermal conditions. It thus appears likely that changes in environmental temperatures will affect host behaviour which could alter their parasite exposure.

1.1.2.2 Host immune response

Host immune defences create a important barrier against parasite infections (Leicht & Seppälä, 2014) and are often activated upon contact with parasites (Wakelin, 1996).

Changes in environmental conditions can affect the efficacy of the immune response. Despite there being important differences between the immune systems of vertebrate and invertebrate aquatic hosts of parasites, the immune response of both generally is up-regulated with moderated increases in temperature (Bowden, 2008; Dang, Speck, & Benkendorff, 2012; Le Morvan, Troutaud, & Deschaux, 1998). However, the response is often non-linear at extreme high temperatures where immune responses, or survival, drop off (Leicht, Jokela, & Seppälä, 2013; Seppälä & Jokela, 2011). Additionally, the length of the period during which increased temperatures occur can play an important role, with short exposures increasing host growth and longer exposure negatively impacting the immune response (Leicht et al., 2013).

1.1.3 Parasite responses to temperature change

1.1.3.1 Parasite survival

The first stages in most parasite life cycles — eggs and free-living infective stages — are generally lecithotrophic (i.e. non-feeding). This means that just like plant seeds they have limited energy stores and need to find a suitable habitat before they can start actively feeding. In the case of plants this is a fertile soil, for free-living parasite stages this is a suitable host. With increasing temperatures the metabolism of ectothermic free-living stages, is expected to increase and therefore energy stores are likely to be depleted more rapidly (Pechenik & Fried, 1995). This can lead to decreases in survival time and thus a shorter available window for infecting a suitable host (Koprivnikar, Lim, Fu, & Brack, 2010; Nollen, Samizadeh-Yazd, & Snyder, 1979; Pietrock & Marcogliese, 2003). Additionally, survival and hatching of parasite eggs can be negatively effected by warmer temperatures, resulting in lower transmission potential (Sakanari & Moser, 1985).

When developing inside the intermediate ecothermic hosts, mortality in these hosts could be significantly increased by short periods of extreme thermal warming. In sticklebacks naturally infected with macro-parasites, mortality during a heat wave was positively correlated with parasite loads (Wegner, Kalbe, Milinski, & Reusch, 2008). Predicted frequencies of heat-waves (Meehl & Tebaldi, 2004) could result in

synchronised clearing of host populations of parasites and thus reduced overall parasite prevalence.

1.1.3.2 Parasite behaviour and infectivity

For parasites that use environmental cues to locate their hosts (Haas, Beran, & Loy, 2008; Haas & Haberl, 1997) temperature could affect their ability to utilise these cues. These effects have not yet been studied enough to make reliable predictions (Lee, Burgess, Sterling, & Lutz, 2013), but there is some evidence that warming temperatures might disrupt endotherm hosts finding by parasites as environmental temperatures become more similar to host temperatures and consequently parasites might be unable to locate hosts based on temperature gradients (Ressurreição et al., 2015).

Although potential increases in metabolism could result in decreases in survival time, an increase in temperature can, on the other hand, also increase activity of free-living parasite stages (Koprivnikar et al., 2010). This could make these stages more visible, and potentially more attractive to visual predators targeting moving prey. As a result the transmission potential of the parasite could be increased. Conversely, increased visibility of free-living parasite stages could make it easier for potential host to select against these stages while feeding.

Again, responses of parasites to temperature are often non-linear, with initial increases but decreases at extreme high temperatures. Additionally, considering increases in activity and infectivity of free-living stages and decrease in survival makes it difficult to make predictions about their combined effects. For example, there could be an overall offset of effects with minimal net change in the transmission of parasites at moderately warmer temperatures (McCarthy, 1999; Morley, 2011). Conversely, changes in the life cycle, including potential increases in host mortality due to parasite infections, could select for lower virulence of parasites to allow parasite transmission before host death (Labaude, Rigaud, & Cézilly, 2015). This makes it hard to predict the results of changing thermal regimes on infective parasite stages without considering multiple steps in the life cycle simultaneously and rigorous experimental testing.

1.1.3.3 Parasite development

As warmer temperatures generally increase metabolism in ectotherm animals it is not surprising that the production of infective stages by trematodes in intermediate hosts if often elevated at higher temperatures (Poulin, 2006). These infective stages are produced asexually and the large size difference between host and parasite allows the parasite to utilise their host resources. However, like other temperature effects, production of infective stages, of parasite species in general, often responds to increases in temperature a non-linear, typically dome-shape, fashion (Paull, LaFonte, & Johnson, 2012). For instance, a helminth ectoparasite, *Gyrodactylus turnbulli*, infecting guppies (*Poecilia reticulata*) shows increased development at elevated temperature, but high levels of mortality at extreme temperatures (Mohammed et al., 2016). This means that with moderate increases of temperature the density of free-living infective stages could dramatically increase. This increased production rate of infective stages could lead to a faster depletion of host resources and therefore a reduction in host survival. For the potential hosts exposed to infective stages, survival could be reduced due to the high number of parasitic stages challenging their immune system and potentially establishing themselves.

1.1.3.4 Parasite distributions

Just like host ranges, parasite ranges are likely to be affected by changing thermal regimes. Local physiochemical conditions (Bozick & Real, 2015), including temperature can restrict parasite ranges (Galaktionov & Bustnes, 1999). Consequently, it seems probably that parasites will experience barriers to their transmission under altered thermal regimes (Lafferty, 2009b; Lõhmus & Björklund, 2015). Parasites with complex life cycles, especially those that are trophically transmitted, are susceptible to environmental disturbances. These parasites do not only rely on favourable environmental conditions, but also the presence of multiple host species and ecological interactions (i.e. predator-prey) between their hosts. At least one of these factors could be easily disturbed by environmental changes (Lafferty, 2009a) and habitats with high levels of environmental disturbance are thus expected to have less healthy parasite biodiversity (Hudson, Dobson, & Lafferty, 2006). As a result overall distributions are likely to

change under changing environments and ranges are expected to be restricted most strongly for parasites with complex life cycles and high levels of sensitivity to environmental variables.

1.1.4 Host-parasite interactions under warming climates

So far, the impacts of environmental changes have been addressed for hosts and parasites separately. Many of these aspects of host parasite interactions have been investigated, including the impacts of temperature on their interactions. The results of these studies often indicate that increasing mean temperatures will favour parasites over hosts. This is often through increases in host ranges (but see Section 1.1.3.4 Brooks & Hoberg, 2007), reduced immune responses of hosts at extreme temperatures (Section 1.1.2.2), and increases in vector ranges (Patz et al., 2003; Purse et al., 2005). Most of these studies have focused on single interactions (e.g. one part of the life cycle). Many parasite have complex life cycles and thus it is difficult to make predictions how the whole life cycle will be affected under changing environmental regimes as each additional host add more interactions and thus permutations of how they could be affected by environmental changes (Barber et al., 2016; Marcogliese, 2008). It is thus paramount to undertake comprehensive studies on parasite life cycles where as many of these interactions are studied to get a full picture of how species could be affected by environmental changes. Additionally, each host in the life cycle will add its own suite of interactions with other species (e.g. predator-prey) this further increases complexity.

1.1.4.1 Host manipulation

Many parasites species are known to alter their host's behaviour for their own benefit. These behavioural manipulations include making host more conspicuous, to facilitate trophic transmission (e.g. Barber & Huntingford, 1995; Joly & Messier, 2004), increased feeding rates to enhance parasite growth (Labaude, Rigaud, & Cézilly, 2016) or reduced feeding rates to avoid predation (Weinreich, Benesh, & Milinski, 2013), and selection of microhabitats with thermal optima for parasites (Bates, Leiterer, Wiedeback, & Poulin, 2011; Macnab & Barber, 2012). In the case of altering the thermal preferences

of the host, climate change may provide more opportunities for parasites to exploit optimal temperatures, this could lead to accelerated parasite development and increase host mortality (Mouritsen & Jensen, 1997). As the effects of parasites on hosts can alter host responses to environmental changes it is important to study these in concert. For example, *Gammarus fossarum* feeding can be increased at warming temperatures, but acanthocephalan infections can reverse this effect, resulting in decreased feeding with increasing temperatures (Labaude et al., 2016).

Consumption of infective stages of the parasite by species in which the parasite can not establish — non-hosts — or from which it can not continue its life cycle — dead-end hosts — can result in a decrease in fitness. Feeding rates of non-hosts and dead-end hosts could also be increased at warmer temperatures, reducing parasite fitness. There is now a suite of studies that shows that free-living parasitic stages can serve as a food source through accidental (Orlofske, Jadin, Preston, & Johnson, 2012; Thieltges, Bordalo, Caballero Hernández, Prinz, & Jensen, 2008) or active (Johnson et al., 2010; Orlofske et al., 2012; Schotthoefer, Labak, & Beasley, 2007; Thieltges, Bordalo, et al., 2008) consumption by dead-end hosts. As with potential host species, increases in temperature are likely to change the metabolic rate of these organisms and potentially alter their mircohabitat selection. This can thus affect the efficiency of parasite transmission (Goedknegt, Welsh, Drent, & Thieltges, 2015), especially if increased activity of free-living parasites stages would make them more attractive as food to non-hosts (Section 1.1.3.2).

Host manipulation can, even in invertebrate intermediate hosts, be timing dependent. *Macrocyclops albidus* copepods infected by *Schistocephalus solidus* or *Camallanus lacustris* (Nematoda) show parasite induced changes in behaviour. Altered behaviour reduces the risk of the copepod being consumed by predators, while the parasites are still uninfective to the next host. This means that infected copepods are less likely to be eaten by sticklebacks than uninfected copepod hosts (Weinreich et al., 2013). Once infective, different species of parasite are able to increase the likelihood that their host is consumed by a prospective next host. The manipulative parasite can for example increase activity (Wedekind & Milinski, 1996), increase the amount of time spend near the top of the water column (Pulkkinen, Pasternak, Hasu, & Tellervo Valtonen, 2000),

or their response to disturbances (Urdal, Tierney, & Jakobsen, 1995). These might increase their chances of transmission, as sticklebacks (*Gasterosteus aculeatus*) show a preference for moving copepods (Wedekind & Milinski, 1996). However, Urdal et al. (1995) failed to find evidence for increase transmission.

Conversely, changes in phenology — the timing of life time events — of host manipulation, because of increased development with warmer temperatures, could also lead to disruptions in the parasite life cycle. Either through mismatches between emerging parasites and the temporal availability of the next host, or increase levels of parasite infections if parasites are able to infect hosts at times when they are more susceptible (Hakalahti, Karvonen, & Valtonen, 2006). As a result parasite transmission could be altered and this could lead to an changes in host population densities, in populations where parasites are able to suppress host numbers. It is thus essential to study the impact on host parasite systems, as parasites in changing environments can both become less or more harmful to their hosts.

Alternatively, altered timing of ontogenetic or body-size dependent shifts in habitat use, could also disassociate hosts and parasites and reduce the transmission potential of hosts. For instance, *Ribeiroia ondatrae* cercariae emerged from snails significantly earlier at warmer temperatures leading to a decrease in overlap with its second intermediate amphibian hosts. In turn, parasite induced limb malformations in amphibians were greatly reduced (Paull & Johnson, 2014). However, changes in phenology could possibly expose hosts to a new set of parasites (Pickles, Thornton, Feldman, Marques, & Murray, 2013). If these are unsuitable hosts for the parasite to complete their life cycle, infections can still have significant negative impacts on the populations of these dead-end hosts if they cause an immune response.

1.2 *Schistocephalus solidus* as a model system for host-parasite interactions

The three-spined stickleback (*Gasterosteus aculeatus*) has been a common species for behavioural studies for more than a century (von Hippel, 2010). Early on (McCaig & Hopkins, 1965; Smyth, 1946), its parasites have also been popular for experimental

investigations. Although probably the most commonly studied parasite of the three-spined stickleback is *Schistocephalus solidus* (Barber, 2013), the impacts and interactions with a range of parasites species have been studied (Wootton, 1976).

1.2.1 *Schistocephalus solidus* life cycle

Schistocephalus solidus (Müller, 1776) is a pseudophyllidean cestode parasite with a complex life cycle (Dubinina, 1980). The life cycle consists of one free living stage and three parasitic stages in different host species (Figure 1.2). At the different parasitic stages it has different levels of host specificity (Dubinina, 1980). The eggs, which give rise to the free living stage, the coracidia, leave the bird definitive host with its faeces. Egg development is succesful in fresh or brackish water, but higher levels of salinity prevent successful development (Dubinina, 1980; Simmonds & Barber, 2016). Egg development takes 17 to 19 days at colder temperatures (16 - 18 °C) and 10 to 12 days at warmer temperatures (22 - 25 °C) (Dubinina, 1980). After hatching, the coracidia are infective to a wide range of cyclopoid copepod species (e.g. *Cyclops strenuus* or *Macrocyclops albinus* Clarke, 1954; Orr & Hopkins, 1969; Urdal et al., 1995; Wedekind & Milinski, 1996), but only for a limited time (1 - 2 days; Dubinina, 1980). Although, further infections have been observed after coracidia have stopped moving (Stewart et al., 2017).

Copepods get infected by ingesting coracidia whilst feeding. After ingestion the coracidia penetrate the wall of the digestive tract and enter the body cavity where they, after developing a cercomer become infective to the second intermediate host, the three-spined stickleback (*Gasterosteus aculeatus;* Clarke, 1954). This development is temperature dependent and takes 13 to 14 days at lower temperatures (16 - 18 °C) and half that at warmer temperatures (7 - 8 days at 22 - 25 °C) (Dubinina, 1980). Presence of *S. solidus* in copepods can increase their mortality, especially at high densities (Wedekind, 1997), and it thus important for the parasite that transmission to the stickleback happens timely. This process is aided by behavioural changes in the copepod when infected by infective *S. solidus* (Hammerschmidt, Koch, Milinski, Chubb, & Parker, 2009; Jakobsen & Wedekind, 1998; Wedekind & Milinski, 1996).

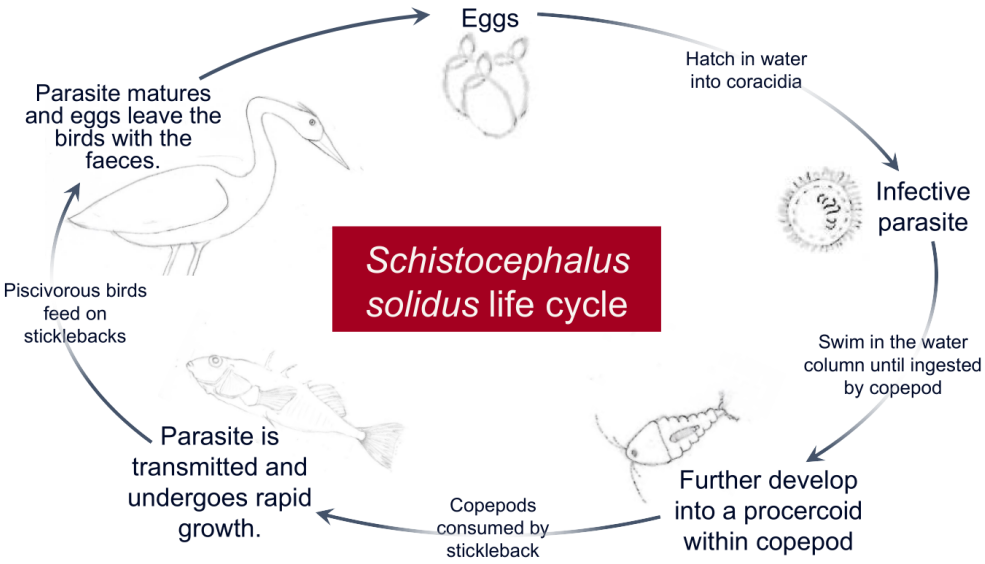


FIGURE 1.2: Schematic overview of the life cycle of *Schistocephalus solidus*. Individual parasites start off as eggs in fresh water which hatch into free-swimming coracidia. These mimic copepod food items (e.g. protozoans) and can be ingested by suitable copepod hosts. In the copepod host the coracidia develop into procercoids and alter copepod behaviour. These behavioural changes facilitate copepod consumption by three-spined sticklebacks. Inside the stickleback hosts procercoids develop into plerocercoids and undergo significant growth. Once *S. solidus* parasites are infective to their definitive bird hosts, host behaviour is affected to facilitate transmission. Inside the bird hosts mature parasites mate or self-fertilise and produced parasite eggs, which leave the bird with the faeces.

Procercoids infecting copepods consumed by a three-spined stickleback will continue to develop into plerocercoids (Figure 1.2), at this stage *S. solidus* is highly host specific and can only utilize three-spined sticklebacks as successful host (McPhail & Peacock, 1983). *Schistocephalus solidus* spends by far most of its life cycle inside the stickleback host (>180 days) and this is where most of the growth occurs. This means that the parasite mass inside a stickleback can rival that of the fish itself (Arme & Owen, 1967). Growth is likely to be this extreme because parasite mass is an important determinant for infectivity and reproductive output in the definitive bird host. Only parasite larger than 50 mg are successful at infecting birds and producing eggs and larger worms produce more eggs (Benesh & Hafer, 2012; Tierney & Crompton, 1992). *Schistocephalus solidus* is known to have a significant detrimental effects on its host's energy reserves (Barber, 2005) and can increase the feeding effort (Milinski,

1985). These parasite infections have serious implications for their host and, among other things, affects their behaviour (e.g. LoBue & Bell, 1993), reproduction (McPhail & Peacock, 1983), and health (Kalbe, Eizaguirre, Scharsack, & Jakobsen, 2016). Possibly the most notable change is the change in predator avoidance behaviour, with infected fish becoming less frightened and swimming closer to the water surface, thus increasing the transmission potential of the *S. soldius* parasite (Barber & Huntingford, 1995; Barber, Walker, & Svensson, 2004; Milinski, 1985). Uninfected three-spined sticklebacks will flee from shapes above the water surface, while fish that carry an infective parasite do not show this behaviour and become more susceptible to predation (Barber et al., 2004; Grécias, Valentin, & Aubin-Horth, 2018). This is likely to increase their potential to be eaten by birds and thus to transmit *S. solidus* to its next, definitive, host.

A wide range of piscivorous birds can be utilized as definitive hosts by *Schistocephalus solidus* and mature worms have been retrieved from more than 40 species of birds in the wild (Cooper, 1918). Additionally, a number of mammals can feed on three-spined sticklebacks, including otters., it is unknown whether these can serve as suitable hosts, but their body temperature might be to low for *S. solidus* maturation. Infection in the bird is almost transient as *S. solidus* can spend as short as one day (Dubinina, 1980) and up to two weeks (McCaig & Hopkins, 1963; Smyth, 1946; Tierney & Crompton, 1992) inside the definitive host. The parasite stays in the digestive tract of its host and never moves into its body cavity (McCaig & Hopkins, 1963). It is therefore unlikely that the parasite has a large impact on its bird host in adults, however anecdotal reports of smaller birds becoming 'stuffed' with plerocercoids exist. As this means that there is low selection on birds to fend off infections, establishment in the definitive hosts has a high success rate (Hopkins & McCaig, 1963). Inside the bird no increase in mass or feeding takes place and only a process of quick maturation occurs (Hopkins & McCaig, 1963). *Schistocephalus solidus* worms are hermaphrodite, but can also self-fertilise. Mature worms will spend some time looking for potential mating partner, to maximise fitness, but otherwise self-fertilizes (Lüscher & Milinski, 2003; Lüscher & Wedekind, 2002; Schjørring, 2004). In either case egg production typically starts within 36 hours after entering the bird (Schjørring, 2004). Worms prefer mating

with worms of similar size (Lüscher & Wedekind, 2002) — probably to maximise fertilisation rates — and worm size correlates positively with egg output (Dörücü, Wilson, & Barber, 2007).

1.2.2 *Schistocephalus solidus* in the lab

Although parasites with complex life cycles are typically hard to study in the lab, *Schistocephalus solidus* provides and amenable study system to study the effects of environmental change on species interactions (Barber & Scharsack, 2010) for several reasons. Many parasites with complex life cycles have hosts that can not be reared in the lab, this is especially true for parasites that cause human diseases. The *S. solidus* parasite model does not have this problem since the whole life cycle can be simulated in the lab; the free-living stages (i.e. eggs and coracidia) and the intermediate hosts can be held in the lab, while the definitive host can be appropriately simulated (Smyth, 1946). The need not to have the definitive host in the lab further increases the strength of the experimental system, as this makes it more reliable. It is possible to rear adult worms in the lab in chickens and other species (McCaig & Hopkins, 1963), but because the time that *S. solidus* spends inside the definitive hosts is exceptionally short compared to other cestode species (Dubinina, 1980) *in vitro* methods provide more practical and reliable methods for egg collection. Most cestode species spend considerable time in their definitive host and do an important part of their growing inside this host (Dubinina, 1980). In *S. solidus* however, this is not the case and the worms only need to mature, which can happen is as little time as the first 24 hours after ingestion by the host or incubation in the lab.

Fourth, apart from being a good model system in the lab, *S. solidus* and its hosts are widespread around freshwater and brackish habitats across the northern hemisphere (Barber, 2007; Kennedy, 1974) that are all likely to be subjected to changing temperatures over the next century (IPCC, 2007). The outcomes of our study will thus be informative for a wide range of ecosystems but can also be easily studied by ecology laboratories around the northern hemisphere.

These features make *S. solidus* an attractive study system for parasites with complex life cycles. The life cycle also allows for convenient studying of individual life

stages. However, when doing this it is important to validate the outcomes of experiments on single steps in the parasite life cycle (e.g. eggs hatching under different temperature regimes or infectivity to first intermediate hosts) in the context of the complete life cycle. This can be done with microcosm experiments in which cover several steps of the life cycle in a single experiment. The outcome of these microcosm experiments can then be compared to the predictions based on the experiments studying the individual steps in the life cycle.

Microcom experiments try to simulate natural conditions in a controlled manner. Often, laboratory experiments focus on a single feature of animal health or fitness, e.g. survival or infectivity, in response to the experimental manipulation. However, in microcosms the aim is to study one or multiple factors in interaction in response to the manipulation under more natural conditions. Microcosms are also used to study longer term patterns. For example, the effects of parasite infection on host feeding behaviour (Labaude et al., 2016), or long term response of copepods to temperature treatments (Maier, 1990) have been studied in microcosms. These experiments are likely to give a more realistic picture of what happens in nature than simpler lab experiments, but are less informative about the underlying mechanisms.

These results can be further combined in mathematical models, to both make long-term predictions about how temperature could affect infection levels and to provide more insight into the most critical stages in the life cycle of the parasite to study.

1.3 Using mathematical models to understand empirical data

For parasites with complex life cycles, predicting the cumulative effects of environmental changes on the whole life cycle can be very difficult based on experimental data. Mathematical models can provide a powerful tool in simulating the life cycle and testing the potential impact of environmental change on the parasite. This holds especially true when combined with experimental data specifically collected to inform model parameters. For example, transmission rates in models are often important but might not often be tested in an ecological relevant experimental manner.

Examples of how mathematical models have been used in host-parasite ecology include models that describe the distribution and maturation of different tick species under future climate change scenarios, as well as recent small changes in temperature (e.g. Estrada-Peña, de la Fuente, Latapia, & Ortega, 2015; Ostfeld & Brunner, 2015). Others have described co-evolutionary principles of host-parasite interactions and attempting the explain the size differences between related parasites of different host species (Haukisalmi, Heino, & Kaitala, 1998).

Previously, mathematical models have often been used to help understand the impact of environmental factors on parasites that can affect the health of humans (Estrada-Peña et al., 2015) or of economically important species (Groner, Gettinby, Stormoen, Revie, & Cox, 2014). This is often successful because for these species a large body of literature is available and mathematical models can be accurately parameterised. The *Schistocephalus solidus* life cycle has also been studied with considerable vigour and with information for the literature, combined with experiments targeting gaps in current knowledge, reliable parameterisation is possible. Therefore the results from the experiments in this thesis will be combined with literature sources in a mathematical model to make predictions on the *S. solidus* life cycle under changing thermal regimes as well as provide a framework for studying the impacts of temperature change on other cestode species.

1.4 Statistical approaches

Recent problems with replication due to low statistical power (Button et al., 2013) or inappropriate use of statistics (e.g. Cramer et al., 2016) have become known throughout the biological sciences and other branches of science. These issues are partially driven by incentives given to scientists to publish 'high impact' papers. Some of these issues are relatively easy to deal with, for example problems with multiple testing in more complicated statistical designs can be mitigated by *a priori* determining the comparisons of interest (Cramer et al., 2016). While others have suggested adjusting the 'significance threshold' (Higginson & Munafò, 2016). In this section I will outline the justification for the general statistical approach taken in this thesis.

All analyses were be carried out in `R` (**?**), and all tests are implemented as linear models with `lm()` or `glm()` from the `stats` package, or extensions thereof. Graphs were produced with the 'ggplot2' package (Wickham, 2016).

They therefore follow the general notation of

$$y = \alpha + x_1 \times \beta_1 ... + x_n \times \beta_n + \epsilon.$$

Where appropriate, random effects are added using the `lmer()` or `glmer()` function from the 'lme4' package (Bates, Mächler, Bolker, & Walker, 2015). When testing for significance, only potential relevant interactions are included, based on biological theory. This is done to reduce the number or tests and thus the risk of false positives (type I errors). Initial models are fitted with all potential relevant biological factors included. Then, step-wise removal of non-significant factors (starting with the highest order interactions) is applied until only significant effects, or at least significant interactions of non-significant main effects remain. More formally, model fitting follows a three step protocol from Zuur, Hilbe, and Ieno (2013):

1. Based on prior knowledge the dependency structure in the data, model structures are selected, including random effects where appropriate, *a priori*.

2. The model is fitted and covariates in the fixed part of the model are inspected for significance, with step-wise removal of non-signficant higher order interactions based on AIC values. Models with a AIC (Akaike Information Criterion) value of at least 2 points lower were considered to have a better fit to the data (Johnson & Omland, 2004).

3. The best model is presented graphically and numerical output is given.

For linear mixed models (LMM's) and generalized linear mixed models (GLMM's) overdispersion was also checked. For GLMM's degrees of freedom (df) are not given as there is still debate about how these are appropriately calculated (Bates et al., 2015).

To assess model fits, model diagnostics are presented. Where diagnostic plots indicate inappropriate models, more robust models are fitted where possible. If this is not possible, limitations of the fitted models are indicated. Normal distribution of data

points are assessed in plots showing theoretical quantiles and standardised residuals, these should fall more or less on a diagonal. To assess the contribution of individual data points to model parameters, Cook's distance for each point is plotted. These points are then inspected for potential disproportionate contribution to the parameter estimates in the model, i.e. values that are much higher on the y-axis than others (Fox, 1991). In diagnostic plots, the alpha (α= 0.3) of points has been set low so that overlapping points show as a darker shade of grey.

Numerical data are generally plotted in box-and-whisker plots ('boxplots' henceforth) to show full distributions of the data. The range of the boxes are the first and third quartiles and the whiskers extend to the largest value that is within the one-and-a-half times the interquartile range from the box. The interquartile range is the distance between the first and the third quartile. Any data points outside of this range are plotted individually as outliers.

All code written for this project, either to record temperature, extract data, or analyse data, is available in the Code Appendix, but not in the printed version of the thesis. This is to ensure the brevity of the thesis. Additionally, all code will also be available as a `.zip` file from `https://www.boriswberkhout.com/thesis/appendices`.

1.5 Objectives and outline

The overarching aim of this thesis is to investigate the impact of differences in mean temperature on the life stages of *Schistocephalus solidus* and its ectothermic hosts and to make estimates about population level impacts of infection of changing mean temperatures. In each chapter the specific objectives of the chapter will be given. Briefly, Chapter 2 describes the development and verification of experimental set-ups used throughout the other chapters. In Chapter 3 the potential impacts of temperature on host and parasite development in isolation (i.e. free-living parasite stages and hosts without parasite exposure) are investigated. Chapter 4 reports parasite transmission to and development inside the first intermediate hosts. In Chapter 5 the effects of temperature combined with host factors are described and repeatable methods to investigate this are proposed. In Chapter 6 a mathematical model of the temperature

dependence of the whole parasite life cycle is constructed and potential effects of temperature change on population densities are described.

Chapter 2

Low-cost methods for studying temperature effects *in vivo*

To ensure the collection of robust and high quality data from empirical scientific research, relevant experimental methods should be used for data collection, along with appropriate statistical analyses. Additionally, it is important to use a relevant range of conditions at appropriate intervals to help understand the biology underlying the processes studied. This is particularly true when studying the effects of temperature (Altizer, Ostfeld, Johnson, Kutz, & Harvell, 2013). Allowing the discovery of potential non-linear relationships and subsequently allows proper parameterization of statistical and mathematical models describing these relationships. Which can be achieved in commercial incubators, but these are often expensive and beyond the financial scope of smaller research labs, especially in times and places where funding is restricted. It can thus be difficult to marry good experimental design with the financial budget. Thus, there is constant development and innovation of experimental methods and setups in a pursuit to improve existing methods. The purchase of new, ready-to-use equipment can be costly, especially if a large range of experimental conditions or field locations are part of the study. A logical alternative is to build setups in-house. This is particularly attractive if in-house workshops are available. Here a reliable low-cost alternative to commercial incubators for small scale temperature experiments on invertebrates is provided, as well as a modification from previous setups for temperature controlled experiments on fish (Macnab & Barber, 2012).

The setups that were developed were used for a range of temperature treatments

(i.e. 11°C, 14°C, 17 °C, 20 °C, and 23°C), which were relevant to the current experimental questions, but with only small modifications the setups could be used for a wider range of experimental conditions.

2.1 Temperature controlled incubators for invertebrates

Temperature variability (Saunders, Tompkins, & Hudson, 2002) as well as mean temperature (Morozińska-Gogol, 2002) can affect biological processes. Hence, when studying one, the other needs to be controlled as much as possible to avoid confounding effects. Here, stable controlled temperatures were needed for the study of the effects of mean temperature on the development of *Schistocephalus solidus* eggs (Chapter 3) and on their first intermediate hosts, i.e. copepods (Chapter 4).

Moreover, for these experiments, controlled day-night light-dark cycles are essential. *Schistocephalus solidus* egg hatching is light dependent (Scharsack, Koch, & Hammerschmidt, 2007) and *Cyclops strenuus* copepods are visual predators and need day-night cycles for normal behaviour (Ludovisi, Todini, Pandolfi, & Taticchi, 2008). Hence, the incubators needed to be designed to house animals under naturalistic light conditions.

2.1.1 Design

The incubators were made from large (50x50x50 cm) Styrofoam boxes, with a thick wall (~7 cm) and close fitting lid (Figure 2.1a). This meant that they were well insulated and heat exchange with the environment was reduced to a minimum. At the bottom of each box were two layers of polypropylene bags, with the inner one filled with water (~3 litre) and an aquarium heater (Betta thermal compact submersible aquarium heater 50W; Figure 2.1b). These bags were specifically selected for their heat-resistance to avoid risks of burning or melting and a double layer was chosen to avoid potential leakage. The heater in each incubator was set to heat the water to one of the desired temperatures (i.e. 11°C, 14°C, 17 °C, 20 °C, or 23°C). Above this a horizontal platform was suspended on which samples could be placed (Figure 2.1c), but it was ensured

that room was left on two sides of the platform to allow for more efficient heat exchange within the incubator.

To allow for the design of these to be consistent and cost effective the incubators themselves were placed in a temperature controlled room which was set at 4°C. This meant that incubators only had to be heated and never cooled, as would have been the case had they been placed in at room temperature (~20 °C).

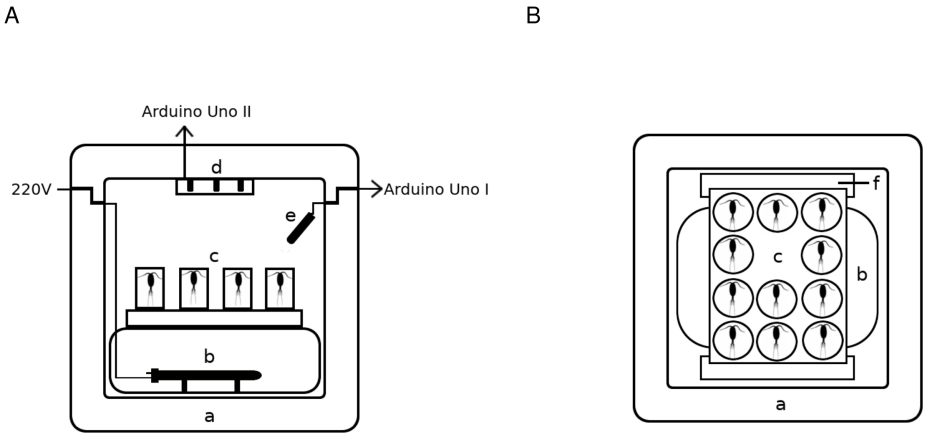


FIGURE 2.1: Schematic overview of the incubators, with a side (A) and a top (B) view. The incubator is made of styrofoam box (a). In this a double layered bag fill with water and an aquarium heater is placed (b). Above this a platform with samples is suspended (c) on two supports (f). Day-night cycles are regulated by LEDs connected to an Arduino Uno microprocessor (d; Arduino Uno II). Temperature readings are made with a temperature sensor connected to a second Arduino Uno microprocessor (e; Arduino Uno I).

To ensure biological processes occurred as natural as possible within the incubators a day-and-night cycle was simulated with three clear white light-emitting diodes (LEDs) in each incubator (Figure 2.1d), to mimic daylight. Over each set of of three LEDs a 9cm Petri dish was placed and sealed with aquarium sealant (Betta premium aquarium sealant) to protect the LEDs from corrosion. A 12:12h day-night cycle was set that largely corresponded to outdoor conditions to not unnecessarily disturbed the invertebrates when handling them. The lights were controlled by an Arduino Uno board (model R3, `www.arduino.org`; Figure 2.1d). Briefly, the board was programmed to switch the lights on and off every 12 hours using relays (for code, see `lights_v1.0_clean.ino` in Digital Appendix). All lights were connected to the same

Arduino board, but on a different circuit to reduce the impact of potential failure. Checking of proper functioning of the lights was done weekly during normal weeks (when only copepod or *S. solidus* egg cultures were kept) and twice weekly during experiments. To monitor temperatures in the incubators stainless steel temperature sensors were fitted in each incubator. These were connected to a second Arduino Uno board (Figure 2.1e), on this a data logging shield (v1.0) and a sensor shield (v4.0) were fitted (for code, see `temperature_v3.1.ino` in Digital Appendix). With the sensor shield temperature sensors could be connected. The data logging shield allowed a regular sized SD card to be plugged in, onto which data was collected. Data could then be analysed using the `incubator_temperatures_v2.1.Rmd` script (see Digital Appendix).

2.1.2 Validation

2.1.2.1 Methods

Before the incubators were used for any experiments they were run for several weeks to monitor the temperature. Temperature mean and range (minimum and maximum) for each incubator was then analysed. Once formal analysis had shown that temperatures were constant within each incubator only weekly visual inspection was done to confirm temperatures had not changed and were stable. During the experimental periods regular checks on functioning of heating and lighting were done.

2.1.2.2 Results

Within each incubator, mean temperatures (i.e. 12.4, 13.7, 17.4, 19.7, and 22.3 °C) were close to their set value and did not show more than 1.1 °C from their mean temperature (Table 2.1). Temperatures followed a predictable cycle, this was caused by heaters switching on and off and slow cooling down of the incubator within the cold room. However, overall temperatures were stable around the set temperatures and thus were deemed suitable for the experiments (Figure 2.2). Functioning of the lights in the incubators was checked weekly by visual observation. No failures of the lights occurred during the nearly three years that the incubators were in operation. However, LED's

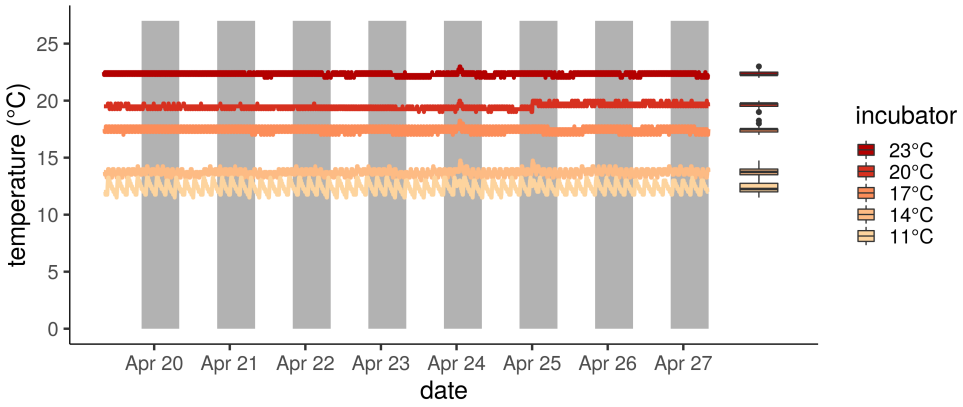


FIGURE 2.2: Representative one week sample of temperature within the incubators. Shading of the background indicates the day:night cycle (12:12h), with grey backgrounds indicating nights. On the right-hand side boxplots per temperature treatment are shown.

were replaced after 1 year as in the original setup the cover was not sealed properly and some rust occurred. After replacement the cover was sealed with aquarium sealant (Betta premium aquarium sealant). See Table 2.2 for a breakdown of the costs per incubator.

TABLE 2.1: Temperatures in incubators. The mean, standard deviation (sd), minimum (min), and maximum (max) temperature in each incubator over a month (same as Figure 2.2) are given. All values are in °C.

	mean	sd	min	max
23°C	22.31	0.13	22.00	23.00
20°C	19.68	0.20	19.00	20.00
17°C	17.43	0.21	17.00	18.25
14°C	13.74	0.26	13.00	14.75
11°C	12.40	0.44	11.50	13.50

2.1.2.3 Discussion

Although some of the temperatures were slightly off the target temperature, the incubators were useful for the current experiments. Temperature control and day-and-night cycles worked consistently during the testing period of three weeks and the subsequent three years of usage. Only small alterations had to be made during use, such as the replacement of the LEDs and additional sealing of the LED cover. Also, aquarium heaters were replaced after two years of use, because some showed a drop

TABLE 2.2: Breakdown of the total costs for each experimental setup for invertebrates. Number indicates the number of each item needed for current experiments.

item	number	cost
aquarium heater	5	£15 each
styrofoam boxes	5	free (recycled)
Arduino Uno	2	£20 each
Arduino wireless shield (including SD card)	1	£15 each
Arduino sensor shield	1	£7 each
sensors and wires	1	~£20
total		~£160
total (each)		~£32

in temperature. For future experiments, it is recommended to replace them every two or three years to optimize performance. Additionally, it would be possible to include daily or seasonal temperature cycles for long term experiments by using Arduino controlled heaters. Day-and-night cycles could be improved by adding gradual transitions which may be important to obtain results in the lab that most closely replicate natural patterns (Nagy et al., 2018). It also has to be noted that care must be taken with the aquarium heaters as they could burn through the polystyrene boxes. It is thus recommended to use heat resistant bags like the ones used here, with a large volume of water to keep the heaters in and to ensure that heaters can not touch the side of the bags or the boxes.

2.2 Temperature controlled recirculating system for fish

In order to study the effects of temperature on fish as well as fish-parasite interactions (Chapter 3) reliable temperature controlled experimental recirculating systems were needed. Ideally fish could be individually tracked in these, so that individual development could be followed, allowing for increased experimental and statistical power. The three-spined stickleback (*Gasterosteus aculeatus*) is a social fish species (Ward, Thomas, Hart, & Krause, 2004) and should not be housed individually for extended periods of time (Home Office, 2014). This means that tracking of individual fish can be difficult without using molecular techniques (Breacker, Barber, Norton, McDearmid, & Tilley, 2017) or tagging the fish. However, tags can be lost and

the monetary and time costs involved in repeated molecular identification of fish can rapidly become cost-inefficient with large sample sizes and number of measurements. It would thus be convenient to have a low cost, time effective method to individually track a social fish species, like the three-spined stickleback. Additionally, the setup needs to allow for experimental manipulation of fish in different treatments. Here, a setup in which fish are housed individually, but with visual and chemical contact is presented. Fish were housed in transparent individual 1 litre tanks, that are embedded in a larger reservoir allowing for visual contact as well as the exchange of chemical cues.

2.2.1 Design

An experimental setup from Macnab and Barber (2012) was modified, so that up to 16 fish could be housed individually in transparent 1 litre tanks embedded in a larger reservoir (Figure 2.3a). This allowed for temperature buffering as well as the exchange of visual and chemical cues which are important for a social species like the three-spined stickleback (Hogan & Laskowski, 2013; Ward & Currie, 2013).

Water from the reservoir drained into a 250 litre sump tank (250 litre mortar tub). In the sump tank a divider was placed (custom made, in-house). On one side the sump tank was filled with gravel (diameter 20mm; Figure 2.3b; left-hand side), this allowed for the growth of denitrifying bacteria which remove waste products out of the water (Van Rijn, Tal, & Schreier, 2006). The water then flowed to the other side of the sump where it was pumped up (PondKraft Solids Handling Pump 3500, flow rate 2800Lh^{-1} at 1 m height; Figure 2.3c) into the header tank (see Table 2.3 for costs details).

For treatments that were below room temperature, a commercial chilling unit (Hailea HC-300A 110V; 395Watt cooling capacity, Waterchiller) was connected in between the pump and the header tank (Figure 2.3d). While, for temperatures above room temperatures, the water was pumped directly into the header tank which was fitted with one or multiple aquarium heaters (Figure 2.3d). Header tanks were wrapped in an insulation jacket (thickness 60 mm) to keep temperatures more stable (see Table 2.3 for costs details).

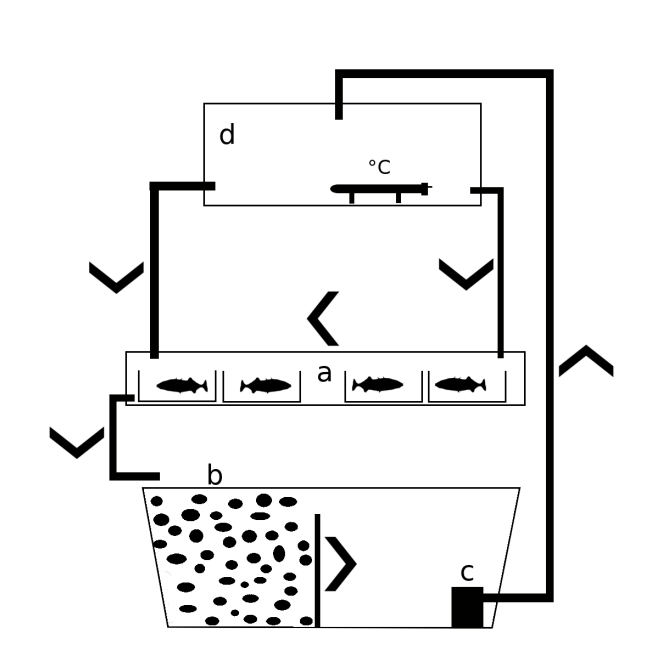


FIGURE 2.3: Overview of recirculating system with temperature control for fish. Fish were housed individually in transparent tanks embedded in a larger reservoir (a). Water from the reservoir drained into a sump tank, where water was biologically filtered through gravel (b). A submersible pump (c) pumped the water to a header tank (d) where the water was heated or chilled to the experimental temperature and recirculated to the fish. Arrows indicate the water flow through the system.

From the header tank, water drained passively into each holding tank to ensure oxygenation and prevent build up of waste products. Additionally, water flowed into the reservoir where the fish tanks were embedded to regulate the temperature.

Connecting pipes were fitted into holes drilled into the header tank and reservoir and aquarium quality sealant (Betta premium aquarium sealant) was used to stop leakage. The room containing the setups was set to a 12:12h day-night cycle.

2.2.2 Validation

2.2.2.1 Methods

Before and during the experiments temperature was recorded daily as required by the Home Office. In each setup, levels of ammonia (NH_3), nitrate, (NO_3^-) and nitrite (NO_2^-) as well as pH were measured regularly using an API freshwater master test kit, to check they fell within the expected range ($NH_3 < 0.50$ ppm, $NO_3^- < 0.25$ ppm, NO_2^-

< 5.0 ppm). Additionally, conductivity was checked using a portable meter (Mettler Toledo, FiveGo^{TM} Conductivity meter FG3). Conductivity values of between 3.0 and 3.5 mS (millisiemens = ω^{-1}) were aimed for, as is common practice in stickleback husbandry (e.g. Bernhardt, von Hippel, & Cresko, 2006; Cresko et al., 2004) to prevent bacterial infections (Bowden, 2008). As some water loss occurred, due to evaporation and spillage, water levels were checked weekly and topped up when needed.

2.2.2.2 Results

Temperatures within temperature treatments stayed constant with standard deviations smaller than 1.0°C within treatments, and most less than 0.5°C (Figure 2.4 and Table 2.4). This means that temperature control was effective. Levels of ammonia, nitrate, and nitrite remained low during the experiment indicating biological filtering was operating succesfully. Conductivity and pH values were also within the expected range.

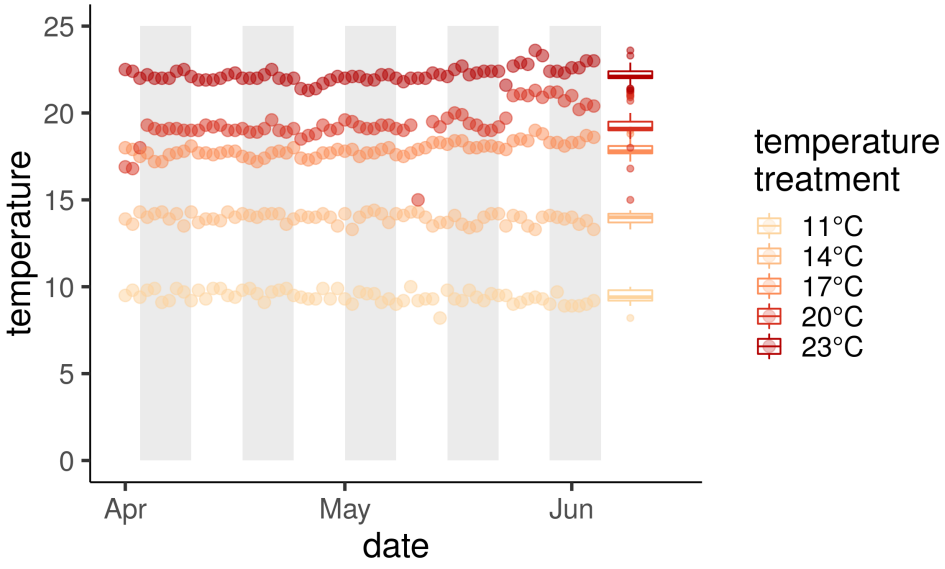


FIGURE 2.4: Daily temperature measurements in five different fish temperature setups. Representative two-and-a-half month sample from manual daily temperature recordings for each setup during 2017. The single outlier for the 20 °C in early May is likely due to miss recording (human error). Boxplots are given for all the dates shown.

TABLE 2.3: Breakdown of the total costs for each experimental setup for fish. Number indicates the number of each item used in the current experiments. While the totals are the total per setup, either heated or chilled.

item	number	cost
aquarium heater	3	£15 each
Hailea HC-300A 110V; 395Watt cooling capacity		
Waterchiller	2	£300 each
250 litre mortar tub	5	£65 each
header tank plus jacket	5	~£50 each
PondKraft Solids Handling Pump 3500	5	£15 each
additional costs (in total)	1	~£50
total (heated)		£155
total (chilled)		£440

2.2.2.3 Discussion

The current setup was used successfully for temperature experiments on fish in a lab setting. It allows for accurate setting of treatment temperatures and for gradual manual change of temperature. An even closer control of temperature could be achieved through the incorporation of a temperature feedback mechanism. For example using a microprocessor (e.g. Arduino Uno) that measures and records the temperature. Based on this the microprocessor could then switch heaters on or off through a simple relay system and thus control temperatures more tightly. This would also allow for experiments investigating the effects of more complex temperature regimes. For example, into the effects of diurnal, seasonal (Morozińska-Gogol, 2002) and stochastic (Saunders et al., 2002) variation in temperatures.

TABLE 2.4: Temperatures in each fish setup. The mean, standard deviation (sd), minimum (min), and maximum (max) temperature in each incubator over a month (same as Figure 2.4) are given. All values are in °C.

	mean	sd	min	max
11°C	9.45	0.34	8.20	10.00
14°C	13.96	0.29	13.30	14.40
17°C	17.86	0.38	17.20	19.00
20°C	19.31	0.98	15.00	21.30
23°C	22.16	0.39	21.30	23.60

In future work dissolved oxygen could be measured, as values will depend on

water temperature and at high temperatures oxygen stress may become a problem as higher temperatures reduce the dissolved oxygen levels in the water (Benson & Krause, 1984).

In both systems daily temperature cycles could be implemented in future to account for, often, lower temperatures during the night which could underlie daily cycles in feeding and metabolism.

Chapter 3

Individual host and parasite development

3.1 Introduction

3.1.1 Temperature effects on development

Temperature is an important regulator of biological processes. A wide range of developmental parameters are affected by temperature; from growth rate in tropical wrasse species (Motson & Donelson, 2017) to survival in lady beetles (Zhang, Cao, Wang, Zhang, & Liu, 2014), and from maturation in gadoids (Tobin & Wright, 2011) to sex allocation in turtles (Tilley et al., 2019). For some of these parameters, there is evidence that responses to temperature are somewhat flexible and they can be adjusted (Laplante, McKinnon, Love, & Vezina, 2019), while others have a fixed response to temperature (Tilley et al., 2019). Given that global mean temperature as well as thermal regimes are rapidly changing (Easterling et al., 2000), it is important to understand which species and populations are likely to be affected.

Changes in mean temperature will affect different organisms differently, but a distinction can be made between endo- and ectothermic animals. Endotherms generate heat via their own metabolism and can maintain their body temperature, whereas ectotherms largely rely on the environment for their thermoregulation. Both types of animals are able to regulate their body temperature to a certain extent through microhabitat selection (Judge, Botton, & Hamilton, 2011) or can adjust their activity in

response to temperature (Rickards & Boulding, 2015). However, physiological functioning of ectotherms is expected to be more heavily impacted by global warming than that of endotherms (Buckley, Hurlbert, & Jetz, 2012).

3.1.2 Temperature effects on ectotherm development

Ectotherm animals can not regulate their own body temperature directly through their metabolism, unlike endotherms. In general, metabolic rates are themselves temperature dependent, due to increased enzyme activity at higher temperatures (Hoffmann, 1983). Thus, the metabolism of ectotherms is partially dependent upon environmental temperatures (Piersma & Van Gils, 2010). Increased metabolism depletes energy stores at a faster rate, consequently requiring stored energy reserves to be used up, or animals to increase their feeding, to sustain the increase in metabolism. For short lived changes in temperature the former strategy will often be sufficient, especially for longer lived animals as these are more likely to have energy reserves (Laplante et al., 2019). However, the only direct way to deal with prolonged or permanent changes in temperature is to adjust consumption. Alternatively, animals could phenotypically adjust to changes in the environmental temperatures, for example by changing the availability of metabolic enzymes in order to restrict metabolism (Hoffmann, 1983). Over the span of generations natural selection can act to select for individuals that are more adapted to warmer temperatures or for individuals that have more phenotypic plasticity.

However, it is not clear whether such adaptations are always possible, as this requires some level of pre-existing genetic variation. In the short term, and in some cases in the long term, increasing temperatures will mean an increased metabolic demand on animals. An increase in the consumption of resources will therefore be necessary and thus a need to feed more often or more intensely (Labaude et al., 2016). Alternatively, feeding behaviour could change to feeding on different food types (Carreira et al., 2016). In turn, these changes in behaviour could lead to increased exposure to predators (Pasternak, Huntingford, & Crompton, 1995). For a detailed discussion of the effects of temperature on feeding, host exposure and parasite transmission see Chapter 4.

Another important factor affected by temperature, and mediated by metabolism, is developmental rate. Many enzymatic and cellular processes are temperature dependent, where increases in temperature can increase the rate of these processes (Hoffmann, 1983). This results in an increased rate of cell division and consequently growth. This increase in growth could result in two scenarios. Increased growth rate, but unchanged developmental time, can result in larger individuals with, as a potential consequence, a greater reproductive output (Wedekind, Strahm, & Schärer, 1998). Alternatively, increased growth rate and similar adult size, result in a reduced developmental time (Milonas & Savopoulou-Soultani, 2000). This could lead to faster maturation of individuals and faster population turnover, all else being equal. The latter only holds if reproductive potential remains similar. However, this could also lead to temporal mismatches between life stages and food availability (Abarca & Lill, 2015; Both, Van Asch, Bijlsma, Van Den Burg, & Visser, 2009) or — for parasitic taxa — host (Paull & Johnson, 2011) availability.

Three-spined stickleback (*Gasterosteus aculeatus*) development and growth, just like that of many other fish species (e.g. Motson & Donelson, 2017), is temperature dependent (Allen & Wootton, 1982; Guderley & Leroy, 2001; Macnab & Barber, 2012; Swarup, 1958).

Although differences between families exist (Guderley & Leroy, 2001), most studies have found that growth rate correlates negatively with temperature at high temperatures (Franke, Armitage, Kutzer, Kurtz, & Scharsack, 2017; Guderley & Leroy, 2001; Macnab & Barber, 2012). While at lower temperatures a positive relationship (Allen & Wootton, 1982) can be seen. When including a wider range of temperatures, a non-linear relationship between temperature and parasite growth becomes apparent (Hovel, Beauchamp, Hansen, & Sorel, 2015; Ramler, Mitteroecker, Shama, Wegner, & Ahnelt, 2014). It is likely that all these studies describe a similar pattern, one where warming temperatures positively affect the growth of fish, but once a thermal optimum is reached a quick drop in growth rate is seen. In some studies (Hovel et al., 2015; Ramler et al., 2014) the whole pattern in seen, while in others, only temperatures on either side of the optimum are tested (Franke et al., 2017; Guderley & Leroy, 2001;

Macnab & Barber, 2012) and in yet other studies, only temperatures below the optimum temperature (Allen & Wootton, 1982). However, it seems likely based on these results that different populations show different thermal optima.

Besides potential negative impacts from increased exposure to predators, through changes in feeding regimes and positive effects of increase population growth, all biological processes have an upper thermal limit (Hoffmann, 1983). This means that as these limits are approached, efficiency is likely to decrease. More negative effects are expected when these limits are exceeded. For example this can lead to increased mortality rates (Mouritsen & Jensen, 1997) and abnormal development (Ottesen & Bolla, 1998). Most thermal limits are likely to be set by the environment that the organism evolved in. In the context of climate change, for many organisms it appears unlikely that upper thermal limits will be reached within the next century (Villar-Torres, Esteban Montero, Antonio Raga, & Repullés-Albelda, 2018). However, it is still important to consider what would happen if these temperatures were reached, to further our understanding of temperature effects on individual organisms and populations.

3.1.3 Temperature effects on non-feeding stages

The uptake of resources is important for anabolism as well as maintenance of the body. Nevertheless, most animal and plant species have at least one stage of their life cycle during which they do not feed. For instance, in plants this is the seed stage and in sexually reproducing animals the sperm and, in most animals, the eggs. Thus all of these life stages need to be equipped with some sort of energy store. The extent of this energy store will determine the potential longevity of this life stage. After which, the organism needs to progress to the next stage of the life cycle and can start to actively feed in order to survive. As the metabolic rate is closely linked with temperature (Piersma & Van Gils, 2010), changes in mean temperature could have important consequences for the fitness of non-feeding stages.

These non-feeding stages typically have a very specific purpose, e.g. sperm must fertilise eggs, eggs must develop into independent feeding stages, and free-living stages of parasitic life cycles must infect a suitable host. Because of their limited energy stores these organisms have a time window in which they need to complete this

task, or they die. For free-living parasitic stages, this window is often restricted to several hours, or maybe days (Dubinina, 1980). Therefore, any shortening of this window could have a substantial impact on the ability to complete the life cycle. An increase in temperature, and consequently in metabolic rate, can lead to a shortening of this time window (Fried & Ponder, 2003; Morley, 2012) and may reduce the fitness of parasites. Conversely, if the time window over which eggs hatch is reduced (i.e. there is a decrease in variance and consequently more eggs hatch over a short period of time), this could mean an increased availability of infective stages in the environment and infection success (McPherson, Friesen, Selbach, & Poulin, 2018), and an increase in mortality of the host (Bates, Poulin, & Lamare, 2010; Sakanari & Moser, 1985), if large numbers of infections challenge the host simultaneously. Having some spread in the hatching of eggs might thus benefit the parasite and increase the likelihood of successfully infecting a next generation of hosts.

Generally, parasite eggs develop more quickly at warmer temperatures (Paull & Johnson, 2011; Sakanari & Moser, 1985; Zakikhani & Rau, 1998), most likely because of increased metabolic rate. However, in the cestode *Lacistorhynchus tenuis* (Sakanari & Moser, 1985) and the trematode *Plagiorchis elegans* (Zakikhani & Rau, 1998), mortality of the eggs is also increased at warmer temperatures. In non-parasitic, free-living stages the same is often seen (Mehlis & Bakker, 2014). The eggs of *Schistocephalus solidus* and the coracidia that hatch from them, are the only parts of the life cycle that are free-living and thus exposed directly to the environment (see Section 1.2.1 for details). This means that these stages could be extra sensitive to environmental changes for example heavy metal pollution and temperature can have an effect on the survival of coracidia (Ismail, 2018). It is thus expected that *S. solidus* eggs and coracidia will respond in a similar fashion to increasing mean temperatures; developmental rate will be enhanced, but survival time will be decreased. To assess the net effect of these two on a population level it is important to quantify the overall impact of temperature on *S. solidus* eggs and free-living coracidia. Here, the aim is to determine the effect of a change in temperature on the developmental rate of *S. solidus* eggs and the effect on the survival of the eggs, i.e. the number of eggs hatching. Additionally, the time period over which eggs hatch at different mean temperature will be determined, to be

able to estimate the effects of temperature on the availability of infective stages in the environment over time.

3.1.4 Temperature effects on the development of endoparasites

For endoparasites, parasites that live inside their hosts, the host can be considered their direct environment (or the inner environment; Figure 3.1), as opposed to the combination of biotic and abiotic factors outside of the host which can be referred to as the outer environment (Figure 3.1). Once the parasite is established inside the host it will only interact with the inner environment, but changes in the outer environment can still be reflected in the inner environment. The inner environment will consist of different factors depending on in what part of the body of the host the parasite lives. If the parasite resides in the gut of the host or any of the organs related to nutrient uptake, it is more likely to directly experience changes in the outer environment through what is ingested by the host. On the other hand, if the parasite hides within the non-alimentary tissues of the host, for example the muscles, it is less likely to be directly influenced by changes in the outer environment. This means that changes in the external environment (i.e. the environment that the host experiences) do not necessarily reach the parasite. For example, endotherm hosts may try to compensate their body temperature for changes in the outer environmental temperature, by increasing or decreasing activity (Johansen, Messmer, Coker, Hoey, & Pratchett, 2014), resulting in higher nutrient levels in the body from which the parasite could benefit (Soutter, Walkey, & Arme, 1980). One could imagine that this inner environment could also act as a buffer against pollutants, like heavy metals. Although the reverse appears to be true in some aquatic hosts, with parasite often accumulating higher levels of heavy metals than their hosts when occupying the alimentary tract (Courtney-Hogue, 2016). These examples illustrate that parasite exposure to the outer environment hinges on the physical niche they occupy inside the host.

Direct impacts of changes in the outer environment are changes that are filtered through to the parasite in the same mode, e.g. changes in temperature in the outer environment lead to changes in temperature in the inner environment. Conversely, indirect effects are factors in the outer environment that cause changes in the inner

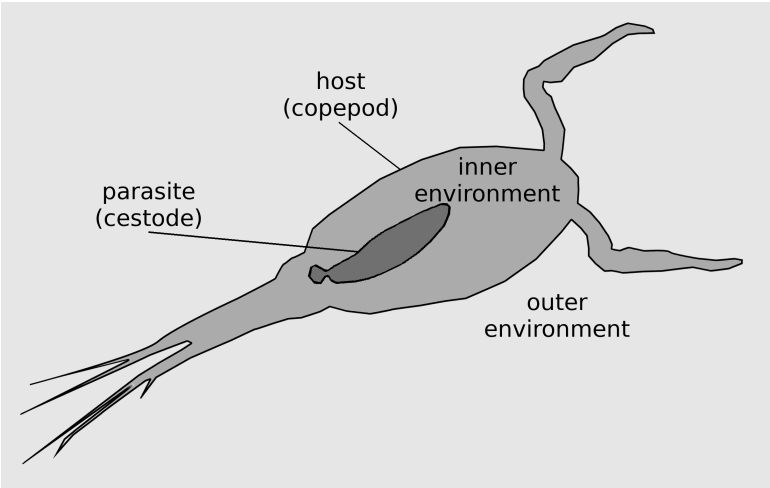


FIGURE 3.1: Conceptual drawing of the inner and outer environment from the perspective of internal parasites (dark shaded shape inside the copepod) in ectotherm hosts. Properties of the outer environment, like temperature and soluble chemicals (e.g. salts and heavy metals) do not affect the parasite directly, but are 'filtered' by the host. Besides these 'filtered' properties of the outer environment, the inner environment also contains immunochemicals and possible other pathogens.

environment in a different mode. For example, at warmer temperatures host activity levels could change (Bauwens, Castilla, & Mouton, 1999) this could lead to a change in nutrient availability to the parasite. The inner environment of the host will also contain factors related to host health and immune response (Press & Evensen, 1999). This means that changes in the outer environment can change the immune system and affect the establishment success of parasites. Thus, the outer environment can have an indirect effect on the parasite as well as a direct effect.

As both direct and indirect effects of changes in mean temperature could affect the development of endoparasites, it is important to get a comprehensive understanding of the interaction between the host and parasite. Previous work has shown that at warmer temperatures, *S. solidus* plerocercoids benefit from increased growth compared to lower temperatures (Franke et al., 2017; Franke, Raifarth, Kurtz, & Scharsack, 2019a; Macnab & Barber, 2012). To gain a full understanding of the system, it is not only important to study mean effects, but also to assess individual differences and thus to track individual host and parasite development. By studying interactions in a range of populations of the same species it will also be possible to draw more general conclusions about temperature effects on the species.

3.1.5 Aims

The first aim is to better understand the effects of temperature changes on the development of *S. solidus* eggs (Aim 3.1; Figure 1.2). More specifically, understanding how the onset of hatching of the eggs (i.e. the developmental time needed until hatching starts) is affected by temperature (Objective 3.1a), what effect temperature has on the total proportion of eggs hatched (Objective 3.1b), and the length of the period of time over which the eggs hatch lasts (Objective 3.1c). The second aim is to quantify the effects of different constant mean temperatures on the development and survival of three-spined sticklebacks (*Gasterosteus aculeatus*) and *S. solidus* plerocercoids developing inside of them (Aim 3.2). Here, the growth of *S. solidus* plerocercoids inside the host (Objective 3.2a) and the number of eggs these worms produce (Objective 3.2b) is studied. Additionally, the growth (Objective 3.2c) and stress response (Objective 3.2d) of juvenile fish hosts infected with *S. solidus* exposed to different temperature treatments is quantified.

3.2 Methods

3.2.1 Stickleback collection and husbandry

Wild sticklebacks were collected with dipnets on one day in December 2015 and on three days between February and May 2018, all from the River Soar in Abbey Park, Leicester, UK (N 52°64'73, W -11°4'34; Figure 3.2) with permission form the Leicester City Council. All fish were collected from the same ~250 metre stretch of river, so are assumed to be from the same population. On each sampling day fishing was carried out at approximately midday for 2 to 3 hours. After collection fish were transferred to the lab in large plastic bags filled with a 1:3 water:air ratio, which allowed for sufficient oxygen exchange. Upon returning to the lab fish were first inspected for *Argulus foliaceus* (ectoparasite) infections (Stewart et al., 2017). Any *A. foliaceus* parasites were removed from the fish using forceps. Fish were screened again the next day. After inspection they were transferred to static stock tanks (25 cm x 30 cm x 40 cm) with a biofilter and a mix (50:50) of river water and enriched copper-free water (here after called 'lab water'). Over the next days the proportion of lab water was increased up to

100%. Within a week of return into the lab fish were treated against fungal infections using Protozin white spot & fungus treatment (Waterlife Research Ind. Ltd.). After treatment for fungal infections, fish tanks were kept on a recirculating system. Fish were kept under constant conditions at 14 ± 1°C and a 12:12 light:dark photoperiod. Fish were kept in these conditions between 3 and 18 months, until they were needed for *S. solidus* parasite culture. Infected fish were visually selected for *S. solidus* based on abdominal swelling.

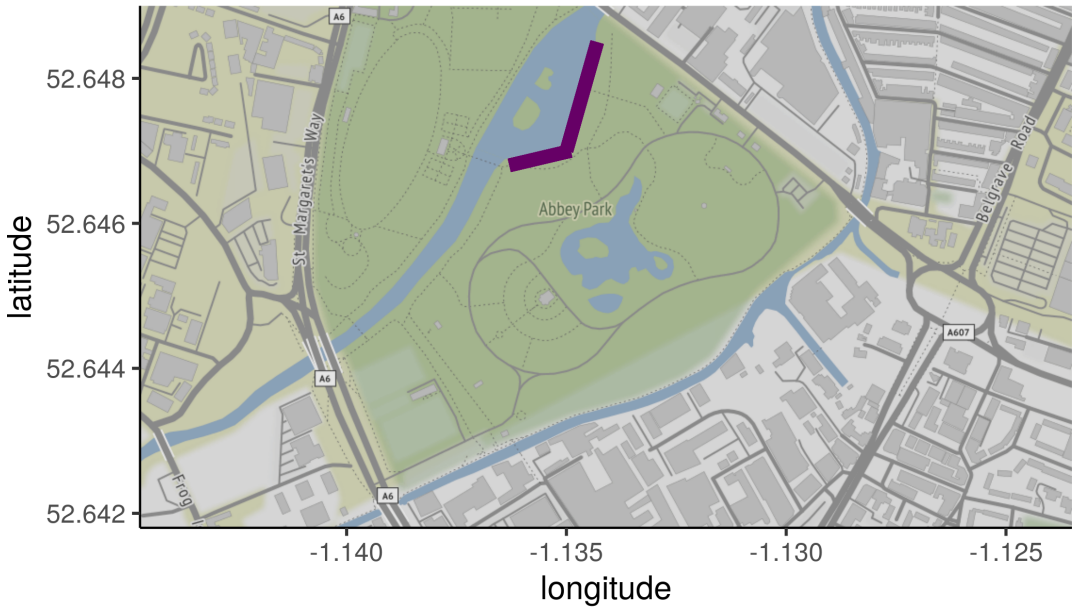


FIGURE 3.2: Map of fishing location in Abbey Park, Leicester, UK. Fishing area is indicated in purple. Sticklebacks were collected on four occasions during two years. The park is a recreational park with, with several species of piscivorous birds present.

3.2.2 *Schistocephalus solidus* egg hatching

After fish dissection, *Schistocephalus solidus* plerocercoids were incubated in pairs in dialysis tubes which were submerged in culture medium (50% heat inactivated horse serum, 50% RPMI medium, 750 µL penicillin), to induce sexual maturation and the onset of egg production (Smyth, 1946). Culturing of worms was done in size-matched pairs, where possible, to maximize fertilization rates as outcrossing makes the eggs

four times as likely to hatch (Benesh, Weinreich, Kalbe, & Milinski, 2014; Lüscher & Wedekind, 2002). After a week the eggs were collected in a Petri dish (⌀= 90 mm) and cleaned. Cleaning was done by adding ddH_20 and swirling the dish, which causes most of the eggs to collect in the centre of the dish, then any worm residue could be pipetted off. This process was repeated several times until mainly parasite eggs remained.

Before the experiment a grid of 15×15 mm (= 225 mm^2, Figure 3.3) was drawn on the bottom of each well of six well plates (surface area approximately 950 mm^2). The grid was used as a counting aid and only eggs inside this grid were counted. To each well 3 ml of ddH_20 was added, then in haphazard order 100 µL of cleaned eggs were pipetted into the centre of each well until all wells had received 300 µL of egg suspension (i.e. each well received three times 100 µL of the egg suspension). Eggs of each worm pair (i.e. parasite family) were used on a set of five plates to ensure each parasite family was tested at each temperature treatment. To get a more accurate estimate, each parasite family was assigned six wells on each plate to create within temperature replicates, so that eggs of each pair filled 30 wells. Each plate was then haphazardly assigned to a temperature treatment (11°C, 14°C, 17°C, 20°C and 23°C) and kept in an incubator set at that temperature (see Chapter 2). *Schistocephalus solidus* eggs need light to induce their hatching (Scharsack et al., 2007), so the incubators were set to a 12:12 dark:light regime to allow eggs to hatch as soon they had matured.

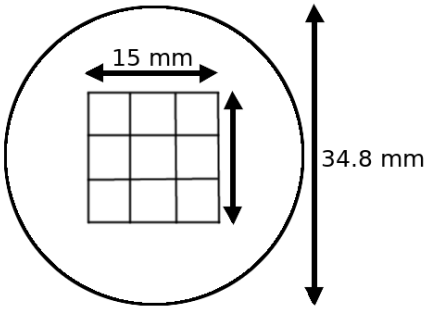


FIGURE 3.3: Illustration of one well with the counting grid. Total size of the well, on the six well plate, is 950 mm^2, surface of the counting area is 225 mm^2. The marking on the wells used for assessing *Schistocephalus solidus* egg hatching.

Hatched eggs can be distinguished from non-hatched eggs by their opened operculum and their clear appearance compared to non-hatched eggs (Simmonds & Barber, 2016). For each well the number of both hatched and non-hatched eggs was counted every 3 days. Eggs within the marked square in each well (Figure 3.3) were counted using a dissection microscope. No distinction was made between non-hatched embryonated and non-embryonated eggs (see Simmonds & Barber, 2016, for details) because the primairy interest was the proportion of hatched eggs. These counts were carried out until the hatching proportion in each temperature treatment had reached a plateau (Figure 3.4, zone C).

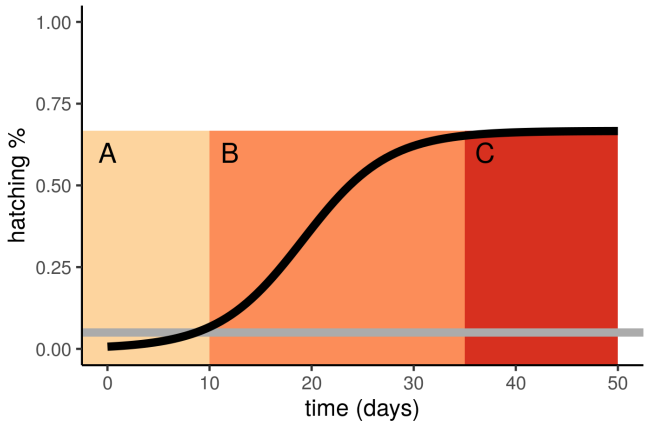


FIGURE 3.4: Hypothesized hatching curve of *Schistocephalus solidus* eggs. Each zone denotes a different phase; A is the minimal developmental time for the eggs, B is the hatching window (e.g. the period over which the eggs hatch), C is the plateau the maximum proportion of hatching. The grey line indicates 5% hatching.

3.2.2.1 Onset of egg hatching

Different parameters were calculated from the count repeats. The time until the onset of hatching was defined as the number of days it took until 5% of the eggs had hatched (Figure 3.4, zone A). This was calculated as the last day that less than 5% of the eggs had hatched for each replicate independently.

3.2.2.2 Proportion of eggs hatching

To determine whether egg hatching had stopped (Figure 3.4, zone C), a linear model was fitted through four observations, using a sliding window approach (illustrated in Figure 3.5). Specifically, starting at the last four observations (m : m-3) a linear model was fitted and if the slope was zero (i.e. no change in proportion hatched) the model was kept (Figure 3.5A). Then the window was shifted by one place, now fitting a linear model to the fifth-last through the second-last observation (m-1 : m-4; Figure 3.5B). If the slope was not different from zero (i.e. non-significant *P*-value for slope) and the r^2 value was higher than the stored model (i.e. a tighter fit to the data) the new model was stored. This procedure was repeated until the data point defined as the onset of hatching was reached. The first day of the model with the best fit (stored model) was then marked as the end of hatching for that replicate.

To confirm that hatching had indeed stopped after the day marked by this method a linear model was fitted through the all data per temperature, taking the median end of hatching day as the first data point. If the slopes of these models do not differ from zero, hatching has indeed stopped, while a positive slope would indicate eggs are still hatching.

3.2.2.3 Hatching window

The hatching window (Figure 3.4, zone B), is the number of days between the end of the minimal developmental time (or 'onset of hatching') until the plateau.

3.2.2.4 Statistical analyses

For all analyses of onset of hatching and length of hatching window, the response was a fitted with Poisson error distribution with a log link function. Proportion of eggs hatched was fitted with a Gaussian error distribution. Parasite family was initially included as a fixed effect to test its contribution to the model, but in the final model it was included as a random effect. Temperature was the only fixed effect in the final model. Models with and without interaction terms were compared using a χ^2-test (see Section 1.4 for details).

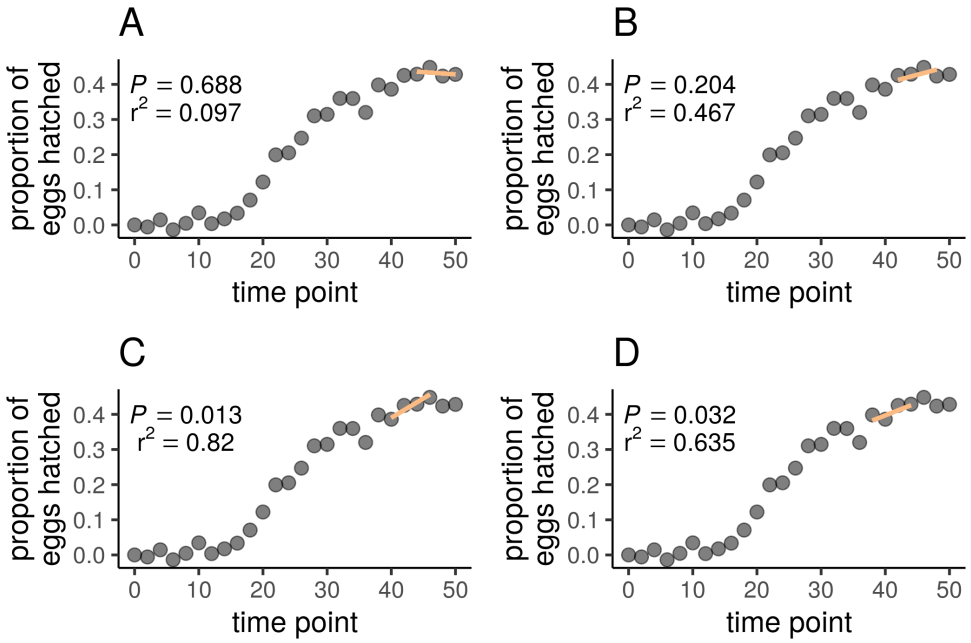


FIGURE 3.5: Illustration of sliding window. For a hypothetical set of hatching proportions a sliding window is applied. When the *P*-value is smaller than 0.05 for the first time (panel C), the hatching is said to still be ongoing. Thus, hatching stops in panel B (has higher r ^{2}-value than panel A), thus on day 42 (a two day sampling interval is used).

3.2.3 Stickleback growth and health

3.2.3.1 Stickleback breeding

Sticklebacks were bred over the summer of 2016 (July-August) from wild fish caught in the winter of 2015, as described in Section 3.2.1, according to the *in vitro fertilization* (IVF) protocol described by Stewart et al. (2017). Briefly, a male and female three-spined stickleback were selected for breeding. Males were selected based on their colouration. Males in breeding condition have a red throat and bright blue eyes (Figure 3.6, bottom fish). Additionally, their overall body colouration is often darker than that of females or non-breeding ready males (Wootton, 1984). Females were selected based on their abdominal swelling (Figure 3.6, top fish). In sticklebacks that have not been exposed to *S. solidus*, this swelling indicates the presence of eggs. To obtain the eggs the abdomen of the female was rubbed gently from the front to the back, in females that are ready to spawn this should induce the release of the eggs. Eggs were collected in a watch glass with a few drops of dH_2O and kept on ice. Males were

killed using an overdose of Benzocaine (10 grams per litre 70% IMS, Sigma-Aldrich) after which death was confirmed by severing the spine,inaccordance with Home Office rewgulatios (Home Office, 2014). Then, their testes were dissected out. The testes of one male were collected in a watch glass and a few drops of dH_2O were added and kept on ice. Using dissection scissors and forceps cleaned with ethanol, the testes were mashed to release the sperm. The eggs of one female and sperm of one male were than mixed together and stored on ice. After 15 minutes the eggs were checked under a dissection microscope to check for the separation of the outer from the inner membrane in the eggs. The eggs were then transferred to 1 litre breeding tanks that were gently aerated. To each breeding tank 1 millilitre methylene blue was added to reduce the chances of fungal infections. After hatching the fry were fed with Liquifry No1 for egg layers (Interpet Ltd) until they were large enough to feed on live *Artemia spp.* (brine shrimp). Females were only bred once to create unrelated crosses. A cross between one male and one female will be called a family henceforth.

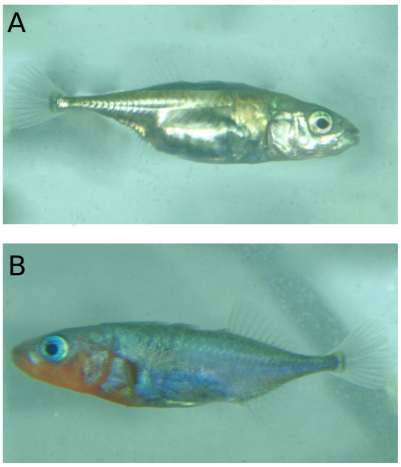


FIGURE 3.6: Photographs showing a female (a) and male (b) three-spined stickleback (*Gasterosteus aculeatus*) in breeding condition. The female shows a clear swelling of the abdomen, indiating the presence of eggs and the male has bright colouration. Out of the breeding season male colouration more or less resambles female colouration. Images are not to scale, females are typically larger than males.

Once they reached > 2 cm sticklebacks were large enough to feed on frozen bloodworm (Stewart et al., 2017) and the diet was switched to bloodworm. Fish were kept in 30 litre tanks (25 cm x 30 cm x 40 cm) per family until March 2017. Five fish families

were then selected based on the number of fish available in each family and fifteen fish from each family were picked. Within each family, three fish were haphazardly allocated to a temperature treatment (i.e. 11, 14, 17, 20 and 23 °C), so that the fifteen fish per family were equally distributed over the five temperature treatments. Fish were housed individually in 1 litre tanks that were on a temperature controlled re-circulating system (as described in Chapter 2). Sticklebacks were fed *ad libitum* with frozen bloodworm.

3.2.3.2 Growth of young sticklebacks

Every two weeks the weight and length of each individual fish was recorded and a dorsal profile photograph was taken. These measures were used to track fish growth throughout the experiment at the different temperatures. To assess fish growth the change in mass was analysed for each fish by fitting a linear regression to the data. From the slope of this model, the mean specific growth rate per day (in mg) was then calculated for each fish. Specific growth rate (Barber & Svensson, 2003) was not used, as this would make interpretation of the absolute change in growth rate with temperature difficult.

Fish that died within a month of the onset of the experiment were replaced with fish from a sixth family. For calculations of fish growth, fish with fewer than 3 data points (due to mortality) were excluded.

3.2.3.3 Stickleback health during development

Fish health was compared between the different temperature treatments using the body condition factor and the splenosomatic index. The body condition factor is the relation between the length and the mass of the fish and a higher index (more mass per unit length) indicates healthier fish (Pennycuick, 1971b). Conversely, a higher splenosomatic index (spleen weight relative to body weight) indicates poorer health as the spleen is involved in the immune response (Handy, Runnalls, & Russell, 2002; Seppänen, Kuukka, Voutilainen, Huuskonen, & Peuhkuri, 2009) and the production of red blood cells (Press & Evensen, 1999). Larger spleens are thus indicative of a larger

turnover of red blood cells or metabolic activity and can thus be used as an indicator of general stress (Ellis et al., 2012). These indices are calculated as

$$I_{BCF} = \frac{M_B}{SL^3} \times 10^5, \tag{3.1}$$

for the body condition factor, and

$$I_S = 100 \times (M_S \times M_B^{-1}), \tag{3.2}$$

for the splenosomatic index. Where M_B is the body mass, SL is the standard length, and I_S is the splenosomatic index, M_S is the mass of the spleen of the fish. All weights are measured in grams and lengths in millimetres.

3.2.3.4 Statistical analyses

Fish weights at the start of the experiment were compared between temperature treatments using a linear model. Temperature was included as a factor and not as a linear predictor as at this point fish were not yet exposed to the treatments.

Daily-growth-rate slopes for each fish (fish mass regressed over time) and the fish mass at the end of the experiment for each treatment were compared with temperature as a linear predictor in a linear model with a Gaussian error distribution. Fish splenosomatic index was analysed in the same way.

Survival of sticklebacks at the end of the experimental period between treatments was compared using a Pearson's χ^2-test. Exact date of death was only available for a limited number of fish, making it impossible to fit survival curves per temperature treatment.

3.3 Results

3.3.1 *Schistocephalus solidus* egg hatching

The effect of temperature on *Schistocephalus solidus* egg development was examined by recording the following non-independent parameters: the onset of hatching, the

cumulative proportion of hatched eggs, and the length of the hatching window. A total of four parasite pairs ('parasite families') were tested.

3.3.1.1 Onset of egg hatching

The rate of *Schistocephalus solidus* eggs development, measured by the time from the start of incubation until the onset of hatching (i.e. the time until at least 5% of the eggs had hatched), was significantly affected by temperature. After removal of the non-significant interaction between temperature and parasite family (χ^2 = 2.1, df = 3, P = 0.5457), there was a significant difference in the onset of hatching between some of the parasite families (χ^2 = 46.3, df = 3, $P < 0.0001$). Thus, in the final model parasite family was included as a random effect. This showed that the time until the onset of egg hatching was significantly reduced at higher temperatures ($GLMM_{Poisson}$, z = -7.936, n = 103, $P < 0.0001$; Figure 3.7).

Visual inspection of the residuals showed no clustering or uneven distribution of the residuals (Figure 3.8). Therefore, model assumptions were not violated. Although there was some overdispersion (dispersion = 2.89), this was not enough to warrant further investigation.

The model shows that with an increase in temperature the developmental time of *S. solidus* eggs is reduced and there are overall differences in the developmental rate of eggs between parasite families. This can be captured more formally by:

$$log(\mu_T) = 3.66 - (0.055 \times T) - (0.032 \times F_p) + \epsilon. \tag{3.3}$$

Where T is the temperature in °C and F_p the parasite family. The standard error for the estimate of the intercept is 0.15 and for the temperature slope is 0.007, indicating that these are both reliable estimates. While for parasite family the error is much larger (0.18). This equation can be rewritten as:

$$\mu_T = e^{3.66-(0.055\times T)-(0.032\times F_p)} + \epsilon. \tag{3.4}$$

From this the difference in time until the onset of hatching could be estimated per degree Celsius temperature change. At the higher end of the range of experimental

temperatures the estimated change was -0.62 days per degree Celsius change (difference between 22 and 23 °C). On the other end of the spectrum the difference was -1.13 days per degree Celsius (difference at 11 and 12 °C). The observed number of days that it took on average before the onset of hatching (i.e. at least 5% hatched) was 24 days at 11°C and 9 days at 23°C, with intermediate values at 14°C (12 days), 17°C (15 days), and 20°C (12 days).

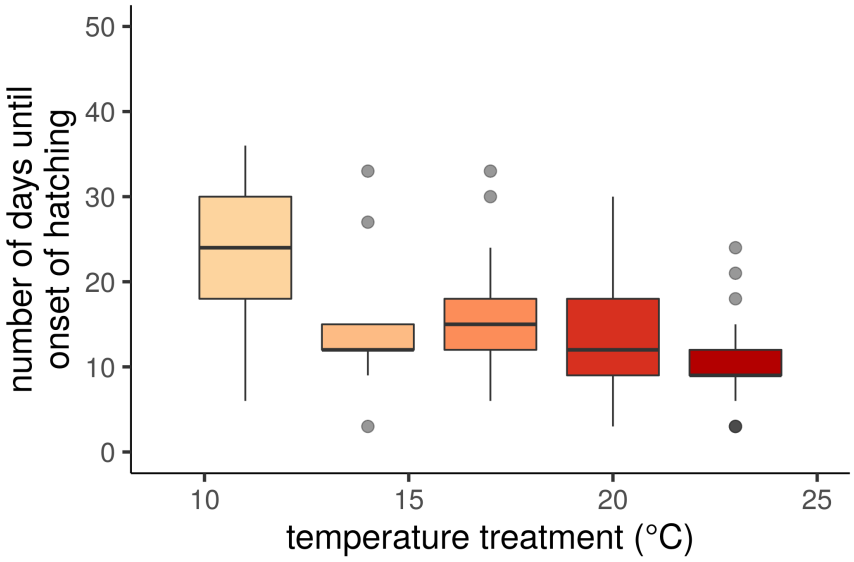


FIGURE 3.7: Boxplots for different temperature treatments showing the onset of hatching in number of days after incubation of *Schistocephalus solidus* eggs. Developmental time until onset of hatching showed a decrease with temperature.

3.3.1.2 Hatching window

The length of the hatching window — the number of days over which the eggs hatch starting at the first day at least 5% of the eggs hatched — increased with temperature ($GLMM_{Poisson}$, z = 8.726, n = 80, $P < 0.0001$; Figure 3.9). There was no interaction effect between temperature and parasite family ($\chi^2 = 0.947$, df = 3, $P = 0.8141$), thus this interaction was removed from the model. There was a significant effect of parasite family on the length of the hatching window by itself ($\chi^2 = 36.1$, df = 3, $P < 0.0001$), so family was included as a random effect in the final model.

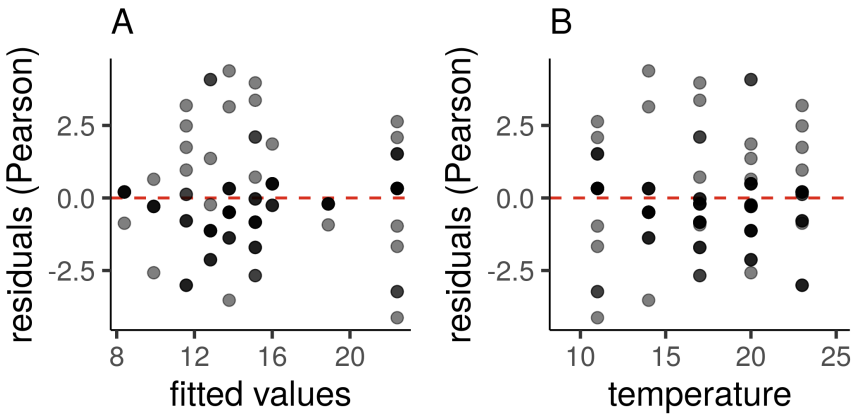


FIGURE 3.8: Diagnostic plots for egg hatching onset. Residuals versus fitted values (A) and residuals versus temperature (B) for $GLMM_{Poisson}$ on temperature and family effects on the onset of *S. solidus* eggs hatching. No violation of assumptions are seen, i.e. even spread of the ponits in both graphs.

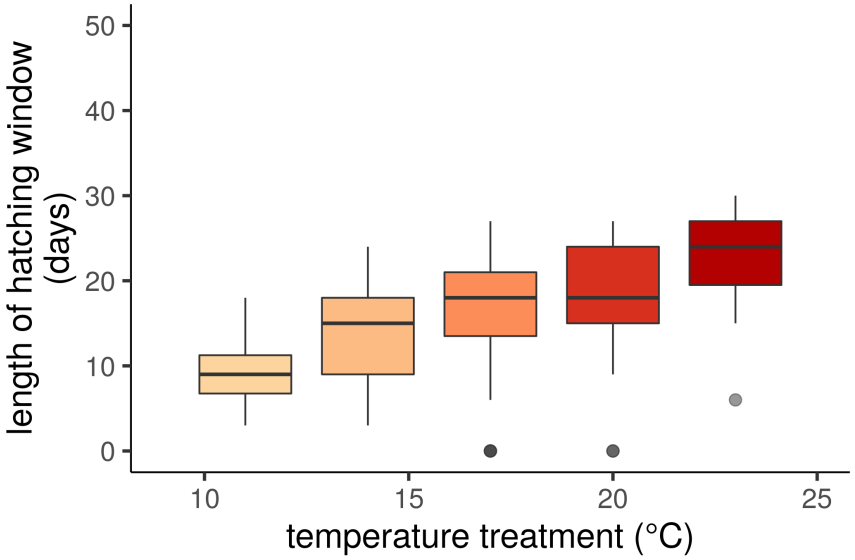


FIGURE 3.9: Boxplot of the length of the hatching window for each temperature. This is the number of days over which *Schistocephalus solidus* eggs were hatching after the onset of hatching. There was a significant increase in the length of the hatching window with increasing temperature treatments.

In the final model, there was a significant effect of temperature on length of the hatching window ($GLMM_{Poisson}$; z = 8.726, $P < 0.0001$), with a positive effect of temperature on the length of the hatching window. Model assumptions were tested and not violated (Figure 3.10). The dispersion parameter was not much larger than 1 (1.97),

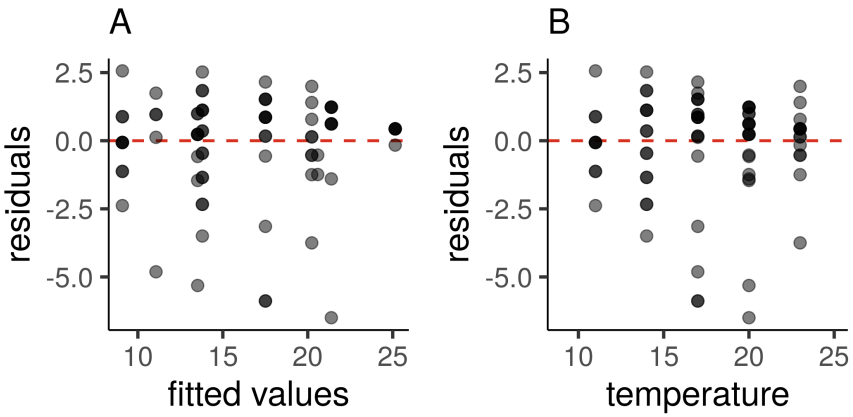


FIGURE 3.10: Diagnostic plots for the length of the hatching window for each temperature. Points are more or less evenly spread in both graphs and thus no violation of the assumption is seen.

thus not warranting further investigation. With increasing temperature the length of the hatching window increased according to:

$$log(\mu_T) = 1.56 + (0.066 \times T) + (0.029 \times F_p) + \epsilon. \tag{3.5}$$

Where the standard errors of both the intercept (0.17) and the temperate slope (0.008) are small, but the error for the slope of family is large again (0.17). Rewriting the equation gives:

$$\mu_T = e^{1.56+(0.066\times T)+(0.029\times F_p)} + \epsilon. \tag{3.6}$$

This shows that the length of the hatching window increased between 0.66 days per degree Celsius on the lower end of the temperature range (difference between 11 and 12 °C) and 1.38 days per degree Celsius at the higher end of the temperature range (difference between 22 and 23 °C).

3.3.1.3 Proportion of eggs hatching

The total proportion of *Schistocephalus solidus* eggs that had hatched after the plateau was reached (i.e. 34 days) was not affected by temperature (LMM, t = -0.745, n = 80, df = 72, P = 0.4585; Figure 3.11). However, the proportion of eggs hatched varied between parasite families (χ^2 = 9.01, df = 3, P = 0.0292). On average 38.9% of *S. solidus*

eggs hatched across families and temperatures, with the lowest median hatching at 14°C (28.2%) and the highest at 23°C (40.2%) (Figure 3.11).

For the final model (LMM) none of the model assumptions were violated; assumption of homogeneity of variance (Figure 3.12A), normal distribution of residuals (Figure 3.12B), and the Cook's distance (Figure 3.12C). Inspection of the Cook's distance led through investigation of three points, removing these did not affect model interpretation and thus these data points were kept in the final model.

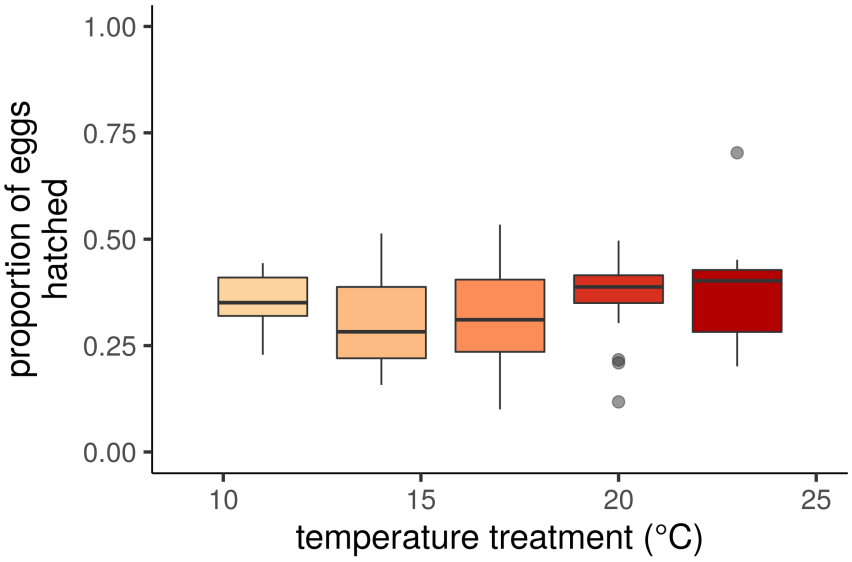


FIGURE 3.11: Boxplots showing the total proportion of *Schistocephalus solidus* eggs hatched 34 days after incubation for each temperature treatment. There were no differences between treatments, i.e. the same proportion of parasite eggs hatched at each temperature.

To verify the cut-off point for egg hatching (34 days) the slope of the hatching curve for each temperature was tested from 34 days onwards. *Schistocephalus solidus* egg hatching did indeed cease after the 34th day after incubation, with none of the slopes of the cumulative proportion of hatched eggs differing from zero (all *P*'s larger than 0.297, see Table 3.1). Until the experimental day prior to this (31st day) slopes were positive (hatching was counted every 3 days).

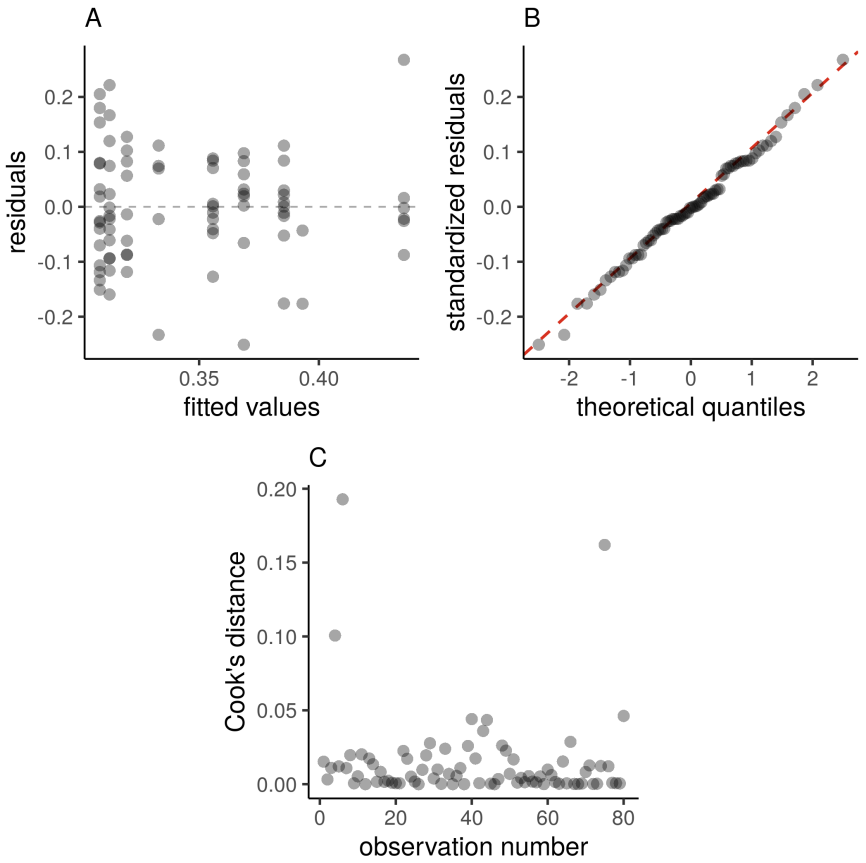


FIGURE 3.12: Diagnostic plots for the model output for the $GLM_{Poisson}$ on temperature and family effects on the total number of *S. solidus* eggs hatching. Subplot A shows the homogeneity of variance, subplot B indicates that the residuals are normally distributed and subplot C shows that three of the points may contribute more to model parameter than others, i.e. points with most extreme y-axis values. Inspection of these individuals points did not lead to concern, as removal of points did not lead to appreciable differences in model parameters.

3.3.2 Stickleback growth and health

3.3.2.1 Growth of young sticklebacks

The mass of young fish (n = 15 in each temperature treatment, 75 in total) did not differ between temperature treatments before the experiment (LM; df = (4, 70), F = 1.111, P = 0.3580; Figure 3.13). Visual inspection of the model parameters showed that model assumptions were met (Figure 3.14). Although one data point had an extreme Cook's distance (Figure 3.13C), removal of this point did not change model interpretation and the data point was kept in the final model.

TABLE 3.1: Output of linear models fitted through cumulative hatching proportion after day 34 of the experiment. None of the slopes differed from zero indicating that there was no change in the number of eggs hatched.

Temperature	slope	df	t	*P*
11	0.001	2, 46	0.252	0.802
14	-0.002	2, 52	-0.322	0.749
17	0.003	2, 86	1.048	0.297
20	-0.001	2, 88	-0.285	0.776
23	0.002	2, 57	0.466	0.643

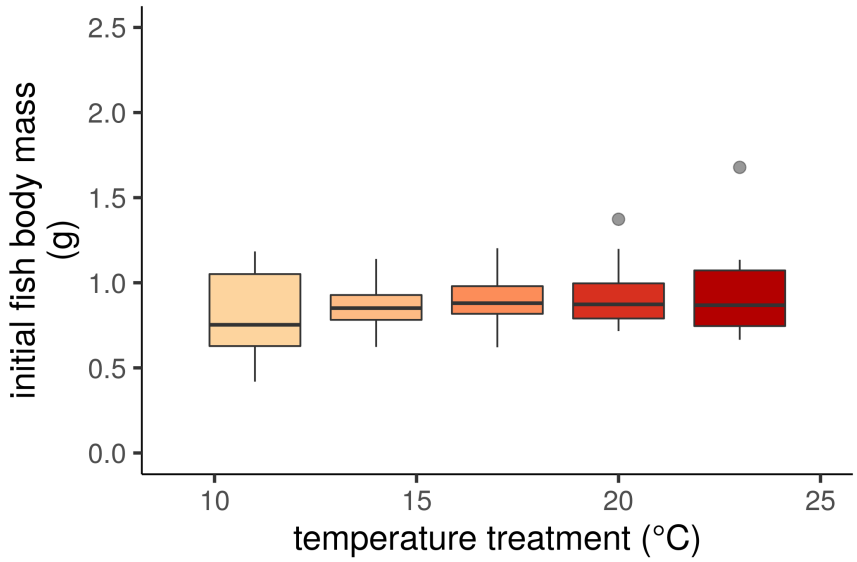


FIGURE 3.13: Individual stickleback body mass at the start of the experimental period for each temperature treatment in grams. Fish grew up under similar conditions and were randomly allocated to treatments and should thus not differ in body mass. Mass did not differ between treatments.

Survival after 4 months was not significantly different between temperature treatments (χ^2 = 6.344, df = 4, P = 0.1748). However, it should be noted that the number of fish that died in the 23 °C treatment was more than as twice as high as in the other treatments (10 out of 15 at 23 °C and 2-4 in the other treatments).

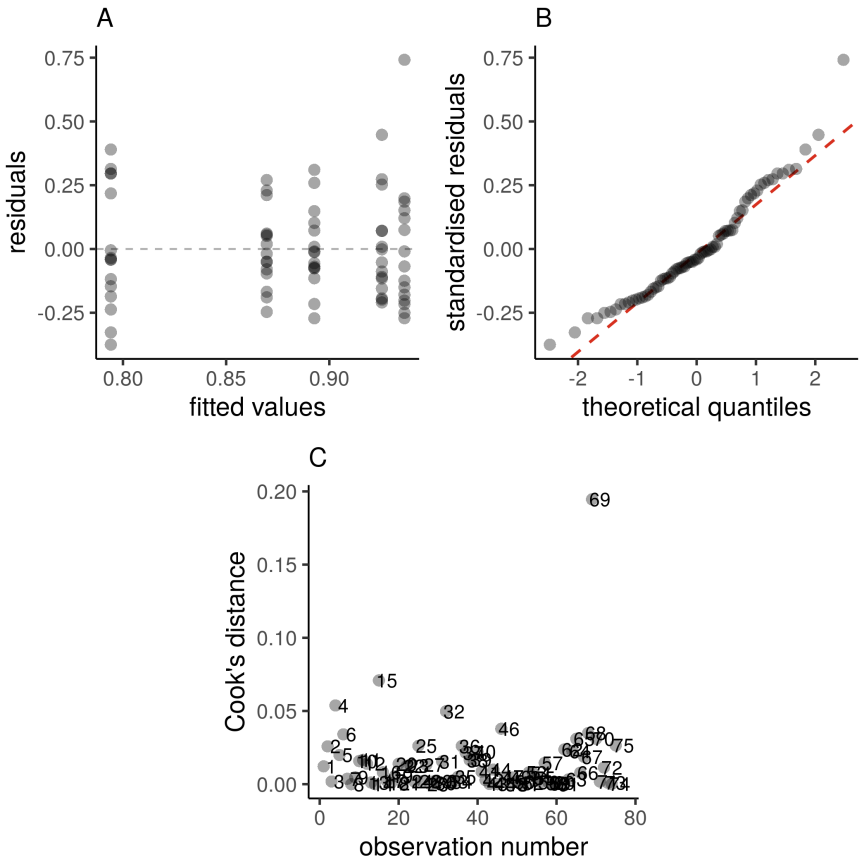


FIGURE 3.14: Diagnostic plots for fish body mass at the start of the experimental period. See Figure 3.13 for model output.

After 4 months of temperature exposure there was no linear effect of temperature on mean fish body mass (LM; n = 51, df = 49, t = 0.407, P = 0.6861; Figure 3.15). The median value was the lowest at 23 °C (902 mg) and the highest for 20 °C (1276 mg).

Visual inspection of model diagnostics and the relation between temperature and fish body mass, indicated that the relationship might be better explained by a quadratic function of temperature. Addition of the quadratic term did not reveal a significant effect of temperature (LM; n = 51, df = 48, t_{linear} = 0.417, P_{linear} = 0.6785; $t_{quadratic}$ = -1.90, $P_{quadratic}$ = 0.0645). For both models, diagnostics showed that the model assumptions were not violated (Figure 3.16 & 3.17).

After exclusion of sticklebacks that had fewer than three data points, the growth rate for 74 individuals was calculated for the analysis of fish growth rate. Here, there was a significant, linear decrease in growth rate (expressed as change in body mass)

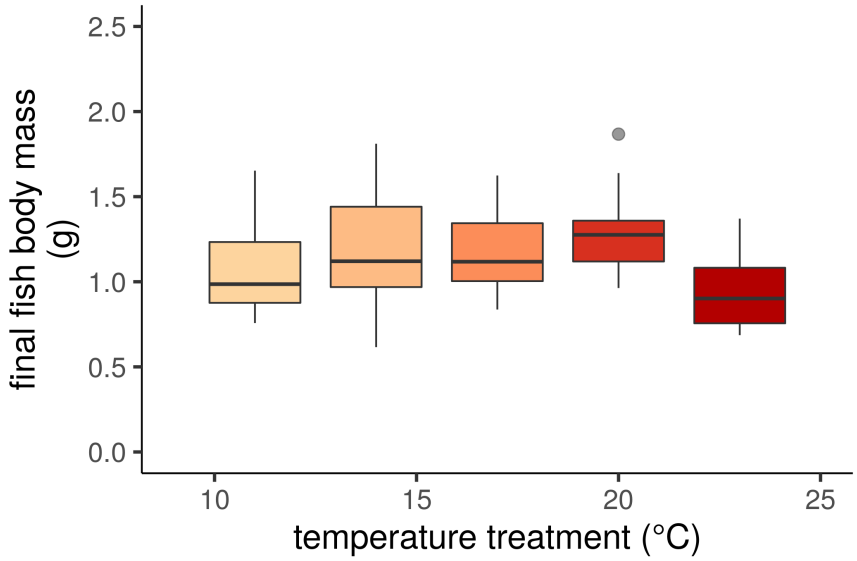


FIGURE 3.15: Fish body mass after four months of growth at different experimental temperatures, in grams. There was no overall effect of temperature on fish body mass at different temperature treatments.

with an increase in temperature (LM: t = -2.913, df = 72, P = 0.0048). This effect was so strong that at the highest temperature, some fish did not gain mass over the experiment (Figure 3.18).

Model diagnostics showed that one extreme data point at 11 °had a disproportionate impact on model parameter estimates (Figure 3.19). This point was thus removed and from this adjusted model the relationship between temperature and individual fish growth was estimated. There was still a significant negative effect of temperature on fish growth (LM: t = -2.471, df = 71, P = 0.0159) that could be described as:

$$\Delta M_F = 7.25 - (0.40 \times T) + \epsilon. \tag{3.7}$$

Where G is the daily growth in mg of the sticklebacks. The median daily growth rate was highest at 11 °C (1.97 mg/day) and lowest at 23 °(– 0.22 mg/day).

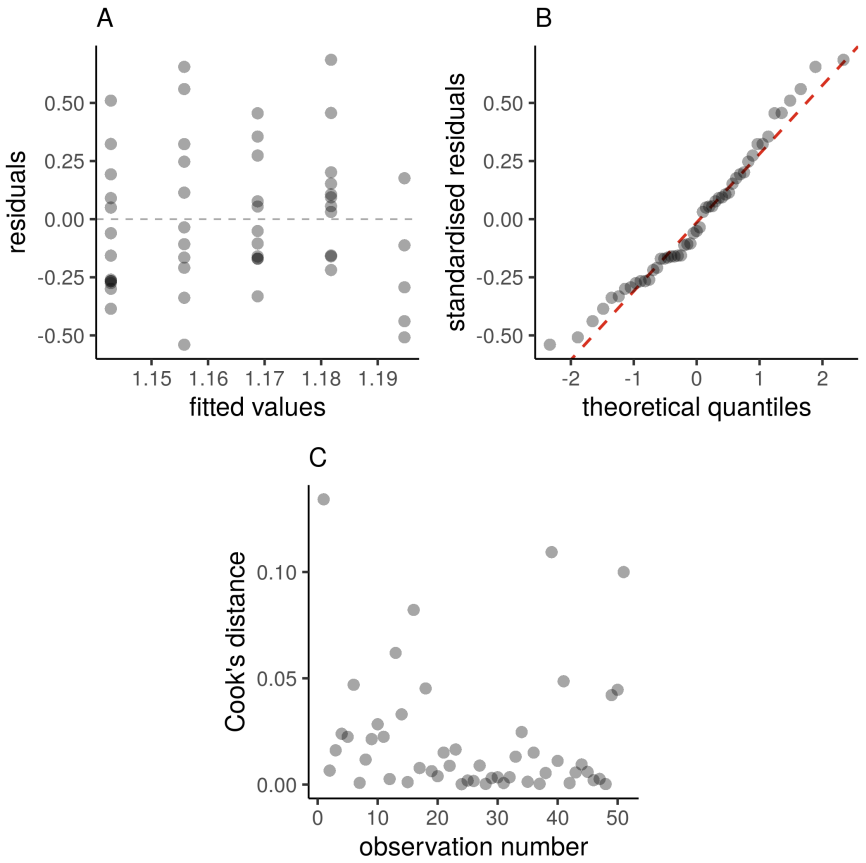


FIGURE 3.16: Diagnostic plots for fish body mass at the end of the experimental period. In plot C there is a large range of Cook's distance values, but none of them warranting further inspection. See Figure 3.15 for model output.

3.3.2.2 Stickleback health during development

As data for 3 fish were available for the splenosomatic index at 23 °C, this temperature was excluded for this analysis. The size of the spleen relative to body size — an indicator of health in fish, specifically immune response — increased with warmer temperatures in a linear fashion (LM: t = 2.560, df = 41, P = 0.0142; Figure 3.20).

Splenosomatic index was approximately 50% higher at 20 °C (0.11) compared to 11 °C (0.07), meaning that for similar sized fish the spleen size was one-and-a-half times the size. At 17 °C the splenosomatic index was even larger (0.13), almost twice as large as at 11 °C. Model assumptions were not violated (Figure 3.22).

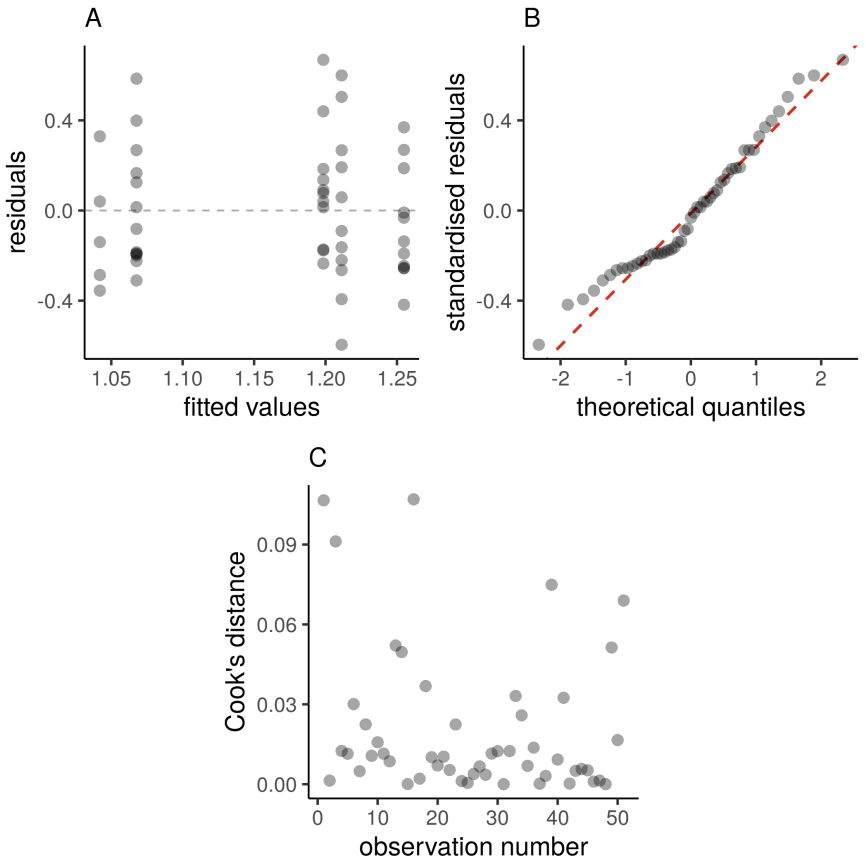


FIGURE 3.17: Diagnostic plots for fish body mass at the end of the experimental period with temperature included as a quadratic predictor. Plots indicate that model assumptions are met, i.e. equal spread of y-values in plot A, reasonably good fit to diagonal in plot B and none of the y-values in plot C are much more extreme than the others. See Figure 3.15 for model output.

3.4 Discussion

3.4.1 *Schistocephalus solidus* egg hatching

Out of the three parameters studied here on *Schistocephalus solidus* egg hatching, both onset of hatching (Figure 3.7) and the length of the hatching window (Figure 3.9) were affected by temperature. On the other hand, the total proportion of eggs hatched was not affected (Figure 3.11).

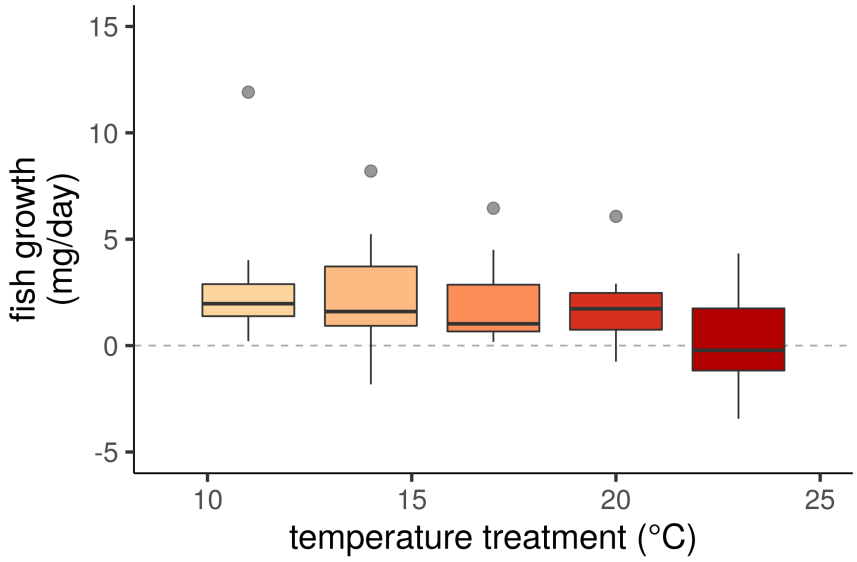


FIGURE 3.18: Stickleback average daily growth rate over the four months experimental period at different temperature treatments. Growth is given as mass increase in milligrams per day. Growth was significantly more at cooler temperatures and at extreme warm temperatures some fish showed a negative growth rate, i.e. a net weight loss. This could be due to high metabolic rates at extreme high temperatures.

3.4.1.1 Onset until hatching

The onset of *Schistocephalus solidus* egg hatching was negatively affected by temperature (Figure 3.7). This means that at warmer temperatures eggs hatch sooner. The shortened period until hatching from incubation, is in line with expectations of temperature effects on development. It is expected that with increased temperatures, developmental rate of ectotherm organisms increases and consequently their developmental time decreases (Smith, 1957). This is seen in the eggs of both the parasitic nematode *Pseudoterranova decipiens* and the digenean parasite *Plagiorchis elegans*. There the eggs develop faster at warmer temperatures and do not develop at all at low (below 4°C) temperatures (Measures, 1996; Zakikhani & Rau, 1998).

Generally, the results also match described developmental times of 10 to 12 days at higher temperatures (22-25°C), and 17-19 days at intermediate temperatures (16-18°C) described in the literature (Dubinina, 1980). Here, at 23°C development took on average 9 days, at 14 and 20°C it took 12 days and at 17°C it took 15 days.

The largest difference in egg developmental time was between 11°C and the other

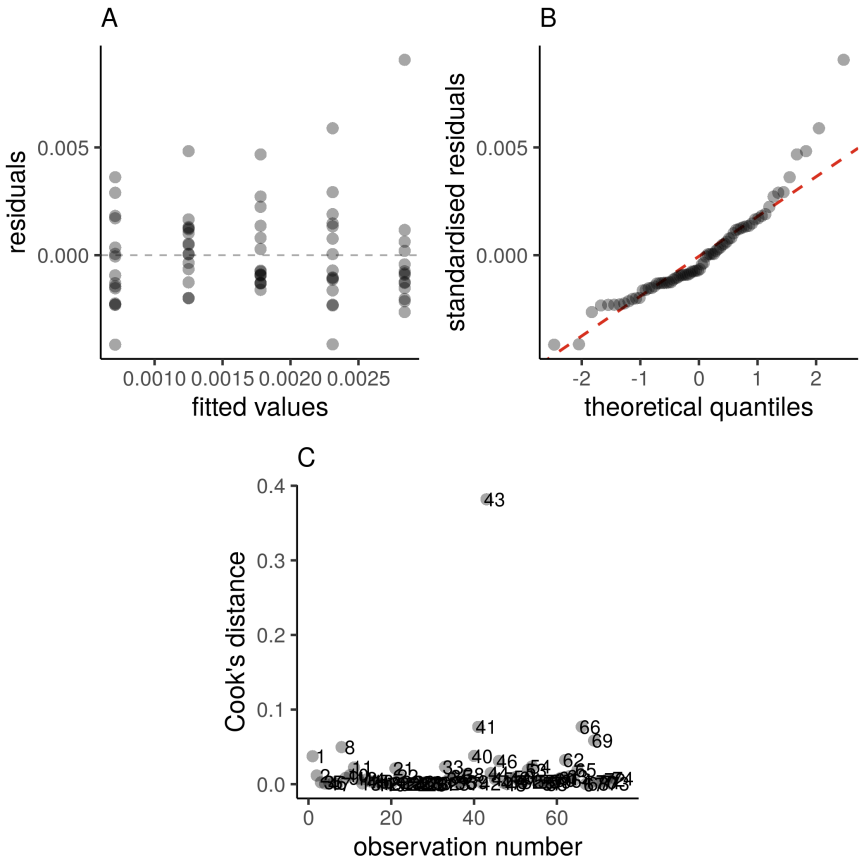


FIGURE 3.19: Diagnostic plot for analysis of temperature effects on stickleback daily growth rate (see Figure 3.18). Inspection of the Cook's distances (C) led to exclusion of one data point from the data analysis as this individual data point substantially shifted model parameters.

temperatures. It is currently unclear, whether there is a linear decrease in the median number of days until onset of hatching between temperatures, or that onset of hatching is later only at 11 °C (Figure 3.7) To untangle this and determine whether the response of *S. solidus* egg developmental time to temperature is linear (i.e. the observed value for 14 °C is lower than in the population), or shows a non-linear response (i.e. developmental time in disproportionately affected at low temperature compared to higher temperatures), studies with higher resolution are needed. For example, tracking individual eggs may reduce the within-temperature treatment variation that is currently observed. Here some of this variation is caused by the potential counting of a different subset of the *S. solidus* eggs present in the sample. Although care was taken on each experimental day to count all eggs, some eggs will have been missed

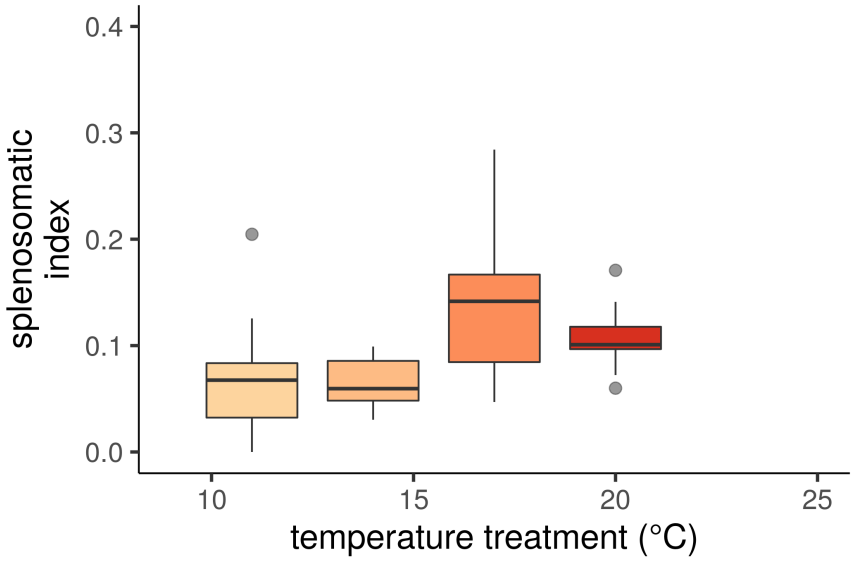


FIGURE 3.20: Splenosomatic index for three-spined sticklebacks exposed to different temperature treatments during four months of growth. Splenosomatic index is the relative spleen size compared to body mass and can be used as an indicator of stress in fish. Larger splenosomatic index indicate higher levels of spleen activity and stress levels. There was a significant positive effect of temperature on splenosomatic index.

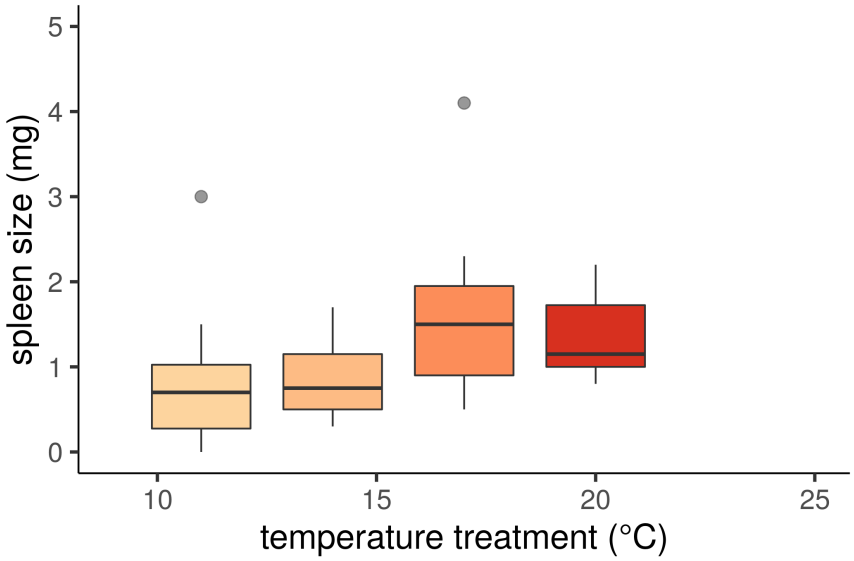


FIGURE 3.21: Absolute spleen size for fish at the different temperature treatments.

as could also be seen from the decreasing number of eggs observed over the course of the experiment. This is due to eggs ending up at the margins of the Petri dish and

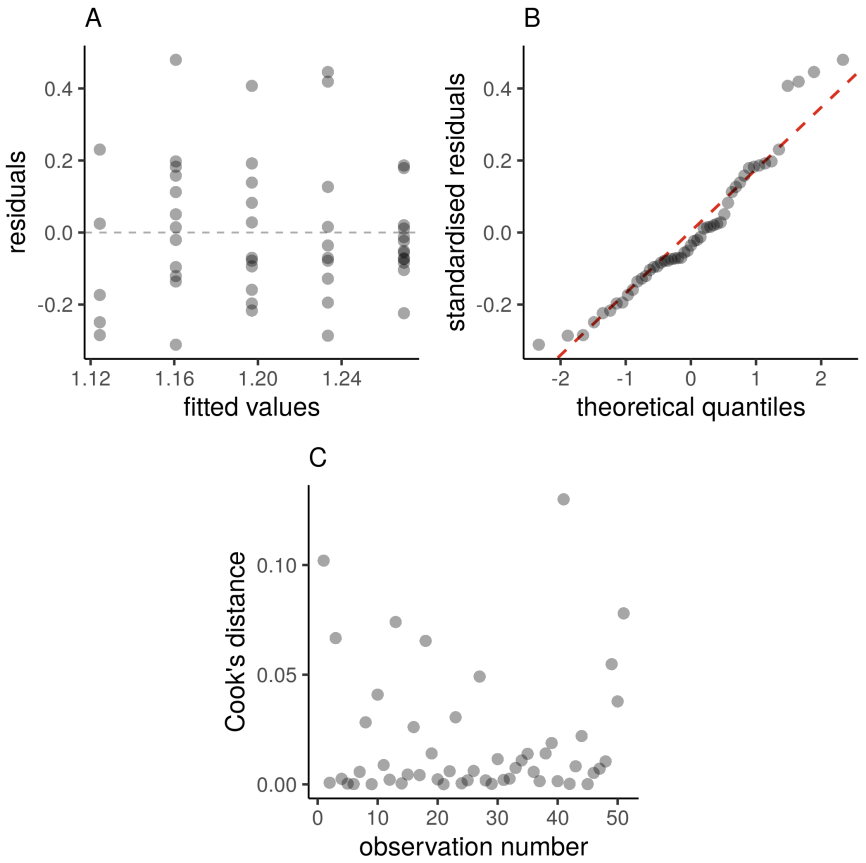


FIGURE 3.22: Diagnostic plot for the analysis of temperature effects on stickleback splenosomatic index (see Figure 3.20). Note that only four temperature treatments are included as the 23 °C had a small sample size (n = 3). Model assumptions were met.

making it impossible to count them.

3.4.1.2 Proportion of eggs hatching

The proportion of parasite eggs that hatch is an important factor in determining the number of infective stages that are available to infect future hosts. Here it was found that the hatching of *S. solidus* eggs stopped after 34 days for all temperature treatments, as the slopes of the cumulative number of hatched eggs was zero (Table 3.1). This means that overall, temperature does not have an effect on the number of parasite eggs that hatch (Figure 3.11), and thus the number of infective stages (i.e. coracidia; Figure 1.2) in the environment.

The survival of free-living, non-feeding stages of some other parasite species has also been found not to be affected by temperature (e.g. Fried & Ponder, 2003). However, these studies typically have looked at the survival time after hatching of non-feeding, free-living parasite stages. Work on *S. solidus* coracidia, has shown that coracidial survival is temperature dependent (Ismail, 2018) and thus in line with other systems. It was found that the different parasite families showed different responses to temperature change and thus parasite family was included as a random effect in the model. Inclusion as a random effect allows for controlling for variance between families without calculating its direct impact as the focus here was on the effect of temperature on the proportion of hatched eggs in the population. Difference in response between parasite families indicate that some genotypes in the population might do better in changing environments (Berkhout, Lloyd, Poulin, & Studer, 2014). Variable responses to changing environments within a population allow natural selection to take place for fitter phenotypes and thus that there is the potential for the population as a whole to adapt.

The method to quantify *S. solidus* egg hatching that was used, is a relatively crude method as it does not track individual eggs. It did however show similar hatching proportions as previous studies when worms are outcrossed (Benesh et al., 2014; Christen, Kurtz, & Milinski, 2002; Simmonds & Barber, 2016; Weinreich, Kalbe, & Benesh, 2014), of 40% to 50% and is thus considered a reliable method of assessing egg hatching. The repeated counting of samples in the lab is likely to cause some stress to the eggs as they are taken out of the incubator into the lab where experimental temperatures can not be matched, but the overall hatching proportion indicates that this most likely not affect the hatching of the eggs. It also seems unlikely that this would make the hatching more similar between the treatments as the relatively short time needed to count the samples (less than 1 hour per plate with samples) is unlikely to have a significant impact on development, compared to the 72 hours between the counting of samples.

3.4.1.3 Hatching window

The length of the hatching window (i.e. the number of days over which *S. solidus* eggs hatched) showed a clear linear relation with temperature (Figure 3.9). This was

caused by two factors, the onset of hatching was earlier at warmer temperatures, while hatching stopped at the same time as at lower temperatures. This meant that the total number of eggs hatching did not change, but that the number of hatching eggs per day did change. This result meant that the number of coracidia (i.e. the infective, free-living stages emerging from the eggs) in the environment was lower on average at the higher temperatures. Resulting in a change in the temporal distribution of coracidia in natural populations.

The length of the hatching window differed between parasite families. This means that differences in the response to temperature between families, could underlie the observation that hatching stopped at a similar time after incubation at all temperatures. If the parasite eggs of some families show a positive response in developmental rate to temperature, but not all, then overall hatching will stop when eggs of the 'slowest' family have stopped hatching. Thus, if some of the families show the same developmental time at warm and cold temperatures, the end of the hatching window will not change with temperature.

Because of the annual life cycle of *S. solidus* (Dubinina, 1980), some synchronisation in the presence of the different life stages in the environment is expected. Although all host species are present year round (Pennycuick, 1971c), procercoids (i.e. parasite stages in the copepod host; Figure 1.2) are most infective to young sticklebacks (Heins, Eidam, & Baker, 2016) and infection rates in sticklebacks spike in early summer (Morozińska-Gogol, 2002). After infecting the sticklebacks, development takes 4 weeks or more, depending on environmental temperatures, to develop to large enough sizes to be infective to the definitive host (Barber & Svensson, 2003; Scharsack et al., 2007). Once consumed by a bird definitive host, the worm produces eggs for a short period of time (Smyth, 1946). Because development of plerocercoids is likely to not only be dependent on temperature but also other environmental factors, like food availability (Barber, Wright, Arnott, & Wootton, 2008), differences in the ability to compete for food in sticklebacks will lead to differences in the developmental rate of *S. solidus* plerocercoids. Consequently this will affect the developmental time until they become infective to the definitive bird host. This is likely to lead to a more or less continuous, but decreasing, supply of infective parasites and from these a flow of

eggs into the environment. Changes in mean temperature which lead to differences in hatching window length are thus not expected to substantially affect the number of coracidia in the environment. On the other hand, if temperature would rise to levels where stickleback hosts could breed year round this would completely change these dynamics (Fenton, Hakalahti, Bandilla, & Valtonen, 2006).

3.4.1.4 Overall effects on *Schistocephalus solidus* egg development

The main effect of temperature on *Schistocephalus solidus* egg development appears to be on the onset of hatching and consequently on the length of the hatching window. The total number of hatching eggs and the number of days after incubation that hatching ends do not change.

As discussed in the previous section, this could lead to a larger spread in the emergence and presence of coracidia emanating from a point-source input (i.e. a bird dropping) in the environment. Given that *Schistocephalus solidus* can reach high levels of prevalence in the fish population that are infected (e.g. MacColl, 2009), it is expected that on a population level the number of infective stages will not change dramatically. Another factor of importance, that may have a larger impact on the number of coracidia present in the environment, is the survival of individual coracidia at different temperatures after they emerge from the eggs. Previous work has shown that at warmer temperatures survival time of free-living stages is decreased (e.g. Ford, Nollen, & Romano, 1998; Pechenik & Fried, 1995), including that of *S. solidus* coracidia (Ismail, 2018). Coracidia hatched from eggs that had developed at 20 °C (i.e. a temperature similar to the highest experimental temperature), on average, only survived half as long when kept at 20 °C than those kept at 10 °C. This indicates that there was no thermal acclimation during this short period of time (Schulte, Healy, & Fangue, 2011) which could help the parasite to phenotypically adapt to changes in temperature over short time scales.

This decrease in survival, could result in lower numbers of infective coracidia and thus a lower exposure intensity of copepods per unit time. Lower exposure rate could mean that copepods might be more successful in resisting infections (Wedekind, 1997) and reduce mortality (Ashworth, Kennedy, and Blanc 1996; Nie and Kennedy 1993,

but see Beintema 1997). However, it is expected that low infection rates of copepods will not disrupt the *S. solidus* life cycle, as stickleback appear to preferentially feeding on more active prey (Wedekind & Milinski, 1996) and infected copepods show increased activity (Urdal et al., 1995; Wedekind & Milinski, 1996). Hence, even when only a small number of copepods is infected, they will be more likely to be consumed by stickleback hosts and thus to transmit the parasite.

However, if local parasite populations go extinct it appears unlikely that they will re-establish because of the populations structure that can be seen in *S. solidus* in Alaska, United States (Sprehn, Blum, Quinn, & Heins, 2015). This structuring of the population means that there is little dispersion between waterbodies of the parasite, even though the bird hosts have the ability to move between the water bodies. As the adult *S. solidus* worms only survive and produce eggs in these hosts for up to two weeks (Dubinina, 1980; Tierney & Crompton, 1992), population structure could be caused by philopatry in bird feeding (Giles, 1981), i.e. individual birds prefer feeding in the same location on, at least, a weekly time scale. This would result in depositing of *S. solidus* eggs, with bird faeces, in the same water body as where the worms were acquired. This would mean that birds do not regularly spread parasites between water bodies and thus limit the potential for the parasite to establish in water bodies where it not yet occurs, or re-establishment in water bodies where local extinction has occurred.

3.4.1.5 Effects on the parasite life cycle

Implications of changes in hatching patterns of *Schistocephalus solidus* eggs for parasite prevalence and intensity will depend on the response of other biological factors, such as host immunity, to changes in temperature. Three general scenarios could be imagined if it is assumed that the number of coracidia hatching from eggs stays equal, but their survival is decreased at higher temperatures. The immune response of the first intermediate host could be enhanced by the increase in temperature (Morley & Lewis, 2014), or the infectivity of the coracidia could be decreased. This would lead to lower infection rates in the copepod host and would thus likely decrease the population size of *S. solidus* further downstream its life cycle. So either an increase in host immunity or

decrease of parasite infectivity, or a combination of these two, could lead to a decrease in parasite population size, with the potential of local extinction (Bush & Kennedy, 1994). Conversely, increased temperature could lead to an increase in the metabolic demand of the host. The increased metabolic rate could either lead to a change in feeding, potentially making the copepods more likely to ingest infective stages of the parasite through less selective feeding, or an inability to compensate for the increase in metabolic demands leading to a less effective immune response (Iles, 2014). Either of these could lead to an increase in the number of copepods infected with *S. solidus*, or to a higher number of infections in infected hosts. Both of these scenarios could lead to a decrease in the copepod population size, with potential cascading negative effects on *S. solidus* populations. The potential increased mortality caused by an increase in infection rates in the host could lead to selection on the parasite for lower virulence of the parasite (Thrall & Burdon, 2003), thus alleviating some of the negative effects.A final scenario is one which is a combination of the first two, where the overall parasite prevalence and intensity remains largely unchanged or would show behaviours that could only be predicted with numerical modelling of the system.

To gain a better understanding of the overall effect of temperature change on the hatching and infectivity of *S. solidus* eggs, and the subsequent infectivity of the coracidia to the copepod hosts, more information on the impact of temperature on the survival and immune response of copepods is needed. It would also be useful to study the interaction between *S. solidus* and the copepod host *in vivo*, e.g. in microcosm experiments. A combination of experiments on single steps in the life cycle combined with larger microcosm experiments could give insights into both the overall effects of temperature on host-parasite interactions and their underlying mechanisms.

3.4.2 *Schistocephalus solidus* development inside young sticklebacks

The initial aim of this study was to infect young three-spined sticklebacks, *Gasterosteus aculeatus*, with *Schistocephalus solids* procercoids and observe the growth of both hosts and parasites over a period of 3 months at different temperature treatments. Because problems were encountered with parasite culture and subsequent infections of the first intermediate copepod (*Cyclops strenuus*) hosts, fish were not exposed to infected

copepods and thus not infected. Instead, fish development at different temperature treatments was studied.

Briefly, experimental copepod infections were unsuccessful for two reasons. Firstly, some of the worms that were incubated did not produce viable eggs. Secondly, infections of copepods themselves were unsuccessful. Both these problems likely had the same underlying cause. Plerocercoids were collected for parasite culture (see Chapter 2) from wild sticklebacks that had been kept in the lab for more than a year, worms used in the egg hatching experiment had only been kept in the lab for up to six months. These fish were originally collected in December and at the point of parasite culture both the fish and parasites were two or three years old. Initially the age of *S. solidus* is positively correlated with egg production as the worms increase in size with time and larger worms produce more eggs (Benesh, Weinreich, & Kalbe, 2012; Dörücü et al., 2007; Wedekind et al., 1998). However, for worms that are older, egg output and especially viability appear to decrease (personal observations). Although this could potentially be attributed the very large size of some plerocercoids and fouling of the medium by the large amount of metabolic products released into the medium. If this is the case it would be possible to circumvent this by changing the medium of the parasite culture (Schjørring & Jäger, 2007). In the field it seems likely that natural infections are transmitted to the bird definitive host within a year in the majority of cases. This would mean there is no selective pressure to retain the ability to produce viable eggs after the first year. The usage of these older infections could have led to a reduced number of eggs produced compared to the egg hatching experiment, as well as low viability of the eggs. Consequently resulting in low numbers of infected copepods.

3.4.3 Effect of temperature on young sticklebacks

3.4.3.1 Growth of young sticklebacks

Although no clear effect of temperature on body mass of sticklebacks was found, the growth rate of individual fish was negatively affected by temperature. The apparent discrepancy between these results can be attributed to the difference in information included in the analysis. In the body mass analysis, only one data point for each fish is included. This ignores between fish differences in size at the start of the experiment.

On the other hand, the growth rate data include regular measurements on the fish and for each fish a linear model of growth is fitted. This captures individual growth rates over the whole experimental period and also accounts for individual differences at the start of the experiment. The analysis is therefore better suited to isolate temperature treatment effects and has higher statistical power.

The overall reduced growth at warmer temperatures, agrees with previous work that used two temperature treatments (Macnab & Barber, 2012) that found that at 20 °C the specific growth rate of sticklebacks was lower than at 15 °C, regardless of whether sticklebacks were infected with *Schistocephalus solidus* parasites or not. These differences can even persist into the next generation (Shama, Strobel, Mark, & Wegner, 2014).

However, when comparing to studies that used a range of temperature treatments (Allen & Wootton, 1982; Hovel et al., 2015; Ramler et al., 2014), the current results for growth rate do not match. Here the peak in growth is not seen at intermediate temperatures. Studies that used a range of experimental temperatures covering a large thermal spectrum found a non-linear response to temperature (Hovel et al., 2015; Ramler et al., 2014). Here a similar pattern is seen (Figure 3.18), i.e. a slight increase in size with warmer temperatures but a drop past the optimal temperature (i.e. around 20 °C in this study). The inability to detect statistical significance might be due to the *ad libitum* feeding and the age of the fish, which may have reduced the effect size. At higher temperatures the efficiency of food conversion into body mass is lower (Guderley & Leroy, 2001), but the feeding regime used here may have obscured this effect. Hovel et al. (2015) estimated growth of fish at different temperature treatments based on respiration and consumption rates, and Ramler et al. (2014) observed growth of sticklebacks for the first three months of their lives. The use of such young fish might produce stronger effects than using slightly older fish like it was the case here. Older fish might have an overall slower growth rate and thus differences due to experimental treatments may be less pronounced (Haddon, 2011).

Although warmer temperatures can lead to increased growth rates in ectotherms (Zuo, Moses, West, Hou, & Brown, 2012), overall they often result in smaller sizes in fish (Baudron, Needle, Rijnsdorp, & Marshall, 2014; Motson & Donelson, 2017). This is

in line with the current results. Additionally, Guderley and Leroy (2001) showed that in three-spined sticklebacks acclimatized to extreme temperature treatments (8 °C and 23 °C) the response in growth rate to temperature depends on fish family. Here, the effect of fish family was not tested as the number of replicates for each family in each treatment was too low. Stickleback family was however included as a random effect to account for its potential effects on the outcome.

3.4.3.2 Stickleback stress response during development

Warmer temperature led to larger spleens (Figure 3.21) and consequently a higher splenosomatic index in sticklebacks (Figure 3.20). An increase in splenosomatic index indicates higher stress levels (Handy et al., 2002). The increased stress levels in fish at warmer temperatures could be due to several factors. First, at higher temperatures the solubility of oxygen in water decreases. To overcome this reduction in available oxygen animals can produce more red blood cells (erythrocytes) which allows them to still take up similar levels of oxygen at lower oxygen saturation levels (Piersma & Van Gils, 2010). Although not the main hematopoietic organ, the spleen is involved in the production of red blood cells in fish (Katakura et al., 2015; Maekawa & Kato, 2015) and thus lower oxygen saturation could lead to a larger spleen if fish start to produce more red blood cells. Unfortunately, dissolved oxygen was not measured in the experiment and this hypothesis could not be tested. Second, warmer temperatures could lead to up-regulation of the immune system, either because pathogen growth benefits from the temperature increase, or because of linkage of the immune response to temperature through metabolism. If the growth of pathogenic microbes benefits from increased temperature, because of faster duplication rates, this might lead to an increased challenge to the immune system of the fish. Then it could be expected that the immune response is up-regulated and organs involved in the immune response, like the spleen and kidney (Press & Evensen, 1999), are enlarged in fish kept at higher temperatures. On the other hand, if the immune response is directly linked to temperature through the base metabolism, then organs related to the immune response are also expected to be enlarged at warmer temperatures. In either scenario the increased

activity of the immune system will be an energy drain thus leading to less available energy for other processes like growth. As splenosomatic index increases, while growth rate decreases at higher temperatures it seems likely that the increased spleen sizes (Figure 3.21) constitutes an energy drain for the fish reducing the resources available for growth.

Increased temperatures had a positive effect on splenosomatic index (Figure 3.21). Previous work did not find such an effect (Macnab & Barber, 2012). The number of fish used in the analysis is similar between the current work (n = 42) and Macnab and Barber (2012) (n = 40; at two temperatures 15 and 20 °). Thus sample size can not explain the difference in finding. However, here a larger range of temperatures was used potentially resulting an increase in experimental power to detect relatively small differences. Most other studies on temperature effects do not report the effects on spleen size (e.g. Franke et al., 2017; Guderley & Leroy, 2001; Ramler et al., 2014; Vézina & Guderley, 1991) and a comparison is thus not possible. Alternatively, if the increased splenosomatic index found in the here is due to an interaction effect of temperature and the experimental set up this would also explain the discrepancy between the results. One such factor could be the ways the fish were housed in the current study. Here fish were housed individually, while the set up allowed for visual contact as well as the sharing of chemical cues as the water between fish tanks was shared and recirculated (for more detail see Chapter 2). Housing the fish in this manner could have led to increased stress levels. However, these effects are expected to be similar for all temperature treatments. The observed difference might thus reflect the net effect of the two stressors (i.e. temperature and housing conditions). This highlights the importance of studying temperature in isolation as well as in combination, as simply combining the results of studies on single stressors may not lead to the same conclusions as studying these in concert. Here, the experiment was designed this way to be able to track individual fish while keeping them in an as natural as possible set up. In future experiments, identification of individual fish could also be possible using molecular techniques (Breacker et al., 2017). Using this technique it would be possible to house fish together to create a more natural setting, but still track growth and development of individual fish. This technique is likely to be more labour intensive and

more expensive.

Cestode infections in fish (Seppänen et al., 2009), including sticklbacks (Kalbe et al., 2016), are known to be able to affect the relative size of fish spleens. This is most likely due to the role the spleen plays in the fish immune response (Kurtz et al., 2007; Press & Evensen, 1999). The combined effects of temperature and *S. solidus* infection on stickleback spleens have yet to be investigated, but the combined effects could worsen the overall effects on spleen sizes and become a significant energy drain for the fish. With potential negative effects on survival. Conversely, the increase in spleen size due to temperature, could also boost the immune response (Ellis et al., 2012) and reduce parasite infection success. This would mean that the combined effect is less than the sum of the two independently. This highlights the need to investigate the real-world effects of temperature on host-parasite interactions.

3.4.3.3 Overall effects on stickleback development

Both the growth rate of fish and aspects of fish health studied decreased with increasing temperatures. This indicates that changes in mean temperatures can put a significant pressure on fish populations in future. This is even without the added impact of parasite infections. Although, parasite prevalence might decrease because of the reduced survival of coracidia (Ismail, 2018), and thus reduced transmission, virulence is likely to increase (Macnab & Barber, 2012). On the other hand, the increase in spleen size at warmer temperatures means that sticklebacks could be better equipped to fend off parasite infections because of increased immune activity (Ellis et al., 2012). Together, this indicates that both the physical size of fish, due to increased energy drains from parasites and temperature, and their population sizes, due to increased mortality, might decrease if environmental temperatures increase. If temperature changes are large enough, annual breeding cycles could disappear and continuous, year round breeding could occur (Hakalahti et al., 2006) which could have far reaching effects on ecosystems. Additionally, at warmer temperatures oxygen saturation levels of water are lower and this can cause ventilation rates in fish (Burton, 1979), in turn increasing parasite exposure with potential increases of parasite co-infections (Mikheev et al., 2014).

3.4.4 Conclusions

The current study shows that developmental time of *Schistocephalus solidus* eggs until hatching is reduced at warmer temperatures (Objective 3.1a) and that this leads to a longer hatching window (Objective 3.1c) as hatching still ceases around the same time. However, there appears to be no effect of temperature on the total proportion of *S. solidus* eggs that hatch (Objective 3.1b). No effect of temperature on fish size was seen, but there was an effect on growth rate, indicating that when acclimatised temperature only has a mild effect on fish growth (Objective 3.2c). There was a clear increase in the splenosomatic index of fish at warmer temperatures, this shows that physological stress is increased by temperature (Objective 3.2d). Unfortunately, the impact of temperature on the growth (Objective 3.2a) and fitness (Objective 3.2b) of *Schistocephalus solidus* in the stickleback host could not be assessed.

Chapter 4

Parasite transmission

4.1 Introduction

Regardless of whether a parasite has a direct or a complex life cycle, transmission to other host individuals is an essential part of their life cycle. Parasites with a direct life cycle theoretically only have to find a host once in their life, but may switch hosts more often when less favourable conditions occur (Connors, Lagasse, & Dill, 2011). On the other hand, parasite species with complex life cycles host finding is an essential step at different stages of the life cycle before reproduction.

Although parasites are expected to have evolved to maximise the probability of transmission, transmission from one host to the next can still confer several problems. First, timing is important (Hammerschmidt et al., 2009; Parker, Chubb, Roberts, Michaud, & Milinski, 2003), the parasite will have to grow inside the current host to become infective to the next host (Tierney & Crompton, 1992). At the same time, longer developmental times increase the risk of the current host dying or being predated upon (Hammerschmidt et al., 2009). Second, transmission of parasites through free-living stages can only be successful if suitable hosts are nearby and the parasite can find them swiftly (Haas et al., 1995), as free-living stages only have a limited energy budget and survival time (Boyunaga, Schmitz, Brouwers, Van Hellemond, & Tielens, 2001). One way around this problem is through trophic transmission, where the current host is consumed by the next host.

Several parasite species can manipulate their hosts, for example through changing the way the host looks or behaves (Bakker, Mazzi, & Zala, 1997). This parasitic manipulation increases the likelihood of trophic transmission (Bakker, Frommen, &

Thünken, 2017) and can even target specific, suitable hosts (Thünken, Baldauf, Bersau, Frommen, & Bakker, 2019).

Third, most host species have evolved mechanisms to prevent parasite infections, such as behavioural avoidance of infected food sources (Hart, 1997), and immune responses against parasites (Marcogliese, 2008). Parasites must thus evolve strategies to overcome this in the continuous arms race between hosts and parasites. Additionally, temperature can play an important role during all these stages by affecting developmental rates, spatiotemporal relationships of hosts and parasites, and impacting host immune systems. Understanding how each of these individually and all of them combined are affected by temperature is one of the current challenges in parasite ecology.

4.1.1 Exposure risk

After sexual (e.g. cestodes and nematodes Dubinina, 1980; Goater et al., 2013) or asexual (e.g. trematodes Goater et al., 2013) multiplication, many parasite species release free-living, motile infective parasite stages, either directly or following the release of developmental eggs. These infective stages must find and infect a susceptible host, either by being ingested by the host and penetrating the gut wall (e.g. the cercaria of cestodes Dubinina, 1980; Goater et al., 2013), or by attaching to the host and penetrating the external tegument (e.g. the cercariae of trematodes Goater et al., 2013).

Free-living parasites that rely on being consumed by their host typically only have a limited time window for transmission (Fried & Ponder, 2003) as they rely on stored energy reserves. Through maximising exposure to potential hosts their transmission is optimised. This can, for example, be achieved through behaving and looking like food items of the potential hosts (Bakker et al., 1997). This increases the likelihood of accidental consumption by the host through being confused for food (Dubinina, 1980). At warmer temperatures free-living parasite stages potentially consume more energy per unit of time because of increased metabolism (Piersma & Van Gils, 2010), and this can lead to a reduction in survival time (Ismail, 2018).

With warming temperatures the feeding rate of hosts may be increased due to an increase in metabolic rate, especially in ecotherms (Roessig, Woodley, Cech Jr, & Hansen, 2004). This is supported by data on the copepod *Mesocyclops pehpeiensis*, for

which the maximum feeding rate is positively correlated with temperature (Sarma, Jimenez-Contreras, Fernandez, Nandini, & Garcia-Garcia, 2013). A similar response of the feeding rate to tempeature is expected in *Cyclops strenuus* and other copepod species. However, feeding rates are also dependent on prey densities, which in most copepod species follow a (Holling) type I or type II functional response (Burns & Gilbert, 1993; Holling, 1959; Jeschke, Kopp, & Tollrian, 2004). The shape of this is dependent on prey species (Wickham, 1995) and environmental variables (Paffenhöfer & Stearns, 1988). Assuming that food densities and free-living infective parasite stages stay proportionally constant (i.e. the proportion of parasite stages mixed in with the food items stays constant), a simple increase in feeding could result in an increased exposure to parasitic stages, and thus an increase in infection rates.

As parasites, by definition, constitute a energy drain for the host, increased parasite infections could lead to a further increase in feeding rates (Pasternak et al., 1995). For example, the feeding rate of *Cyclops strenuus* copepods recently infected by *Diphyllobothrium* spp. increases temporarily (Pasternak et al., 1995). This might be due to increased metabolic demands of either their immune system or the parasite. Although increases in feeding due to increased temperatures might only be ephemeral. In between host transmission, internal parasites generally go through a period of growth during which they are not infective to the next host. To minimise the risk of premature mortality of the hosts, parasites are expected to reduce host exposure through behavioural manipulation to predators during this period and thus maximising parasite fitness (Hammerschmidt et al., 2009). Furthermore, under warming environmental temperatures, growth rates of parasites can be increased, making the burden on the host even larger (Macnab & Barber, 2012).

Changes in temperature could alter host exposure to infective parasite stages. Host exposure is determined by the the interactions between hosts and parasites. Factors such as host behaviour, parasite behaviour, and dilution effects play key roles in determining the outcome of the interactions.

4.1.1.1 Host behaviour

A change in host behaviour under warming temperatures could be through a change in microhabitat usage, either in general or during feeding (Henshall, Sadler, Hannah, & Bates, 2011; Macnab & Barber, 2012). This can either be in response to changes in food availability (Henshall et al., 2011) or because of optimisation of the balance between metabolism and feeding rates. If the behaviour of free-living parasite stages is not altered accordingly this could lead to a reduction in the exposure to parasites, and thus a temporary parasite release (Prenter, MacNeil, Dick, & Dunn, 2004). However, it could also lead to exposure to new parasite species and thereupon new host-parasite interactions. In the case of parasites acquiring new host species, successful establishment within that host and, for parasites with complex life cycles, transmission to the next life stage are required.

For many species, the timing of behaviour and life history events is dependent upon environmental cues, such as thermal regimes (Jonzén et al., 2006) or photoperiod (Coppack, Pulido, Czisch, Auer, & Berthold, 2003; Shostak & Esch, 1990). Changes in temperature regime could thus impact the feeding regime of host species. Feeding regime could be specifically impacted in crustacean intermediate hosts, which often show diurnal patterns in behaviour (Ringelberg, 1991). Feeding could shift to other parts of the day if temperatures become more extreme (Ludovisi et al., 2008) and mortality is increased during the warmest parts of the day (Studer, Thieltges, & Poulin, 2010). For parasite species that rely on environmental cues, other than temperature, to time their emergence (e.g. *Schistocephalus solidus* coracidia use light as a cue for their emergence from their eggs; Scharsack et al., 2007) a mismatch between parasite timing and hosts presence could develop (Hakalahti et al., 2006). This mismatch could result in disruption of the parasite life cycle. Furthermore, more extreme scenarios of temperature change suggest that species distributions will change on a macro ecological scale (Beaugrand, Edwards, Raybaud, Goberville, & Kirby, 2015) thus potentially futher upsetting the availability of suitable hosts in space. However, there is some evidence that infective stages can also adjust the time of their emergence based on environmental factors (Shostak & Esch, 1990).

4.1.1.2 Parasite behaviour

Similar to ectotherm host metabolism, free-living parasite metabolism may be affected by changes in temperature. This could have detrimental effects as, like egg-bound embryos (see Section 3.4.1), they are lecithotrophic and contain a finite amount of energy to sustain life and fuel locomotion. At warmer temperatures, levels of activity of free-living stages typically increase (Koprivnikar et al., 2010), and thus their energy stores are depleted more rapidly (Pechenik & Fried, 1995).

Thus, the longevity of the free-living stages could be reduced with increases in temperature (e.g. Koprivnikar et al., 2010; Nollen et al., 1979; Pietrock & Marcogliese, 2003), and consequently their opportunities to infect a suitable host, if host behaviour is unchanged. However, not all species show a simple linear relationship between survival and temperature. For example *Centrocestus formosanus* and *Haplorchis pumilio* cercariae show a dome-shaped response to temperature in their survival (Lo & Lee, 1996). These non-linear responses are typically only uncovered when a wide range of experimental temperatures is used. The usage of a more moderate range of temperatures, which is generally argued to be the ecologically relevant range, in other studies might explain linear responses to temperature that are typically reported (Jeschke et al., 2004).

Additionally, the host-finding behaviour of parasites with active host searching might be affected by changes in temperature. The motile, free-living infective stages of many aquatic parasites, including trematode miracidia and cercariae, have evolved adaptive behaviours to locate their potential hosts (Haas & Haberl, 1997). These parasites have evolved a range of sensory and physiological mechanisms, including phototropism (Haas et al., 2008) and chemotaxis (Haas et al., 1995) to aid host-finding. Although temperature does not appear to have a direct effect on host finding in some species (Lee et al., 2013), it is unclear what potential changes in parasite sensory physiology could mean for host finding by free-living parasites (Thieltges, Jensen, & Poulin, 2008).

4.1.2 Host susceptibility

After host-finding, the next step in the parasite life cycle is infecting the host. This involves successful penetration of either the gut or tegument of the host and establishment in a suitable tissue. This will generally elicit an immune response from the host (Press & Evensen, 1999). Some parasite species use immunosuppression (Cornet, Franceschi, Bauer, Rigaud, & Moret, 2009), while others mimic their host's tissues to avoid detection by the immune system (Hammerschmidt & Kurtz, 2005).

In ectotherm species the activity of the immune system is often correlated to the environmental temperature and thus changes in temperature affect the strength of the immune response (Dang et al., 2012). With warming temperatures, it is regularly observed that in the immune response becomes more effective and as a result it becomes more difficult for parasitic species to establish themselves. For example, in three copepod hosts (*Cyclops* spp.) the establishment of the nematode *Anguillicola crassus* is negatively correlated with temperature (Ashworth et al., 1996).

Conversely, when temperatures reach extreme values a decrease in the immune response can be seen (Seppälä & Jokela, 2011). This could be either due to a disruption of biochemical processes, or the increased energetic costs of the immune system at high temperatures and the inability to sustain these. It is thus expected that for most invertebrate, ectotherm host species the immune response follows a dome-shaped response to temperature. A similar dome-shaped response can be seen in the activity and ability to infect the host for a range of parasite species (e.g Evans, 1985; Morley, 2011; Studer et al., 2010), it is thus important to investigate the precise shape of these responses to environmental temperatures and finding the optimal temperatures for different stages of the parasite life cycle as well as for host responses.

4.1.3 Parasite development

As temperature can affect metabolic rates in ectotherms, environmental temperatures could play an important role in the rate at which larval stages of parasites with complex life cycles develop within the bodies of their hosts. Among the different groups of parasites with complex life cycles which involve a free-living stage, the trematodes

have probably been studied the most extensively. Trematodes undergo asexual multiplication in their first intermediate, gastropod host. After this the cercariae emerge from their host to search for their next host (Goater et al., 2013). Warming of environmental temperature has been shown to increase the number of cercariae produced in the intermediate host per unit time, due to an increase in their production rate and the triggering of emergence from snails by warmer temperatures (Ataev, 1991). Although generally a positive effect on cercarial production of warmer temperatures is seen (e.g. Evans, 1985; Paull, Raffel, LaFonte, & Johnson, 2015; Shostak & Esch, 1990), this does not hold true in all cases (Koprivnikar & Poulin, 2009). Also, time scale (Paull et al., 2015) and between individual variability (Berkhout et al., 2014) may play a role in the patterns observed.

In crustacean intermediate hosts, several examples are known of increase parasite development rate at warmer temperatures (e.g. Sakanari & Moser, 1985; Studer et al., 2010). The trematode *Maritrema novaezealandensis* shows a dome-shaped response of development in response to temperature in its amphipod host *Paracalliope novizealandiae*. In *M. novaezealandensis* temperatures up to about 10°C above the mean temperature experienced in the field enhance parasite development, but at higher temperatures increases in host mortality are seen and overall parasite output of infective stages and infectivity of these stages is reduced (Studer et al., 2010). In another crustacean host, the acanthocephalan *Polymorphus marilis* shows a positive response in development rate to changes in environmental temperatures (Tokeson & Holmes, 1982). However, a more restricted range of temperatures was used here and, again, if warmer temperatures had been included a dome-shaped response might have been found. Generally, an increase in developmental rate is seen at warmer temperatures (Lv et al., 2006; Tokeson & Holmes, 1982), but a drop in development is expected at extreme temperatures (Studer et al., 2010).

An additional advantage of a decrease in developmental time within the host, is that this leads to less time spent during which pre-transmission mortality of the host can occur (Poulin, 2010), thus increasing the chances of successful transmission. Yet, when increases in temperature lead to an increase in development, but not in an earlier transmission to the next host due to whatever restriction, the parasite might still

benefit. *Schistocephalus solidus* plerocercoids that develop inside the stickleback host grow faster and larger at warmer temperatures and reach infectivity to their next host earlier (Macnab & Barber, 2012). The larger worms show an increase in reproductive output in the next host (Dörücü et al., 2007), the parasite thus still benefits from the increase in growth because of warmer temperatures.

Conversely, there is some evidence that, given the opportunity, hosts can use microhabitat selection for lower temperatures to retard parasite development (Moore & Freehling, 2002). It appears likely that this behaviour increases when environmental temperatures increase.

When considering a longer time scale, a reduction in developmental time within the host could mean that parasites are able complete more life cycles per year. This is especially true for parasites that can already complete multiple life cycles per year. However, for parasites that complete one a life cycle per year (monocyclic) and rely on the presence of their hosts at specific times of the year, may experience mismatches between development and host presence. On the other hand, more extreme environmental temperatures could affect the length of the suitable season for development of hosts for the parasites and thus affect the overall output of infective stages (de Montaudouin et al., 2015).

Although previous work indicates that generally development is faster at warmer temperatures. It is unknown whether the development of *Schistocephalus solidus* inside the copepod host *Cyclops strenuus* is affected by temperature. Development and infectivity of *S. solidus* in the copepod host is easily assessed by size measurements, or observing the cercomer respectively. Although the function of the cercomer is debated — it is possibly involved in attachment in the fish host before penetration of the digestive tract — it does mark the development from coracidium into the procercoid. After formation of the cercomer the parasite can successfully transmit to the stickleback host (Smyth & McManus, 1989).

4.1.4 Aims

In order to determine whether copepod feeding changes in response to temperature, and thus the exposure risk to *Schistocephalus solidus*, the feeding rate of *Cyclops strenuus*

at different temperatures was investigated (Aim 4.1). For this feeding was assessed at different densities at different temperatures (Objective 4.1a) and functional responses were fitted (Objective 4.1b). Then, to assess the effects of temperature on the transmission of *Schistocephalus solidus* to the copepod host in a semi-natural setting, microcosm experiments were carried out (Aim 4.2). In those the survival of exposed and control copepods was tested (Objective 4.2a). Also, the infection rate of copepods (Objective 4.2b), and development of the procercoids inside the copepods (Objective 4.2c) was investigated. It was also checked that the concentration of copepod food in the microcosms was not affected by temperature (Objective 4.2d).

4.2 Methods

4.2.1 Impact of temperature on copepod feeding rate

Maximum feeding rate of *Cyclops strenuus* copepods at different temperatures was assessed to understand the potential impact on exposure to trophically transmitted, infective parasite stages. Cultures of *C. strenuus* were kept at the respective experimental temperatures (i.e. 11, 14, 17, 20, and 23 °C) for at least three weeks before the experiment to acclimatize them to their experimental temperature treatment. They were kept in the incubators described in Chapter 2. During both acclimation and experimental feeding trials, copepods were fed with the ciliate protozoan *Colpidium striatum* (henceforth 'protozoans'). Before each feeding trial protozoan concentrations ranging between 5 and 200 protozoans per millilitre were prepared. The protozoan stock was mixed by pipetting it up and down, then 500 µL of the stock was added to 24 wells (volume = 1.6 mL) on a 48 well microtiter plate. These were divided into eight triplets of experimental condition, positive and negative control. To each experimental condition one copepod (size range 250 - 390 µm) was added, while the positive control was left with just the protozoans, and the negative control was fixed with 500 µL ethanol. The negative control served as a control for the number of protozoans at the start of the experiment, while the positive control accounted for the natural increase in protozoan densities without predation. The copepods were then allowed to feed for 1 hour at their experimental temperature and the positive control was kept

under the same conditions. Afterwards, both the experimental condition and the positive control were fixed with 500 µL ethanol. All samples were allowed to settle and then counted. Mean values were taken for each density-temperature combination, as there was some stochasticity in densities. So for each experimental density all replicates were averaged, this prevented negative counts for some samples due to random variations in densities (i.e. some experimental samples had higher densities than their respective positive controls). Large variability between samples was present due to heterogeneous protozoan mixtures. These mean values were then used to fit the functional responses for each temperature treatment.

Copepod species are known to show both type I and type II functional responses (Jeschke et al., 2004), thus both were fitted to each temperature treatment. Out of these two, the functional response with the best fit was selected based on the Akaike Information Criterion (AIC) per temperature treatment. The type I response fitted was a traditional Holling type I response (Holling, 1959);

$$N_e = aN_0T. \tag{4.1}$$

Here N_e is the number of protozoans eaten, N_0 the number of protozoans at the start of the experiment, a the capture rate or area cleared (i.e. consumption rate), and T the total time available. For the type II functional response a Roger's type II decreasing prey function was used, as prey items were not replaced during the experiment and significant depletion of the resource was expected, particularly at low densities (Rogers, 1972);

$$N_e = N_0(1 - e^{a(N_eh-T)}). \tag{4.2}$$

Here the additional parameter, h, is the handling time.

The `R` package `frair` (Pritchard, 2017) was used to run these models. Equation 4.2 has N_e on both sides, a description of how this is dealt with in fitting the model see Bolker (2008).

Starting parameters for both models were set to the same values, with area cleared to $a = 1$, handling time in filter feeders is expected to be low (Jeschke et al., 2004) so

this was set to $h = 0.015$. The obtained functional responses were plotted against the data for each temperature treatment. Where possible maximum feeding rates, and protozoan densities at which satiation occurred were determined. The parameter T was required for the `frair` package, but as the time available was the same in each experiment, 1 hour, so $T = 1$ was used.

4.2.2 Microcosms

In order to assess *Cyclops strenuus* copepod survival and infectivity of coracidia to copepods at different temperatures (i.e. 11, 14, 17, and 20 °C), in a semi-natural setting, microcosm experiments were carried out. For the *Cyclops strenuus* copepod microcosm experiments the 23 °C treatment was excluded as normal activity of the copepod stops above 21 °C (Verbitsky, Grishanin, Malysheva, Medyantseva, & Verbitskaya, 2017). All the other treatments were within the range of temperatures for normal behaviour of the copepod. Initially, it was attempted to establish the microcosms at the 23°C treatment as well, but mortality levels were high and variable and longer term experiments therefore proved difficult.

Microcosms were kept in 100 mL jars, with an inner diameter of 54 mm. Each microcosms consisted of 40 mL of dH_2O and 5 mL of protozoan (*Colpidium striatum*) stock. This allowed the copepods to feed naturally. To maintain protozoan levels, 1.5 mL of protozoan stock was added every three days. This was based on pilot data on survival of the copepods (data not shown). The food stock was filtered through a 150 µm sieve to remove alfalfa (protozoan food) lumps, before adding the food stock. Each batch of the experiment consisted of three microcosms at each temperature, two of which were experimental (i.e. copepods were exposed to the parasite) and one served as a control (i.e. no parasite eggs were added; see Figure 4.1). For each batch of three microcosms it was haphazardly and independently decided which of the three served as a control.

After adding protozoan stock, in experimental microcosms 1.5 mL of *Schistocephalus solidus* egg solution was added. Egg solution was made by gently shaking egg stocks (see Chapter 3 for details on egg culturing) so that densities were homogeneous. Homogeneity was checked under a dissecting microscope before adding the eggs to the

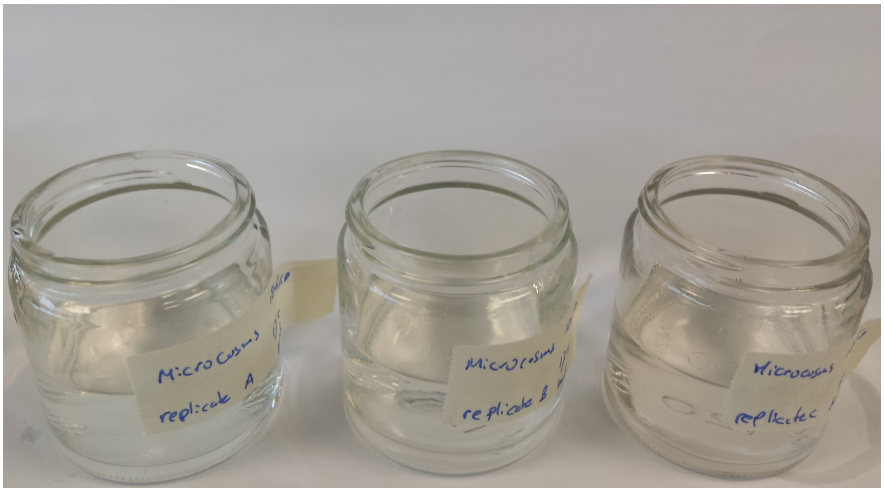


FIGURE 4.1: Example of one set of three copepod microcosms. Two experimental jars (five copepods + *Schistocephalus solidus* eggs) and one control (five copepods). Each microcosm started off with a total volume of 45 mL and was topped up with 1.5 mL of protozoan food stock every three days.

microcosms. The volume of egg stock added equated to approximately 150 *S. solidus* eggs. In control microcosms the equivalent volume of dH_2O was added. Eggs were sourced from different parasite families, with each family used once at each temperature treatment. Then, in each microcosm, control and experimental, five copepods were placed. Copepods were collected from stock cultures kept at the experimental temperatures and were size selected using a set of sieves. Only copepods from 250 µm and 390 µm were used. This ensured the exclusion of gravid females as these are less susceptible to cestode infections (Nie & Kennedy, 1993).

After adding the copepods the jars were incubated for 12 days. At the end of this period the copepods in each microcosm were collected and screened for infections. In each microcosm, it was recorded how many copepods were alive and how many of those were infected. For infected copepods it was also noted whether the procercoid had developed a cercomer. The cercomer indicates that *S. solidus* is infective to the next host, the three-spined stickleback (Smyth & McManus, 1989). Copepods that were unaccounted for or had died and were decomposing, were counted as dead and uninfected. This approach was taken as it is not possible to reliably detect parasites inside dead copepods. This thus provides a conservative measure of infection rates.

4.2.3 Statistical Analyses

4.2.3.1 Survival

To analyse the effect of temperature and exposure to *S. solidus* on the fitness of copepods, a generalised linear mixed model was constructed. Copepod survival was used as the response variable and was a binomial variable (alive or dead). The fixed effects were temperature (continuous) and exposure to the parasite (binomial; exposed or not exposed). Finally, microcosm number (factor) was included as a random effect in the model. Additionally, a model was run without control copepods to estimate the effect of parasite family, as different parasite families can differentially affect the host (Van der Veen & Kurtz, 2002).

4.2.3.2 Infection rate

For *S. solidus* fitness was calculated as the number of infected copepods at each temperature treatment. The response variable in the model was thus the infection status of each copepod (binomial). Temperature and parasite family were included as a fixed effect, with microcosm number (factor) was included as a random effect. This gave the effect of temperature on observed infection rate.

4.2.3.3 Protozoan numbers

To account for potential effects of microcosm conditions on the outcome of the experiment, the number of protozoans in each microcosm was estimated at the start and the end of the experiment. The count (continuous) at the start of the experiment was then used to fit a linear model, with temperature (continuous) to test their effect on copepod survival (binomial).

4.3 Results

4.3.1 Copepod feeding

For all temperature treatments, the Roger's type II functional response had a lower AIC value than the Holling type I (Table 4.1) and thus a better fit.

TABLE 4.1: AIC values for functional response Holling type I and Roger's type II for each temperature treatment. Models with lowest AIC values were considered to have the best fit. Values are rounded to the nearest integer.

functional response	11 °C	14 °C	17 °C	20 °C	23 °C
type I (Holling)	53	112	36	49	30
type II (Roger's)	47	86	35	40	29
ΔAIC	6	26	1	9	1

The intended range of protozoan densities was not obtained for all experimental temperatures (Figure 4.2), and consequently the maximum feeding rate can't be estimated accurately. Based upon visual inspection of Figure 4.2 and Table 4.2 it appears that at 14°C feeding rate is highest, while it is lower at more extreme temperatures (i.e. 11, 20 and 23°C). This indicates a dome-shaped relationship between temperature and feeding rate. Based on the fitted curves, estimated handling time was similar between treatments, but capture rate (or, area cleared) showed a peak at 14 °C (Table 4.1). No mortality was observed in the feeding experiments.

TABLE 4.2: Parameter values for the Roger's type II functional response for each temperature treatment. Estemated parameters are area cleared (a), handling time of prey (h) and length of the ezperiment (T). The latter was always one hour.

	a	h	T
11°C	0.630	0.041	1.000
14°C	1.791	0.024	1.000
17°C	0.571	0.019	1.000
20°C	0.767	0.063	1.000
23°C	0.719	0.031	1.000

4.3.2 Microcosms

4.3.2.1 Copepod survival

No effect was found of temperature ($GLM_{binomial}$, z-value = -0.218, df = 236, $P = 0.8270$; Table 4.5), exposure (z-value = -1.204, $P = 0.6763$; Table 4.5), or their interaction (z-value = -0.510, $P = 0.6100$) on the survival of copepods.

When including parasite family as a fixed effect (explanatory variable) in the analysis of the survival of exposed copepods, there is a significant effect of parasite family

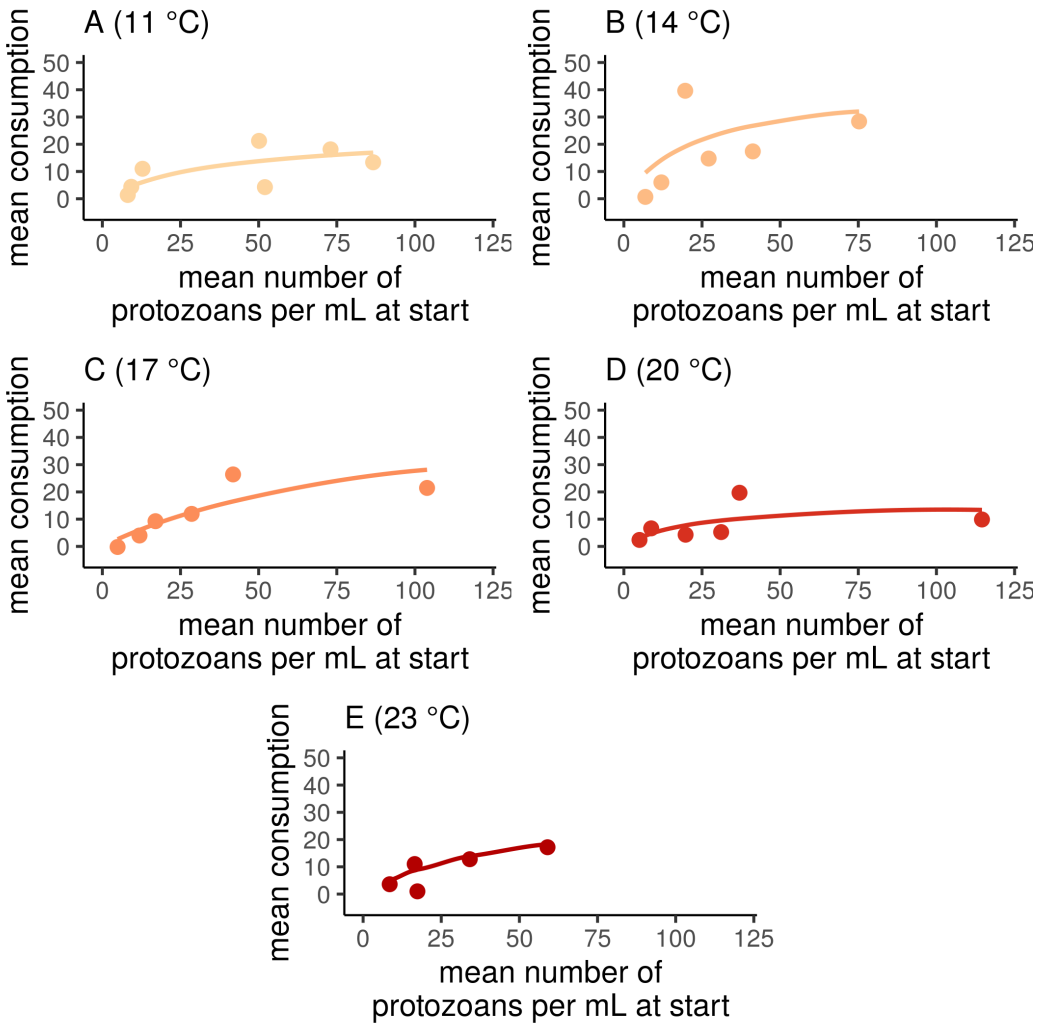


FIGURE 4.2: Mean consumption of protozoans by copepods at mean protozoan densities. Consumption rates and food densities were used to estimate the shape of the functional response at each temperature treatment, in this case a Roger's type II functional response. Plots show incremental temperature treatments.

(χ^2-test, P = 0.0305), while the effect of temperature remains unchanged ($GLM_{binomial}$, z-value = -1.243, df = 153, P = 0.2138). Comparisons between the individual parasite families, using the Tukey correction for multiple testing reveals that copepod survival was not significantly different between individual parasite families (Table 4.4).

TABLE 4.3: Number of surviving and dead copepods for each parasite family from the microcosm experiments. Survival was quantified at the end of the experiment, i.e. after twelve days.

	T37b	T38a	T38b	T39	T53b	T54
dead	1	12	7	14	9	9
alive	19	28	13	26	11	11

TABLE 4.4: *P*-values for pairwise comparison of copepod host mortality between parasite families at the end of the microcosm experiment.

	T37b	T38a	T38b	T39	T53b
T38a	0.36				
T38b	0.29	1.00			
T39	0.24	0.85	0.97		
T53b	0.13	0.85	0.97	0.13	
T54	0.13	1.00	1.00	0.13	1.00

4.3.2.2 Infection rate

There was an overall effect of parasite family on infection rate in copepod hosts (χ^2-test, $P = 0.0350$). Pairwise comparisons using a Tukey correction shows that there were no differences between the individual parasite families (Table 4.6).

Temperature did not have an effect on the infection rate of the copepods ($GLM_{binomial}$, z-value = 1.784, df = 107, $P = 0.0744$; Table 4.5). Only single infections were found in the copepods and because of the low number of infections the number with and without cercomer were not compared between temperatures.

4.3.2.3 Protozoan numbers

To correct for potential biases in survival caused by food availability, mean survival for each microcosm was tested against the protozoan density at the start of the experiment. There were a main effect of the number of protozoans (LM; t-value = -3.404, $P = 0.0019$), as well as of temperature (LM; t-value = -3.626, $P = 0.0010$), and an interaction effect (LM; t-value = 3.553, $P = 0.0012$) on the survival of the copepods (Figure 4.3). Overall, both the number of protozoans (β= -0.050 per protozoan added) and the experimental temperature (β= -0.055) had negative effect on the mean survival of the copepods. The interaction effect, on the other hand, was positive but much smaller (β= 0.003; Figure 4.3).

TABLE 4.5: Percentage of copepods surviving at the end of microcosm experiments, for control (non-exposed), exposed, control and exposed combined, and percentage of exposed copepods that got infected.

temperature	survival control (%)	survival exposed (%)	survival all (%)	infected (%)
11	80	70	73	7
14	70	78	75	0
17	45	60	55	8
20	85	62	70	20

TABLE 4.6: *P*-values for pairwise comparison of parasite family infectivity to copepod hosts during the microcosm experiments. Only few copepods got infected, possibly explaining the lack of differences between families.

	T37b	T38a	T38b	T39	T53b
T38a	1.00				
T38b	0.61	1.00			
T39	1.00	1.00	1.00		
T53b	0.74	1.00	1.00	0.74	
T54	0.48	1.00	1.00	0.48	1.00

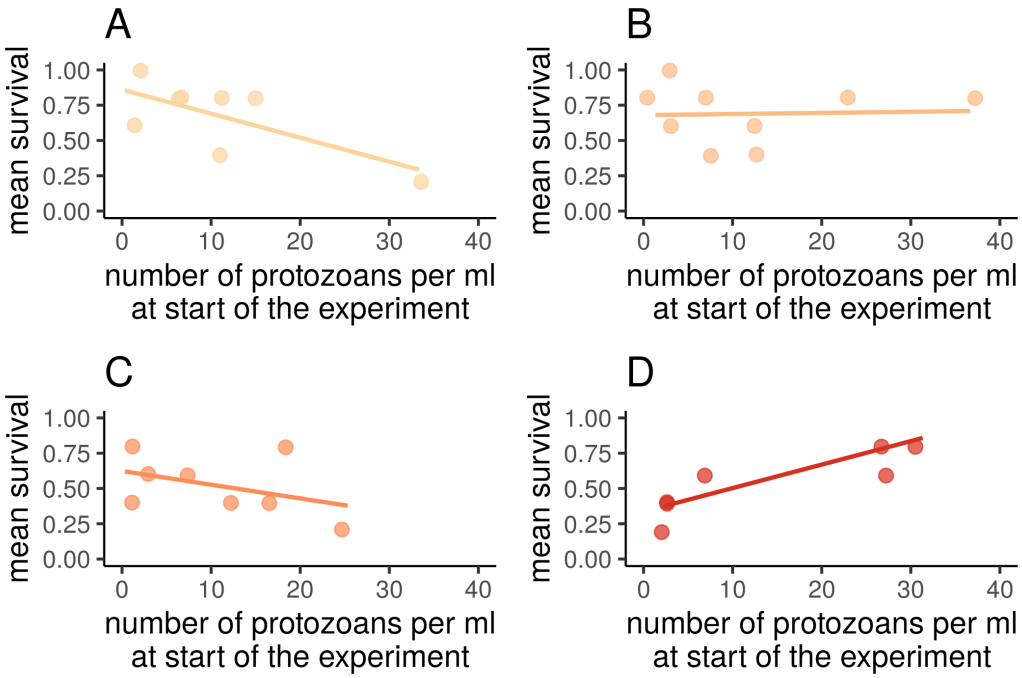


FIGURE 4.3: Mean copepod survival in each microcosm for the number of protozoans per mL at the start of the experiment. Regressions are fit per temperature treatment as there were main effects of protozoan count and temperature, as well as an interaction effect (see main text for details). Plots show incremental temperature treatments

4.4 Discussion

Copepod feeding showed a general dome-shaped response to temperature, but the data was not fine scaled enough to reliably estimated the optimum feeding temperature. Overall infection rates in microcosms were low and no effect of temperature on survival or infection rate was found.

4.4.1 Copepod feeding

As environmental variables can affect the shape of the functional response (Jeschke et al., 2004; Paffenhöfer & Stearns, 1988), the type of functional response could have been different for the different temperature treatments. Both a type I and type II functional response were fitted for each temperature treatment to account for this. Type III functional responses were not fitted as these are not typically seen in copepod species (Jeschke et al., 2004). For all temperature treatments the Roger's type II functional response (Rogers, 1972) had a better fit to the data than a type I response based on AIC values (Table 4.1). This indicates that *Cyclops strenuus* copepods feeding increases in a curve-linear manner with increasing densities of *Colpidium striatum* protozoans.

Copepod feeding caused a significant reduction in the number of protozoans in the solution (Figure 4.3), additionally supporting the use the Roger's type II functional response (Rogers, 1972). This function thus appears more appropriate than the more traditional Holling's type II functional response (Holling, 1959) to model the relationship between protozoan density and copepod feeding. However, it should be noted that without accurate data it can be hard to distinguish between type I responses with satiation and type II functional responses (Jeschke et al., 2004).

The estimated rate of attack (a) of the copepods appears similar between most temperature treatments (Table 4.2), except for 14°C. At this temperature feeding rate increases between two- and threefold compared to the other temperature treatments (Figure 4.2; Table 4.2). There thus appears to be an optimal feeding temperature for *C. strenuus* copepods. On the other hand, the estimated handling time (h) at the different temperature treatments appears similar from the analysis (Table 4.2). This is in line with the expectation that handling time is negligible in filter feeders (Jeschke et al.,

2004) and indicates that it is mainly the attack rate between copepods and protozoans that changes with temperature.

The peak in feeding at intermediate temperatures matches previous studies on optimal feeding temperatures in both vertebrate and invertebrate predator-prey and parasitoid-host interactions (Englund, Öhlund, Hein, & Diehl, 2011). However, except for the 20°C treatment it appears that the satiation point had not been reached for the copepods. This makes it hard to accurately compare between the temperature treatments and determine the precise optimal temperature for the feeding rate.

The peak in feeding activity at 14°C indicates that at intermediate temperatures the copepods are more successful feeders. Copepod activity is positively correlated with the number of infective stages consumed (Van der Veen, 2003). Thus, copepods at the intermediate temperature are at a higher risk of getting infected by parasites such as *Schistocephalus solidus*. Although no differences in egg hatching were found (Section 3.3.1), coracidia can survival longer at lower temperatures (Ismail, 2018). Previous work and theory suggest that for active foraging ectotherms, food consumption may increase with warming temperatures (Roessig et al., 2004), but for sit-and-wait predators feeding is likely not be affected by temperature (Novich, Erickson, Kalinoski, & DeLong, 2014). Copepod species can employ both feeding methods. Based on this it appears that in the current setup copepods were feeding through a sit-and-wait strategy. In the field, temperature change might increase selection pressure for sit-and-wait predation as opposed to active predation. This would mean exposure to free-living parasites is likely to remain relatively similar. If, however, no such selection occurs parasite exposure might increase with increased feeding. Interestingly, the peak in feeding was seen near current environmental mean temperatures.

4.4.2 Microcosms

4.4.2.1 Copepod survival

Only an effect of temperature on the survival of copepods at 23 °C was seen in the microcosms. No effect was seen either in the exposed or control group, or those two combined for the range 11 - 20 °C (Table 4.5). This indicates that there was no effect on survival of the copepods of parasite infection, temperature, or the interaction between

these two. However, there may only be a small effect on survival of infections (Sakanari & Moser, 1985) that would not have been picked up with current small number of infections. Increased mortality at higher temperatures was expected because of increase metabolic rates of invertebrates at higher temperatures (Piersma & Van Gils, 2010) and with infection (Pasternak et al., 1995). Stochastic mortality at 23 °C confirmed this. Increased metabolism could lead to quicker depletion of resources and if adequate feeding is not possible increased risk of mortality. However, the highest feeding rate was seen at intermediate temperatures (14°C). This is in line with previous work that shows that invertebrate attack rates often show a dome-shaped response to temperature (Englund et al., 2011).

Parasite family had a significant effect in the copepod survival analysis for the exposed copepods. However, after correcting for multiple testing no significant differences were found in survival between copepods exposed to individual parasite families (all *P*-values >0.05; Table 4.4). Although there were some overall differences in survival between families, no individual parasite family was different from the others in affecting host survival.

To overcome temperature shock effects, copepods were acclimatised to their experimental temperature for at least three weeks before any experiments. This also more realistically simulates the way copepods would be exposed to potential future mean temperatures then transferring them to the experimental temperatures on the day of the experiment, as mean temperatures are expected to rise gradually (Easterling et al., 2000). Copepods may initially have had higher metabolic rates at higher temperature and lower metabolic rates at lower temperatures. The acclimation may have allowed the copepods to adjust their metabolism to the new temperature regime. To test for the effect of acclimation, the feeding of copepods after different periods of acclimation could be tested. Alternatively, copepods could be tested in respirometers at different time intervals (see e.g. Pasternak et al., 1995). This would allow to answer important questions regarding the ability of, and mechanisms through which species adapt to increasing mean temperatures.

As some parasite species are restricted in their growth by the nutrient availability inside the host (Benesh & Hafer, 2012; Parker et al., 2003), copepods in this experiment

were selected for size. Thus, copepods use were likely to be copepodites (C4 or C5) of either sex or adult males. If higher temperatures do not affect survival at young ages, but reduce the maximum longevity that would not be seen in this experiment but could still have significant impacts on the population.

4.4.2.2 Infection rate

In the microcosms experiments 6.9 % (11 out of 160) of the exposed copepods got infected. This is slightly higher, but in the same order of magnitude as cestode infections in wild copepod populations (e.g. Hanzelová & Gerdeaux, 2003; Marcogliese & Esch, 1989). From this it was concluded that the microcosm simulated the natural environment adequately for the current experimental goals. However, the low number of infections made it hard to find statistical differences between the different temperature treatments. This could explain the absence of any temperature effects on *Schistocephalus solidus* infections in *Cyclops strenuus*. Alternatively, it is possible that temperature does not affect the total infection rate in copepods in a semi-natural context.

At warmer temperatures the immune system might be better at fighting off new infections (Dang et al., 2012), but is also likely to be a larger energy drain. It could thus be expected that when challenged with a relatively large number of parasites, survival of the hosts actually decreases because of the amount of energy required to mount the immune response to the incoming infections. Hence, it is not unlikely that some of the mortality was caused by the parasite infections, as infections can facilitate further infections (McPherson et al., 2018), and higher levels of infection increase the probability of copepod host mortality (Michaud, Milinski, Parker, & Chubb, 2006).

In the full model parasite family had a significant effect on infectivity. When comparing between parasite families no significant differences between the families were found. Parasite family was thus retained in the models as a random effect but was not further treated. Similar differences between parasite families have been observed in other studies (e.g. Ismail, 2018).

Warmer temperatures can have a negative effect on the survival of *S. solidus* coracidia (Ismail, 2018), which is in line with other studies on free-living parasite stages (e.g.

Pechenik & Fried, 1995). However, it is likely that if lower temperatures were included survival might have showed a dome-shaped relation with temperature (Lo & Lee, 1996). If survival of infective stages is negatively affected by warmer temperature, but total infection rate appears unchanged (Table 4.5), either exposure to infective stages must be increased, or the infectivity of the infective coracidia is higher at warmer temperatures. For the exposure of copepods to be increased the contact rate between copepods and coracidia must increase. As feeding of copepods appears to be highest at intermediate temperatures this seems unlikely. On the other hand, it is possible that because of the higher temperatures the metabolism of the coracidia was increased and consequently the activity of the coracidia. This would have reduced their energy stores more quickly, potentially increasing mortality rate (Ismail, 2018). However, in Ismail (2018) eggs and coracidia were kept at room temperature in the 24 hours before the experiment, and not acclimatized to the experimental temperatures. It is thus possible that acclimation would have offset this effect of temperature on the survival of the *S. solidus* coracidia. Higher motility of coracidia as a result of an increase in metabolism would increase the likelihood of contact between the copepod and the parasite and thus parasite exposure. Alternatively, the immune system of the copepods could be affected negatively by warmer temperatures. This is for example seen in snails, where long-term exposure to warm temperatures reduces their ability to resist a trematode parasite (Leicht & Seppälä, 2014). However, establishment of a nematode parasite within several copepod intermediate host species was negatively affected by temperature (Ashworth et al., 1996), indicating that the immune response against some metazoan parasites of copepods is actually up-regulated by warmer temperatures. This means that regardless of the potential decreased survival of the coracidia and the potential increased immune response in the copepod the infectivity stays unchanged. This implies that the encounter rate between *C. strenuus* copepods and *S. solidus* coracidia increases at warmer temperatures to offset these effects.

Here only young copepods were considered. When assessing the whole host life span, temperature has the potential to decrease the life span of copepods and thus the time window in which they can get infected, but also are able to transmit the

parasite. This could have negative impacts on the life cycle of *S. solidus*. Additionally, no differentiation was made between male and female copepods, but infection risk is higher for males (Wedekind & Jakobsen, 1998) and this may have implications for future populations as well.

In the cestode *Lacistorhynchus tenuis* egg hatching happened earliest at the higher temperature, while survival was favoured by intermediate temperatures. Additionally, no clear effect on prevalence in the first intermediate host was found (Sakanari & Moser, 1985). This indicates, that just as in this study, there may be opposing effects of temperature on different traits of a life stage of the parasite (Koprivnikar et al., 2010; Studer et al., 2010), consequently these can largely cancel each other out when taken together. In a natural environment, where other species are present, transmission of free-living parasitic stages may be further inhibited by predators and dead-end hosts (Orlofske et al., 2012). Given that for most ectotherm species consumption increases with warming temperatures the mortality of infective stages is expected to increase. Additionally, selection for cooler microhabitat by hosts could reduce transmission and slowdown parasite development inside the host in future climates (Moore & Freehling, 2002)

4.4.2.3 Parasite development

Parasite development could not be accurately assessed due to the small number of infected copepods. However, data from previous studies show that development is likely to be sped up by warmer temperatures (e.g. Sakanari & Moser, 1985). On the other hand, parasite growth inside intermediates host can be restricted by host size (Parker et al., 2003), including *Schistocephalus solidus* (Wedekind, 1997). This means that even at warmer temperatures final parasite size might not change drastically. Nevertheless, *S. solidus* that developed faster are better able to infect fish hosts (Benesh & Hafer, 2012).

4.4.2.4 Protozoan numbers

The number of protozoans at the start of the experiment had a overall negative effect on the mean survival of the copepods (Figure 4.3). Additionally a positive interaction

effect of temperature and starting number of protozoans was seen. This indicates that at warmer temperatures higher densities of protozoans facilitated a larger number of copepods to survive. Copepod survival was thus potentially restricted by food availabillity, even though overall protozoan numbers increased during the experiment at all temperatures (Figure 4.3).

4.4.3 Conclusions

A Roger's type II functional response had the best fit to *Cyclops strenuus* feeding data at all temperatures (Objective 4.1b). An increase in feeding was seen at intermediate temperatures (Objective 4.1a), indicating a dome-shaped relation of feeding and temperature. No effect of temperature on infection rate of the *Schistocephalus solidus* parasites (Objective 4.2b), or copepod survival (Objective 4.2a) was seen in microcosm experiments. Due to a low number of infections the effect of temperature on procercoids inside the copepods (Objective 4.2c) was not investigated. Additionally, there was a positive interaction between temperature, starting number of protozoans in the microcosm and the survival of the copepods (Objective 4.2d).

Chapter 5

Temperature effects on parasite infections in sticklebacks

5.1 Introduction

Changes in thermal regimes are expected to affect vertebrates and invertebrates in different ways. The immune systems of vertebrates and invertebrates show some differences. These differences mean that their response to changes in temperature regimes may also vary. Differences in immune function in response to temperature can affect which parasites can successfully invade the host (Mouritsen & Jensen, 1997; Seppälä & Jokela, 2011). Aquatic vertebrates generally are larger and more mobile than their invertebrate counterparts and so have more opportunity to select different microhabitats or to migrate in response to adverse thermal conditions (Capra et al., 2017; Hoffacker, Cecala, Ennen, Mitchell, & Davenport, 2018). Changes in (micro)habitat selection could then result in altered food availability and, consequently, different feeding patterns (Bevelhimer, 1996; Freitas, Olsen, Knutsen, Albretsen, & Moland, 2016). In turn, such changes could lead to exposure to a different assemblage of parasites (Marcogliese, 2002). Additionally, alterations in phenology caused by changes in thermal regimes can further affect the assemblage of parasites that vertebrate hosts are exposed to through changes in feeding behaviour (Both et al., 2009).

5.1.1 Impacts of individual host differences on parasite development

Once established inside the host, not all host individuals within the same species will provide the same environment for the parasite. Traits associated with the specific host, and with the environment are likely to affect the development and survival of the parasite. Some environmental factors that could impact the growth and development of endoparasites include temperature, heavy metals (Lefcort et al., 2002) and acidity (MacLeod & Poulin, 2016a, see also Section 3.1.4 and Figure 3.1). Host-related factors include prior exposure to parasites (McPherson et al., 2018), host behaviour (Poulin, 2011), and host growth and development (Barber, 2005).

Previous exposure to disease can impact the susceptiblity of potential host species. Depending on the host and infection it may make the establishment of parasites more (McPherson et al., 2018), or less successful (Beemelmanns & Roth, 2016), or have not effect on infection success (Karvonen, Rintamäki, Jokela, & Valtonen, 2010). The variation in these responses probably reflects the selective pressure that different parasites exercise on their hosts as well as the stage of the host-parasite arms-race. In the three-spined stickleback host, *S. solidus* does not activate the antibody-mediated adaptive immunity (Scharsack et al., 2007). This is most likely because plerocercoids 'hide' themselves from the host immune system, as a strong immune response would be expected given the detrimental effects of *S. solidus* infections on their stickleback hosts.

The behaviour of potential hosts can also affect their exposure to parasites, or affect the ability of parasites to settle on, or infect potential hosts (Poulin, 2011). Depending on the interaction between the negative impact of the parasite on host fitness and the potential for the host to adjust its behaviour, natural selection sould favour hosts to adopt behaviours that help avoid infection. For example, social individuals could avoid infected conspecifics to reduce the risk of vertical transmission or spending time in microhabitats where they are likely to be exposed to trophically transmitted parasites (Stephenson, Perkins, & Cable, 2018). In changing habitats these trade-offs could change.

Development of stickleback hosts, specifically growth rate, is positively correlated with *S. solidus* development (Barber, 2005). Variability in host growth rates could be due to different factors, food conversion efficiency, difference in how well fish can

compete for food, or base metabolic rates. Regardless of which of these underlies differences in growth rates between individual differences between fish, all of these would probably increased nutrient availability to the growing plerocercoids.

5.1.2 Non-invasive techniques for assessing parasite development

In order to complete the *Schistocephalus solidus* life cycle in the lab it is commonplace to substitute the definitive bird host with a culture technique. This allows for the convenient culturing of adult worms without the use of animal facilities (i.e. keeping birds) yet producing similar results (Stewart et al., 2017). Currently, one of the constraints of this system is the ability to determine whether (wild) fish carry parasite infections and to identify the size and number of parasites when a fish is infected. Recently, a technique to non-lethally assess the presence or absence of *S. solidus* infections in its fish host has been established using qPCR of 'environmental DNA' (eDNA; Berger & Aubin-Horth, 2018). By sampling the body cavity of the fish, the presence or absence of parasite DNA can be reliably established.

These eDNA techniques do not, however, give any information on the size of the parasite infection and require invasive techniques which are only allowed under a Home Office licence (Home Office, 2014). Plerocercoids that have not reached their infective size (i.e. >50 mg) are less likely to produce viable eggs (Tierney & Crompton, 1992), making this an essential piece of information. This is particularly important when (wild) fish are killed purely for parasite culture. It is possible to estimate the size of the parasite (in males and non-gravid females) quantifying the abdominal swelling of the fish (Barber, 1997). Basing the decision to kill or not kill fish on visual observations to obtain their parasites can be relatively straight forward for trained experts, but more difficult for the untrained eye. For this reason Barber (1997) developed a non-invasive method to estimate parasite size from abdominal swelling. This uses the difference in abdominal swelling between infected and non-infected fish, allowing the size of the parasite to be estimated relatively reliably. The method can also be used to track parasite growth *in vivo* throughout an experiment (Barber & Svensson, 2003). However, it requires the manual analysis of the abdominal swelling based on dorsal profile photographs of the fish, making it a labour intensive process. Consequently

the screening of large groups of fish (>30) is impractical. By developing a computer algorithm that automates the photograph analysis, it would become possible to screen large groups of fish for infective parasites. Additionally, it supports a more objective and repeatable manner of estimating fish swelling and thus parasite size, as well as removing the risk of human-error. Combined with the recently developed eDNA techniques in *S. solidus* this could enable new avenues for research that were previously impossible because of they were too labour intensive.

5.1.3 *Schistocephalus solidus* growth inside the stickleback host

Under natural conditions, growth of *Schistocephalus solidus* inside the stickleback host varies between regions and latitudes, with temperature playing a notable role in predicting growth rate (Franke et al., 2019a). However, other host-related factors might also play a role, such as host size, food availability (Benesh, 2010a), and concurrent infections (Meakins & Walkey, 1973). Although there is no direct effect of absolute fish host size on *S. solidus* growth, fish growth rate is positively correlated with the *S. solidus* growth rate (Barber, 2005). In the same cohort of fish, size will correlate positively with growth rate as faster growing fish will be larger. This correlation allows the distinction to be made between faster and slower growing fish in studies that only report fish sizes from the same cohort. On the other hand, there appears to be no effect of size in juvenile fish on their immune response (Gradil et al., 2014) and thus on the likelihood of parasite establishment.

The three-spined stickleback-*Schistocephalus solidus* system is now commonly used to test hypotheses regarding hosts parasite interactions (Barber & Scharsack, 2010), and has also been used to test several hypotheses regarding temperature effects on growth and parasite component community (e.g. Karvonen et al., 2013; Morozińska-Gogol, 2002). To estimate the effects of temperature on the health and fitness of *S. solidus*, data was collected from previous studies that included different temperature treatments. Across ectotherm species growth rates following a quadratic function in responds to temperature are seen (Labaude et al., 2016; Paull et al., 2012) including *S. solidus* (Sinha & Hopkins, 1967). Thus, temperature was fitted both as a linear and as a quadratic predictor in the statistical models.

5.1.4 Aims

In this chapter the possibility to more reliably and conveniently predict the size of *Schistocephalus solidus* parasites inside its second intermediate host, the three-spined stickleback (*Gasterosteus aculeatus*), is investigated (Aim 5.1). This is done by implementing the previously existing theory on parasite size inside its host based on abdominal swelling as a computer algorithm (Objective 5.1a). This algorithm is then verified by comparing it's predictions against manual measurements (Objective 5.1b). This will support a non-invasive method of parasite screening that is also high- throughput. Second, the growth of *Schistocephalus solidus* inside the fish host at different temperature regimes is established from literature sources (Aim 5.2). For this previously published data (available online and obtained from the authors) were collated (Objective 5.2a) and a linear mixed model (LMM) is fitted to the data (Objective 5.2b), allowing the relationship between environmental temperature and plerocercoid growth rates to be investigated. Additionally, the optimal temperature for *S. solidus* development could be estimated (Objective 5.2c).

5.2 Methods

5.2.1 Fish analysis code

A simple pipeline was set up to estimate parasite size in a non-invasive manner (all code available in the Digital Appendix). Briefly, photographs were taken of individual sticklebacks. From these the length and area of the fish was measured in an `ImageJ` script (Rasband, 2016), then the parasite mass in the fish is estimated in an `R` script.

5.2.1.1 Photographing fish

Fish were placed in a circular dish (diameter = 12.0 cm) in a layer of 2 - 3 centimetres of water to allow fish to move freely, whilst restricting vertical movement as much as possible and so minimising parallax. The dish was placed on a back-lit white background with a pink square (20x20 mm). This square was later used to scale each image so that the analysis was independent of photographic zoom level. Back-lit, dorsal profile photographs were taken of the fish manually. Each fish was photographed at least

twice to ensure a good photograph was taken of each fish (e.g. a photograph in which the fish was not moving and consequently not blurry). In between fish a photograph of the empty dish was taken, this aided the process of assigning photographs to each fish later on. After photographing, each fish was blotted dry and weighed to the nearest milligram and individually put into labelled one litre holding tank.

5.2.1.2 Photograph analysis, `ImageJ` script

After selecting individual photographs for each fish and assigning fish identification numbers to the title of each, these could then be run through an `ImageJ` script that extracts fish sizes from the photographs. First, the sizing square is found and extracted by splitting the colour channels of the images and subtracting the green from the red channel which only leaves the pink size reference square. From this the scaling of the image is set. Then, using colour thresholding, the fish is selected from the red channel and measurements are taken. See Figure 5.1 for a schematic overview of the process. The measurements include the area and Feret's diameter (distance between two parallel tangents on opposite sides of the object; Merkus, 2009). The Feret's diameter is used to get the length of the fish as this allows for accurate length measurements even when the fish body is bent. All measurements are exported to a csv file which can then be imported into `R` for further processing and analysis of the data.

5.2.1.3 Parasite size estimation, `R` script

For data processing and analysis an `R` script is used. This script also allows `ImageJ` code to be run directly from `R`, so facilitating easy data loading. Briefly, the `ImageJ` data and manual weight measurements are loaded and collated. Parasite size in infected fish is estimated as well as the parasite index (i.e. the proportion of the host weight comprised by the parasite Arme & Owen, 1967). The parasite index (I_p) relates to the size of the infected fish as:

$$I_p = 24.75 \times A_r^{0.293}, \tag{5.1}$$

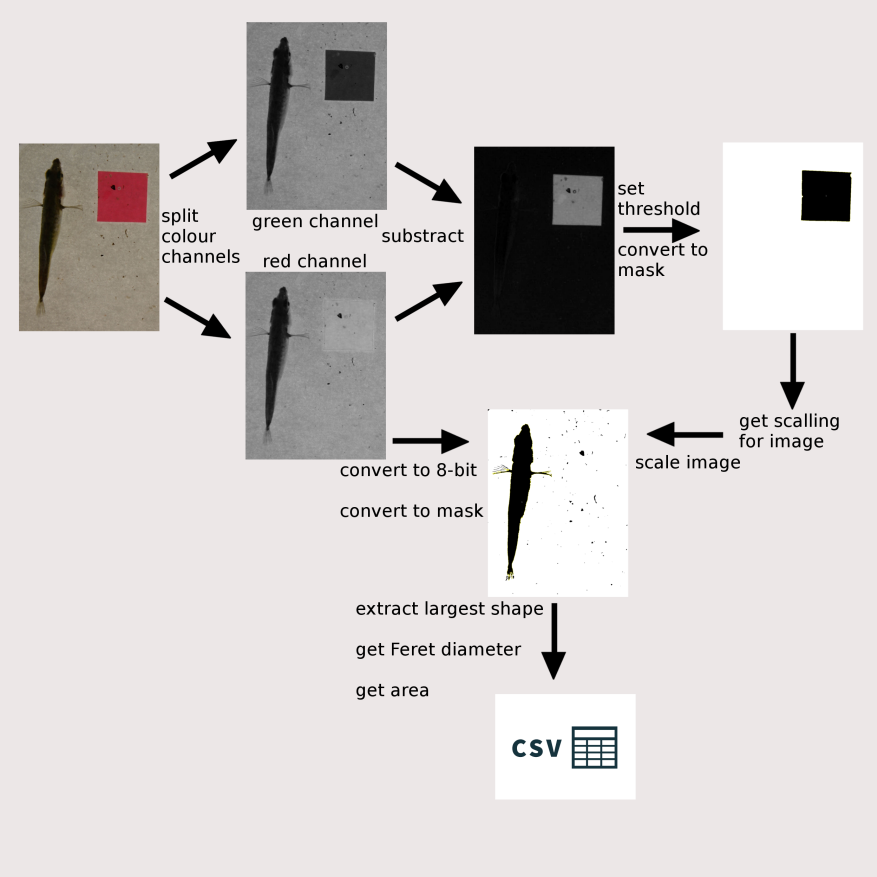


FIGURE 5.1: Visual summary of fish analysis `ImageJ` script. First the photograph is split into different colour channels and the red channel is subtracted from the green channel. This leaves only the size calibration square. From this the image is scaled. Using the red channel the area and length are estimated after scaling. Data are stored in a csv file that can be directly imported into a R script for analysis. The photograph analysis script can also be run straight from R.

(Barber, 1997) where A_r is the difference (residual) between the estimated square root of the area of the fish predicted based on fish length, and the observed area.

Using a population of uninfected fish a linear regression model between the length and the square root of the area of fish is calculated, where possible calculated with fish from the same population to increase accuracy. Then inputting the observed length for an infected fish gives a predicted (square root of the) area. The difference between this value and the observed value is then calculated (Barber, 1997) giving the residual area. From this the parasite mass can be estimated from the total mass of the fish including parasites according to (Barber & Svensson, 2003):

$$W_p = \frac{W_{f+p} \times 24.75 \times A_r^{0.293}}{100}. \tag{5.2}$$

In this, W_p is the mass of all parasite inside a single fish combined and W_{f+p} is the combined mass of the fish and parasite(s) (as multiple infections are possible). A reliable minimal size for *Schistocephalus solidus* parasites to produce viable eggs is given as 50 mg (Tierney & Crompton, 1992) for single infections. Because wild fish were used and multiple infections are thus likely (Arme & Owen, 1967), a threshold of an estimated parasite mass (W_p) of 100 mg was used to make it likely that at least one parasite would produce eggs.

5.2.1.4 Code validation

To assess the accuracy of the `ImageJ` script in estimating length and area of the fish, photographs of 56 uninfected were run through the developed script and then manually analysed. For both measurements produced by the script (i.e. length and area of each fish) the respective value was compared to the manual measurement in a linear mixed model (LMM). In the models the computer measurements were taken as the response variable, the manually measurements as a predictor variable and fish ID as random affect.

Additionally, the predicted parasite mass was compared to observed parasite mass in infected fish. For this, fish for which the combined predicted parasite mass exceeded 100 mg were killed (as described in Section 3.2.3.1). For each fish, length to the nearest millimetre was taken using vernier calipers and for both fish and parasite the weight to the nearest milligram was taken. The predicted combined parasite mass of each fish was than compared to the obtained values using a similar LMM with fish ID as random effect.

5.2.2 Estimating parasite growth from the literature

5.2.2.1 Literature search

All available databases on the Web of Science website were searched using the search string `'("Schistocephalus solidus" OR "s.  solidus" OR "s solidus") AND`

`(temperature OR heat)'` in the topic field using. The last search was done on May 16, 2019. The aim was to find all papers that described the growth of *Schistocephalus solidus* at two or more temperature treatments. This yielded 41 results. These papers were exported following which the search was further narrowed on the basis of their title. Twenty papers and books remained for which the abstract was read. Of these 17 were rejected for various reasons (Table 5.1), resulting in two papers containing adequate data (Franke et al., 2017; Macnab & Barber, 2012). Upon request of the data from Franke et al. (2017) the authors informed us of a second dataset (Franke, Raifarth, Kurtz, & Scharsack, 2019b) they were working on for Franke et al. (2019a) and were happy to share both datasets. This resulted in a total of 3 useable datasets.

TABLE 5.1: Reasons for exclusion of studies from analysis. In total 41 studies matched the search. Based on the titles 21 were excluded, and of the remaining 20, two met the criteria to be included. The full overview of papers is available in the Literature Search Appendix.

reason	n
on a different species	4
not on temperature	3
review paper	2
field observations	2
not on growth	2
not possible to convert data	2
presentation	1
paper not available	1

5.2.2.2 Data preparation

Experimental periods in the different studies differed, thus the growth period had to be standardized to allow comparisons. Growth of plerocercoids over time is not linear (Barber & Svensson, 2003), so the standardized growth period was taken to be as close as possible to the original growth periods. In Macnab and Barber (2012) all fish were infected for 56 days, whereas Franke et al. (2017) used infection periods of 56 and 58 days. Franke et al. (2019b) does not mention the number of days growth for each individual plerocercoid, but the text mentions 62-64 days. For the analysis of the data from Franke et al. (2019b) 63 days is used on the basis that, after this many days of growth, daily growth is expected to small given the size of the worm (see results

Sinha & Hopkins, 1967). The size of the plerocercoids was standardized to 60 days of growth for all datasets. This allowed for a fair comparison between studies.

The size of the fish at the start could play a role in the growth of the parasite as larger hosts may have more nutrients available for growth of the parasite (Barber, 2005). Therefore, fish size at the start of the experiment was included as an explanatory variable. Both Macnab and Barber (2012) and Franke et al. (2019a) had this variable included in the dataset. However, for Franke et al. (2017) it was possible to estimate the size of the fish at the start of the experiment based on their length and Equation 5.3 obtained from the photograph analysis experiment.

5.2.2.3 Data analysis

Relevant parameters from these data sets were selected and a linear mixed model (LMM) was fitted with estimated *S. solidus* size at 60 days post infection (Gaussian, numeric) as response and temperature and fish weight at the start of the experiment (both numeric) as predictor variables. The data source (i.e a three level factor) was included as a random effect. Initially, two models were constructed, one with temperature just as a linear term, and a second with temperature as a quadratic term given that temperature leads to a quadratic response (Sinha & Hopkins, 1967). However, it was unknown whether the maximum of this curve fell within the range of temperatures in the dataset so a linear model could be more appropriate. These models were then compared based on log-likelihood scores using a χ^2-test. Generally, the protocol outlined in Section 1.4 was followed. Additionally, the relation between fish length and weight was estimated.

5.3 Results

5.3.1 Fish analysis code

Length of uninfected fish measured manually from photographs and measured by the computer (`ImageJ` script) were not statistically different (LMM, t-value = -0.818, df = 55, P = 0.417; Figure 5.2). Additionally, when fitting this model the slope was nearly 1 (β= 0.95) indicating highly similar values.

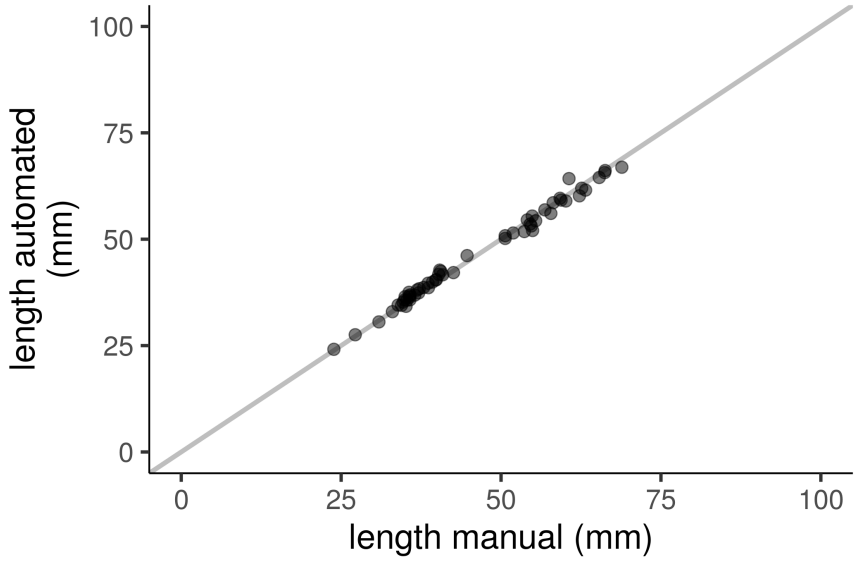


FIGURE 5.2: Length of individual uninfected fish measured by hand (x-axis) and through automation (y-axis), both in `ImageJ` (n = 56). The grey diagonal represent 1:1, thus if measurements are the same with both methods they fall on this line.

Similarly, the values for area of uninfected fish did not differ between the manual and automated (`ImageJ` script) analysis of the photographs (LMM, t-value = 0.363, df = 55, P = 0.718; Figure 5.3). Again the slope through the two measurement methods was close to 1 (β= 0.97).

The parasite mass was predicted based on dorsal profile photographs of a small number of infected fish for which parasite weight was available using the pipeline. Predicted and obtained parasite mass were compared and plotted (Figure 5.4).

The weight (from manual measurements) of uninfected three-spined sticklebacks (*Gasterosteus aculeatus*) was predicted by their length (t = 29.93, df = 54, $p < 0.001$; Figure 5.5) in a non-linear fashion (AIC = -199) better than by a linear equation (AIC = 22), or a cubic relationship proposed in Pennycuick (1971b, ; AIC = 17). This relationship can be described as:

$$W_f = 10^{(0.027 * L_f - 1.230)}. \tag{5.3}$$

Where the weight of the fish is given by W_f and is solely dependent upon fish length (L_f). This formula estimates the weight of individual sticklebacks based on their length.

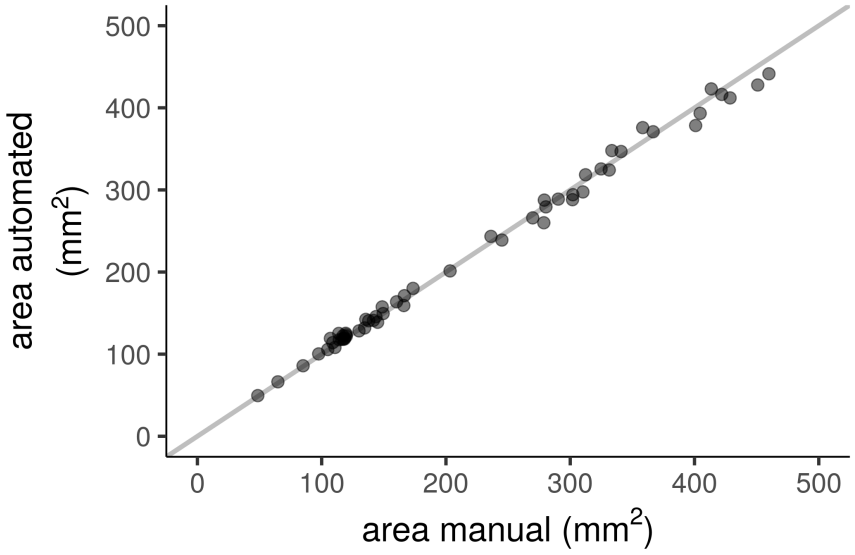


FIGURE 5.3: Area of individual uninfected fish measured by hand (x-axis) and through automation (y-axis), both in `ImageJ` (n = 56). The grey diagonal represent 1:1, thus if measurements are the same with both methods they fall on this line.

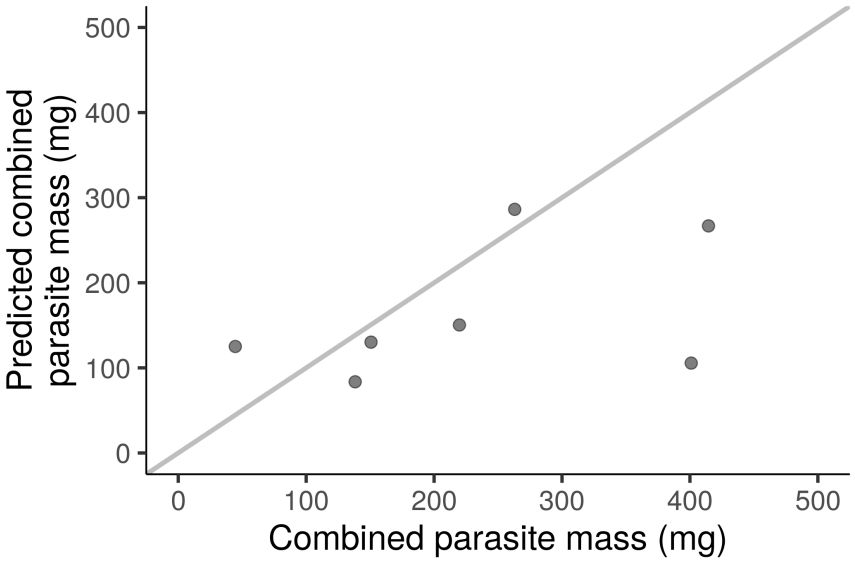


FIGURE 5.4: Combined parasites mass of *Schistocephalus solidus* in sticklebacks (x-axis) plotted against their estimated size (y-axis) based on measurements on fish (n = 7). The grey diagonal represent 1:1, points above this line indicate a smaller parasite mass than predicted, while points below the diagonal indicate larger parasite mass than predicted.

To test this relationship, the weight of fish in (Macnab & Barber, 2012) was estimated based on their length. Although the predicted values match the observed

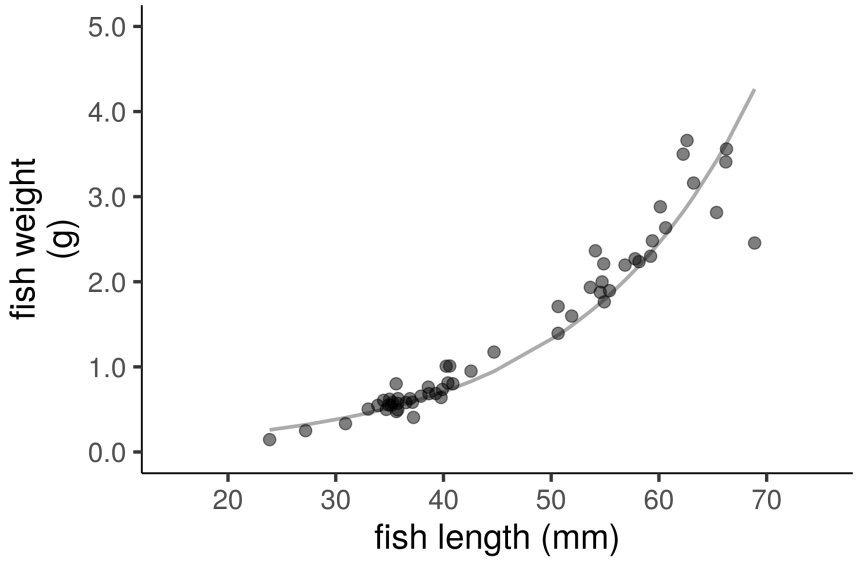


FIGURE 5.5: The relationship between length and weight for uninfected three-spined sticklebacks (*Gasterosteus aculeatus*) based on fish used in the experiments.

values well, the model appears to overestimate weight for small fish and slightly underestimate the weight for larger fish (β= -0.312, t = 39.27, df = 190, $p < 0.001$; Figure 5.6).

5.3.2 Estimating parasite growth from the literature

The model with the best fit to the data of the three datasets included temperature as a quadratic term and parasite weight at the time of infection as well as their interaction as predictor variables. It also included fish population as a random effect, but not parasite population (Table 5.2). Assessing the model fit shows that there is heteroscedasticity in the data (Figure 5.8A). Fortunately, heteroscedasticity does not affect the parameter estimates, but can lead to a bias in significance tests. Commonly this increases the type II error and can be resolved by correcting the covariance matrix and recalculating *P*-values (Long & Ervin, 2000). Thus, using the `R` packages `sandwich` (Zeileis, 2004) and `lmtest` (Zeileis & Hothorn, 2002), a new, corrected covariance matrix and new *P*-values were calculated. For the calculation of the covariance matrix using the function `vcovHC()` the default settings were used. However, these methods have not been developed for mixed effects models and thus the random effect was

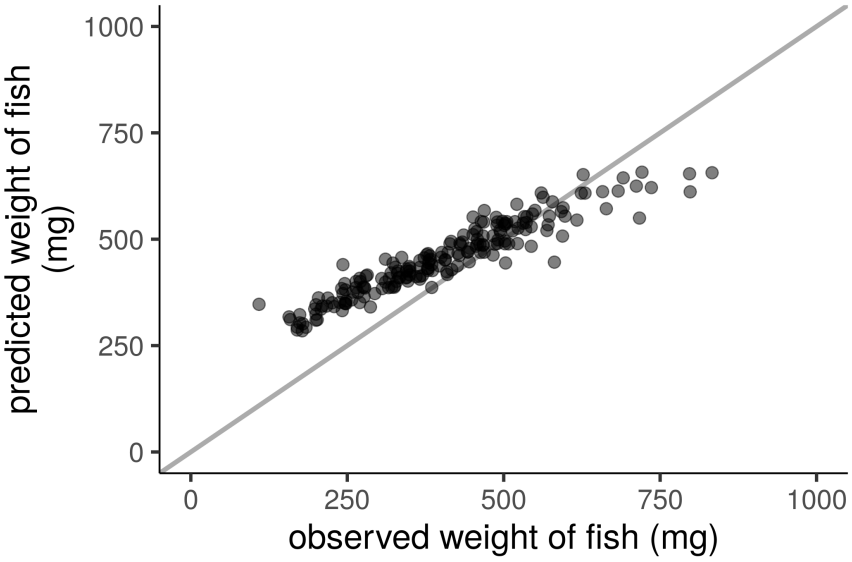


FIGURE 5.6: Predicted weights for sticklebacks based on their length plotted against the measured weight. Data obtained from (Macnab & Barber, 2012). The grey line represents a perfect model fit. Intermediate sizes are predicted well, while for more extreme — smaller or larger fish — estimates become less accurate.

dropped from the analysis. As the random effect only slightly improved the model fit (Table 5.2) it was considered more important to correct for heteroscedasticity.

Inspection of the leverage plot (Figure 5.8B) shows that there are three points that potentially have large leverage (three free standing points on the right hand side of the plot; values >0.075). Dropping these points from the model shows that the change of parameter estimates is between 30% and 59% of their standard error. It was thus considered prudent to rerun the analysis without these points and results are based on analysis without these points.

This resulted in a model where there was a significant quadratic (β= -0.79, t = -8.23, $P < 0.001$) and linear (β= 26, t = 6.53, $P < 0.001$) effect of temperature on the growth of *S. solidus* plerocercoids in sticklebacks. There was also a significant negative effect of fish weight at the start of the experiment (β= -0.13, t = -5.12, $P < 0.001$) and a positive interaction effect between the linear temperature term and the fish weight at the start of the experiment (β= 0.013, t = 7.71, $P < 0.001$; Figure 5.7).

This can be summarized as:

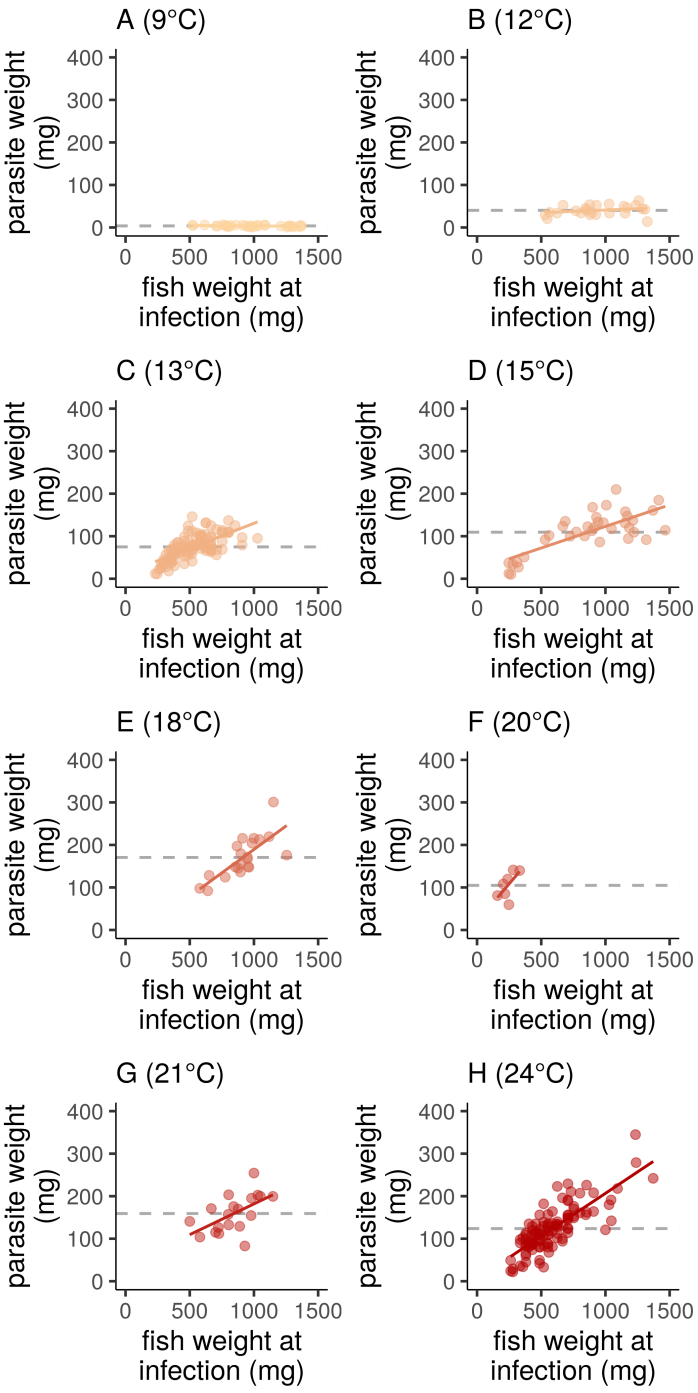


FIGURE 5.7: Each plot show the estimated plerocercoid size after 60 days of growth on the y-axis. Fish host weight at the time of infection is shown on the x-axis. Mean growth rate responds in a dome-shape manner to temperature (represented by the dashed grey line in each plot). Plerocercoid growth is linearly dependent on the temperature-host size interaction.

TABLE 5.2: AIC values for sequential statistical models. Models with an AIC value of at least 2 points smaller are considered to have a better fit. W_p is weight of the plerocercoid, W_{f0} is the weight of the fish host at the start of the experiment. P_p and P_f are the parasite and fish populations respectively. Finally, T and T^2 are the experimental temperature and the experimental temperature squared.

model	AIC
$W_p \backsim T$	3747.98
$W_p \backsim T * W_{f0}$	3514.88
$W_p \backsim T * W_{f0} + (1\|P_p)$	3503.87
$W_p \backsim T * W_{f0} + (1\|P_f)$	3502.57
$W_p \backsim T^2 * W_{f0} * T + (1\|P_f)$	3446.00
$W_p \backsim T^2 * W_{f0} + T * W_{fp} + (1\|P_f)$	3444.01
$W_p \backsim T^2 * W_{f0} + T * W_{fp}$	3460.94

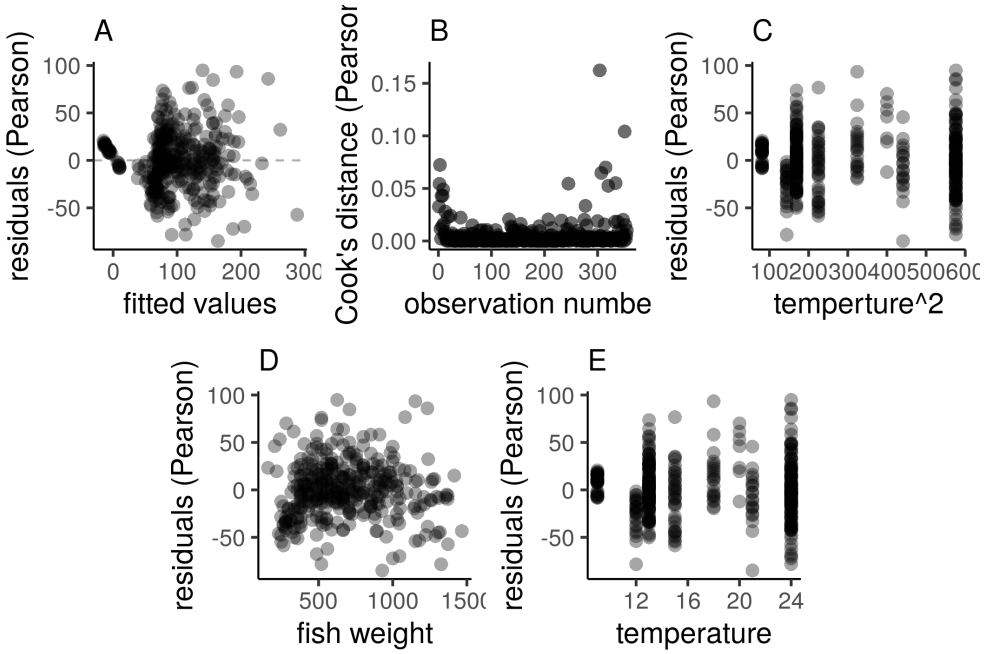


FIGURE 5.8: Diagnostic plots for plerocercoid growth dependent on host size and temperature. Inspection of the residuals and fitted values plot (A) shows some clustering of points at the low end of the fitted valued. These are due to low variance at the lowest temperature (9 °C) treatment. In plot B a few potential influential points are seen — more extreme y-values — inspections showed that none of these substantially changed model predictions. The spread of the points for the predictors showed no reason for further investigation, i.e. the points are more or less equally spread on the y-axis.

$$W_p = -0.79 * T^2 + 26 * T - 0.13 * W_f + 0.013 * T * W_f - 164. \tag{5.4}$$

Describing the dependence of plerocercoid weight on temperature and fish weight at the time of infection (Figure 5.7, see Table 5.2 for parameter description). From this the optimal temperature for plerocercoid growth can be estimated. Taking the first derivative and setting this equal to zero gives the temperature at which the parasite achieves the largest size after 60 days of *in vivo* growth under laboratory conditions. Setting fish weight at the start of the experiment to the mean fish weight from the studies used here (793 mg) this gives 22.9 °C (Figure 5.9) as the optimal temperature for growth.

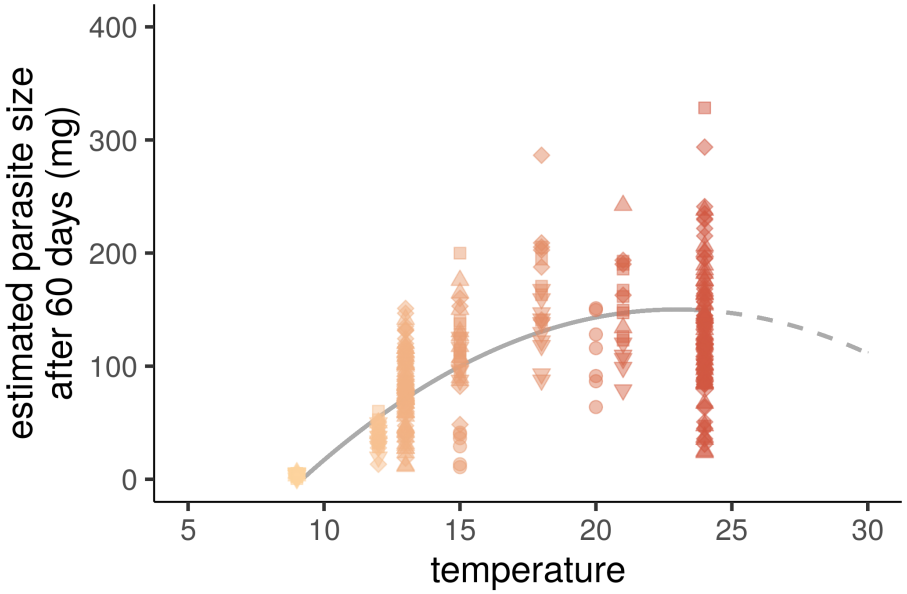


FIGURE 5.9: Estimated plerocercoid weights obtained from the different literature sources. Grey line is the fitted curve for the best estimated of parasite size response to temperature (see Equation 5.4). The dashed part of the line is the prediction beyond the data, this dome-shaped response is supported by other studies, see main text for details.

5.4 Discussion

5.4.1 Fish analysis code

The comparison between the manual and automated (`ImageJ`) measurement methods of the fish photographs showed that this script obtained the same results as manual analysis, but was much more time efficient when measuring length and area of the

fish (although not formally quantified here). Using the methods from Barber (1997), it was then possible to estimate *Schistocephalus solidus* mass inside the fish without killing the fish. Combined these methods allow for fast screening of large numbers of second intermediate host fish infected with cestode parasites. However, parasite size estimation based on Barber (1997) might have to be optimised, as for the small number used here predictions were not very accurate (Figure 5.4). It is unclear whether this was due to small sample size or inaccuracy in the method.

Using this automated method analysing one photograph took only a few seconds, compared to several minutes doing this by hand. Additionally, using automated methods makes the process more reproducible and objective. For this reason, Loot, Giraudel, and Lek (2002) presented a different technique with a similar aim. A neural network was used to predict the size of *Ligula intestinalis* plerocercoids inside roach (*Rutilus rutilus*). Roach, like sticklebacks, serve as the second intermediate host and the *L. intestinalis* life cycle is similar to that of *S. solidus* (Dubinina, 1980). However, the technique used requires the measurement of up to 39 physical traits of the fish by the algorithm and the understanding by the researcher of such algorithms (Loot et al., 2002). Conversely, the method proposed here uses an `ImageJ` script (macro) that can comfortably be executed by researchers with no experience with this program. Additionally, `ImageJ` is a free and open-source program and thus stimulates open science (Vicente-Saez & Martinez-Fuentes, 2018).

The technique presented here allows for fast screening of parasite mass for a large number of fish and also for the area of the fish itself. Using the relation between abdominal swelling and parasite growth this technique can, therefore, be used to track the growth of both the fish and cestode parasites developing inside its body cavity (Barber, 1997). This allows for experimental designs where non-invasive tracking of large numbers of hosts and parasites is required. For example, combined with behavioural assays it would be possible to closely monitor the size of the parasite and any changes in behaviour related to this (e.g. at what exact parasite size is host behaviour manipulated to facilitate transmission to the next host). This technique can

be applied not only to the stickleback-*S. solidus* system, but also other similar host-parasite systems where currently less is known about host manipulation, for example *L. intestinalis* in the roach (*R. rutilus*; Boulange-Lecomte, Geraudie, Forget-Leray, Gerbron, & Minier, 2011) or lake Malawi sardine (*Engraulicypris sardella*; Gabagambi, Salvanes, Midtoy, & Skorping, 2019).

When tracking the growth of cestode parasites in the body cavity of fish, the swelling of gravid females can obscure the parasites. Although *S. solidus* largely reduces reproduction of females in some populations (Heins, Singer, & Baker, 1999; McPhail & Peacock, 1983) thus removing this problem, this does not happen in all populations (Heins & Baker, 2014). Additionally, not all cestodes infecting fish as their second intermediate host might reduce their host's reproductive output. Host egg development is thus a factor that needs to be kept in mind when designing these experiments. On the other hand, this technique could also be used to track females and determine their optimal breeding time. By mounting a permanent camera above the tank that automatically takes one or multiple photographs each day, that are then automatically analysed, it would be possible quickly to assess whether any uninfected females are ready for breeding based on their abdominal swelling.

In the data run through the script developed here, sticklebacks from several different backgrounds were used to ensure the generality of the results. As consistent morphological differences exist between populations of stickleback (McKinnon et al., 2004) and other fish species (e.g. Sigurjónsdóttir & Gunnarsson, 1989), it is essential to test that parameterisation of the model is appropriate for the population under study. Still, the model appears to overestimate the weight of small fish and underestimate the weight of large fish when applying it to data from Macnab and Barber (2012). Therefore it could be useful, especially when studying large numbers of animals, to use a small training dataset for which measurements are initially made both by hand and automated. Then parameters could be changed to fit the population under study after which all analysis can be automated. Currently, only a size calibration square is added to the photos. However, it is possible to also add an identification number for each animal and further automate the linking of this number to the results table that is outputted. Another step in the analysis that is currently manual, is the selection of

the best photo; this too could be automated. Either by averaging the measurements of multiple photographs on the same individual or by selecting the photograph with values closest to the median value. Finally, further expansion could be to analyse multiple fish in the same photograph, either in the same tank or in neighbouring tanks in experiments where this would be appropriate.

5.4.2 Estimating parasite growth from the literature

Analysis of three datasets containing data on the growth of more than 350 *Schistocephalus solidus* plerocercoids at 8 different experimental temperatures, showed that the growth rate depends quadratically on temperature and that the weight of the host fish at the time of infection has a negative linear effect. The optimal temperature for growth of plerocercoids is approximately 23 °C (Figure 5.9). The dome-shaped response had a better fit to the data than a linear response, but when visually inspecting the data this shape is not obvious (Figure 5.9). However, looking at data from a study growing *S. solidus* plerocercoids in *in vitro*, this dome-shaped response is justified. This study included temperatures up to 40 °C at higher temperatures a gradual decrease in plercoid growth was seen (Sinha & Hopkins, 1967).

All published previous studies on the temperature effects on *S. solidus* growth found some temperature effect. In Macnab and Barber (2012) a steep increase in growth was seen with temperature increase (15 °C and 20 °C). Franke et al. (2017) found a similar effect of an increase in growth at warmer temperatures (13 °C and 24 °C). They additionally found that fish were better able to resist parasites from populations other than their background population, indicating local adaptation of the sticklebacks. In the current model this was captured by the random effect of fish population in the model. Franke et al. (2019a) considered the largest number of experimental temperatures and found that with increasing temperatures, parasite growth, egg production and offspring hatching increased strongly while host immunity and growth were impaired. A parabolic response of growth rate to temperature was seen by Sinha and Hopkins (1967), and the optimal temperature for growth was similar (23 °C found here and 23-27 °C in Sinha & Hopkins, 1967). Quadratic responses to temperature in

growth rate are common in biological systems (Li & Jackson, 1996; Ratkowsky, Olley, & Ross, 2005) and indicate a trade-off between multiple (physiological) processes. For *S. solidus* this trade-off is likely to be for increased cell division and thus growth at warmer temperatures and increased metabolic demands at warmer temperatures (Buckley et al., 2012) reducing the energy available for growth.

None of the studies mentioned explicitly took into account the impact of weight or growth rate of the stickleback host on parasite growth rate. Previous work showed that increased growth rate of the fish host can positively impact the growth of the plerocercoid (Barber, 2005). In the studies considered here, fish grew up under (semi) competitive conditions and thus obtained different body sizes which were likely to be related to their specific growth rates, thus the measured size at the start of the experiment could be seen as a proxy for specific growth rate. The statistical model (Equation 5.4) indicates a negative effect of fish host size on the plerocercoid growth. This effect should not be seen without the positive effect of the interaction between temperature and fish hosts size. Overall, the effect of the interaction will be stronger for temperatures above 10 °C and thus offsetting any negative effects on parasite growth (see Equation 5.4). This is illustrated by the absence of a fish host effect at 9 °C where growth of plerocercoids did not differ between host sizes. Conversely, with increasing temperatures the mean plerocercoid growth rate is increased (indicated by the grey dashed horizontal lines in Figure 5.7) as well as the host effect (indicated by the slope in each plot).

Overall, both temperature and fish size at the time of infection play an important role in determining the outcome of the interaction between *S. solidus* and the stickleback host. Other factors that modulate this interaction and impact the growth and fecundity of worms include host and parasite origin (Franke et al., 2019a). Other factors could also play a role, such as any concurrent infections (Meakins & Walkey, 1973) and food availability (Benesh, 2010a). It remains to be seen whether parasites are able to adjust to these conditions and prevent premature host mortality through resource competition between the host and parasite (Benesh, 2010b).

5.4.2.1 Estimating effects for UK populations

Here 60 days was chosen as the time after which the size of the plerocercoids was estimated. Dubinina (1980) states that the time spent by *S. solidus* in the stickleback host is approximately 180 days and in cold water populations the parasite might even live beyond 1 year (Heins et al., 1999). This is much longer than the time allowed for development inside the fish in most experiments (e.g. Franke et al., 2017; Hammerschmidt et al., 2009; Macnab & Barber, 2012), including those used here to fit the models to. Even at the low temperatures studied here (i.e. 12 °C) the current experimental time was enough for the plerocercoids to become infective to the definitive hosts. Based on the current results if growth is be expected to continue at the same rate, plerocercoids would reach large (up to ~1000 mg depending on the temperature) sizes after 180 days. However, growth of plerocercoids is not linear (McCaig & Hopkins, 1965; Sinha & Hopkins, 1967) and caution must be taken to make estimates about growth beyond the experimental periods observed. Although these sizes are possible (e.g. Pennycuick, 1971a), they are not commonly observed in the field (e.g. Pennycuick, 1971a, and Figure 5.4). Additionally, growth rates of plerocercoids are likely to be reduced overall under field conditions, due to more variable temperatures (Groner et al., 2014) and lower growth rates of fish due to lower food availability in the field (Barber, 2005). Indeed, it appears that parasite growth is reduced in winter (Pennycuick, 1971c) when food availability and temperature are reduced. This growth reduction makes sense from an evolutionary perspective of the parasite and is advantageous for both the stickleback and the parasite. Sustained growth of the parasite would likely increase fish mortality rates during a time when predation by birds appears less likely (i.e. predation is likely to be highest during bird migration and breeding periods due to the increased metabolic demands of the birds Giles, 1981; Piersma & Van Gils, 2010) and thus transmission. Individuals in populations of three-spined sticklebacks in the United Kingdom can live for up to four years (Pennycuick, 1971a), unlike some populations in more northern regions (McPhail & Peacock, 1983). This means that *Schistocephalus solidus* plerocercoids that infect young-of-the-year fish in theory have a long time to develop. Yet, it seems unlikely that many fish harbouring *S. solidus* infections survive that long, as mean sizes of plerocercoids drop in early

winter, indicating that infected fish died or were consumed by predators (Heins et al., 2016; Pennycuick, 1971c).

5.4.2.2 Data quality and availability

Meta-analyses and systematic literature reviews can be used as a powerful tool to use the available literature to answer new questions or revisit old ones. From this a deeper understanding of underlying processes can be gained. Fortunately, for the current question several datasets were available and their authors were happy to share them or had already made them available online (Franke et al., 2019b). However, more sources for which the data was of potential interest were found (Ismail, 2018; Sinha & Hopkins, 1967) As some of these are old retrieving the original data is likely to be impossible. This does show clearly that it is important to properly archive data for the long term.

Additionally, requirements for datasets include a clear description of the parameters recorded and how they were obtained to ensure the accurate use of the data in future. It is also important that no data are excluded as it can be difficult *a priori* to decide which parameters may be important to include into data analysis in future.

5.4.3 Conclusions

An efficient, easy to use method was developed to assess fish length and area (Objective 5.1a). Based on this the size of *Schistocephalus solidus* plerocercoid infections could successfully be estimated (Objective 5.1b). Data on the temperature dependent growth of *S. solidus* plerocercoids were successfully obtained (Objective 5.2a) and from this it could be confirmed that the development is affected by fish host population (Objective 5.2b). The growth response of *S. solidus* to temperature was found to be quadratic, with the optimum temperature at 23 °C (Objective 5.2c). The temperature dependence is modulated by host size at the time of infection.

Chapter 6

Modelling the life cycle of *Schistocephalus solidus*

6.1 Introduction

Parasites can play important roles in marine (Mouritsen & Poulin, 2002), freshwater (Shields et al., 2002) and terrestrial (Maruyama, Nahas, Moura-Neto, & Santos, 2012) ecosystems. This is particularly evident in the way parasites can regulate host dynamics (Hudson, Dobson, & Newborn, 1998) and alter food-web structures (Dunne et al., 2013). On the other hand, it is evident that changing environmental temperatures can impact parasite fitness (Thomas & Blanford, 2003). Consequently, the role of parasites in ecosystems may be altered with continued changes in environmental temperatures, in particular in parasites with complex life cycles (Harvell et al., 2002). It is, however, unknown what the precise long-term impacts of changing environmental temperatures on parasites with complex life cycles are. Collection of long-term data on internal parasites is difficult as it requires the killing of many host organisms, which in itself could have negative impacts on populations. As an alternative to long-term data, mathematical modelling the known effects of temperature on the different life stages can also help predict past and future impacts of temperature on the role of parasites within ecosystems. This technique is particularly attractive for systems where the impact of temperature on individual life stages is largely known but different life stages respond to changes in temperature in contradictory ways.

Mathematical models have previously been used study the effect of environmental

temperatures on a range of host-parasite interactions. For example, in sea lice, models based on individual life stages show that overall densities of infections on fish are likely to increase with warming temperatures due to a decrease in generation time (Groner et al., 2014). For *Schistocephalus solidus* similar results might be expected as, at least, plerocercoids grow faster at warmer temperatures (Chapter 5). Conversely, in marine trematode parasites infecting amphipods, increases in temperature could lead to local extinctions of the amphipod hosts, followed by local extinction of the parasite (Mouritsen, Tompkins, & Poulin, 2005; Studer, Poulin, & Tompkins, 2013). These examples show that appropriate mathematical models can provide a powerful tool in understanding the combined potential impacts of environmental changes.

The mathematical model used is based on models presented in Mouritsen et al. (2005) and Studer et al. (2013). In the current study the focus of the model is shifted from the host to the parasite and the impact of environmental temperatures on the latter. Estimates for each parameter in the model are either based on results from previous chapters or on literature sources. For each parameter a mean, default value is estimated and then a range around that value is set. By testing a range of likely values for each parameter the impact of that parameter on model outcomes can be assessed and important parameters in the life cycle can be established. Simulations run for the model were be done for temperatures from 10 °C to 20 °C. These temperatures are chosen for two reasons. First, this is the range of mean temperatures that *S. solidus* is likely to experience in British ponds and lakes. Second, most experimental data, in this study and the literature, were collected in approximately this range and can thus most reliably be interpreted in this range.

6.1.1 Aims

The aim of this chapter is to describe the full the life cycle of *Schistocephalus solidus* in a mathematical model and to make predictions on the potential impact of temperature on this life cycle (Aim 6.1). First, a model of the life cycle is constructed, using a set of coupled differential equations (Objective 6.1a). This model is parameterised using the outcome of the experiments described in this thesis as well as values taken from the literature (Objective 6.1b). Then, temperature dependence is added to the model

(Objective 6.1c), and potential equilibria are investigated (Objective 6.1d). Finally, the combined effects of temperature and varying other parameters on the different life stages is investigated (Objective 6.1e). These results will be discussed in the framework of changes in environmental temperatures and compared to field observations.

6.2 Model formulation and parameters

6.2.1 Model Formulation

The whole life cycle of *Schistocephalus solidus* was described by a set of coupled differential equations. This means that for each stage in the life cycle an equation is formulated and that these equations feed into each other (e.g. free living parasites transition to procercoids at a rate based on copepod feeding). Changes in populations are described as the mean density of parasitic stages per square metre in a typical freshwater body in the United Kingdom. Parameters are therefore based on UK data where possible. For simplicity the immature stages of the parasite — the procercoid in the copepod hosts and the plerocercoid in the stickleback hosts — have been combined into a single stage in the model. This makes interpretation of the output and understanding the impact of different parameters more straightforward. Hence, the life cycle is described by three equations:

$$\frac{du}{dt} = hk(T)s - \frac{\alpha_v(T)u}{1+\beta_v u}C - \mu_u(T)u, \tag{6.1}$$

$$\frac{dv}{dt} = \epsilon_v \frac{\alpha_v(T)u}{1+\beta_v u}(C-v) - \frac{\alpha_s v}{1+\beta_s v}B - \mu_v(T)v, \tag{6.2}$$

$$\frac{ds}{dt} = \epsilon_s \frac{\alpha_s v}{1+\beta_s v}B - \mu_s s, \tag{6.3}$$

where free-living stage in the life cycle (i.e. eggs and coracidia) is described by u, the combined immature stage (i.e. procercoid and plerocercoid) is given by v, and the adult worm in the bird definitive hosts is described by s (summarised in Figure 6.1). All temperature dependence in the model is indicated by (T). The number of eggs produced by each mature parasite is described by k and is temperature dependent. The

likelihood of successful hatching of parasite eggs is given by h. The transition from one parasite life stage to the next is described by a Holling type II functional response (i.e. $\frac{\alpha_{(i+1)}(T)i}{1+\beta_{(i+1)}i}$; Holling, 1959), where i indicates the stage in the life cycle and $(i + 1)$ the next stage. In this, α is the encounter rate between the parasite stage and the next host. The handling time of the host when consuming the parasite is given by β, either of the free-living stage (i.e. β_v) or the intermediate host (i.e. β_s). The average density of hosts is described by C for intermediate hosts and B for definitive hosts. For simplicity, it is assumed that copepods can only get infected by one parasite as copepod survival of is reduced with subsequent infections (Michaud et al., 2006; Nie & Kennedy, 1993) and does not lead to completion of the life cycle. Consequently, the number of susceptible copepods is given by $(C - v)$; the total number of copepods minus the number the number of already infected copepods. Mortalities of the different stages in the life cycle are given by μ_i. Not all parasites that are ingested by a suitable host are successful at establishing within the host, hence infectivity is estimated by ε_i.

6.2.2 Parameter estimation

Parameters in the model were estimated based on experimental data from previous chapters and from the literature. An overview of the estimates are given in Table 6.1, including references. A more detailed description of each parameter and how its fixed values and range were derived is given in the following sections.

6.2.2.1 $\mu_u(T)$: Mortality of free-living stages

Hatching success of *S. solidus* eggs, the inverse of mortality, is temperature independent (Section 3.3.1). Thus, egg survival is assumed to be temperature independent. Mortality of *S. solidus* coracidia is temperature dependent (Ismail, 2018), with shorter survival at warmer temperatures. To calculate the relationship between temperature and survival for coracidia, data were extracted using Engauge Digitizer (version 10.4; Mitchell et al., 2019) from Ismail (2018) as raw data were not available. At each temperature the time until 95% mortality (= 5% survival) was estimated. Although it is possible that mortality of infected copepods follows a type I — concave — mortality function (Demetrius, 1978; Pietrock & Marcogliese, 2003), for simplicity a linear

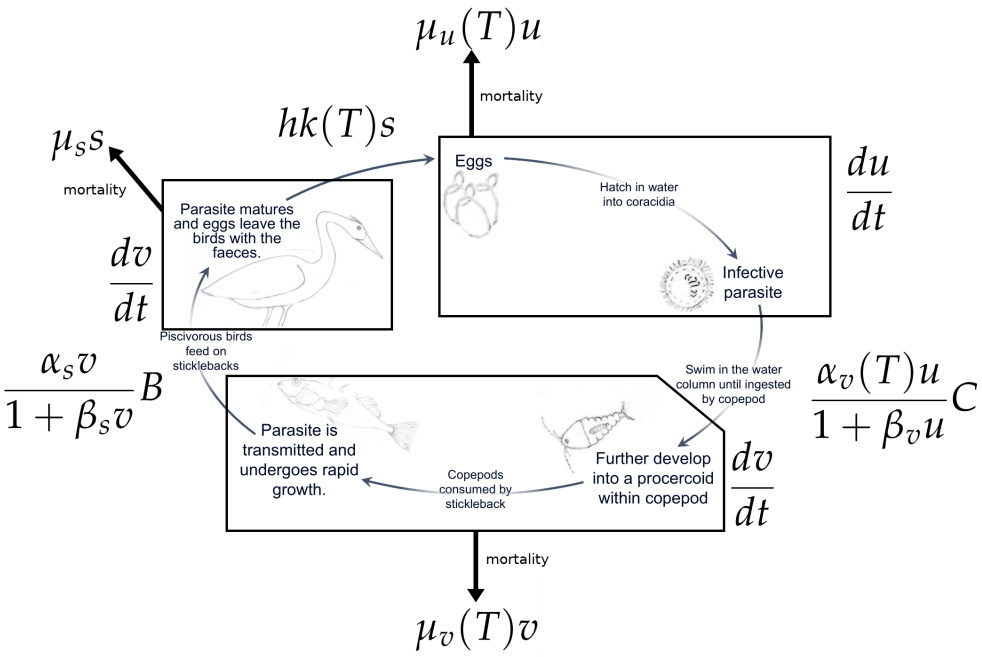


FIGURE 6.1: Linked differential equations describing the *Schistocephalus solidus* life cycle. The free-living, egg stage of the life cycle is denoted by $\frac{du}{dt}$, with inflow of new eggs from the definitive hosts ($hk(T)s$), mortality ($\mu_u(T)u$) and transmission to intermediate hosts ($\frac{\alpha_v(T)u}{1+\beta_v u}C$). In the intermediate hosts ($\frac{dv}{dt}$) mortality ($\mu_v(T)v$) and transmission to definitive hosts are described ($\frac{\alpha_s v}{1+\beta_s v}B$). For the parasites in the definitive hosts mortality rate is also given ($\mu_s s$). See Section 1.2.1 for biological details on the life cycle.

mortality curve was used to calculate μ_u. Temperature dependence was calculated using a linear regression, resulting in the temperature dependent survival curves for coracidia. This results in a daily (day^{-1}) mortality rate of coracidia:

$$\mu_u = 0.22 - 0.030 \times T, \tag{6.4}$$

where T is the experimental temperature. The intercept (0.22) and the slope (0.030) were varied between 0.05 and 0.40 and from 0.005 to 0.70 respectively.

TABLE 6.1: Model parameters with sources. Temperature is indicated by T and ranges from 10 to 20 °C.

Parameter	Definition	Default value [range]	Units	Source
$\mu_u(T)$	*S. solidus* eggs and coracidia mortality rate	$0.22(\alpha) + 0.030(\beta) \times T \begin{bmatrix} \alpha = 0.05 - 0.40 \\ \beta = 0.005 - 0.070 \end{bmatrix}$	day^{-1}	[20, 24]
$\mu_v(T)$	*S. solidus* intermediate host mortality rate	$0.99 - 0.002 \times T$	day^{-1}	[19-21]
μ_s	*S. solidus* mortality rate in bird host	1	day^{-1}	[3, 8]
$\alpha_v(T)$	Free-living stages-intermediate hosts encounter rate	$25(\alpha) - 0.5(\beta) \times T \begin{bmatrix} \alpha = 12 - 50 \\ \beta = 0.1 - 1 \end{bmatrix}$	day^{-1}	[4-5, 12, 17, 21-22, 24-26]
α_s	Intermediate-definitive hosts encounter rate	$1[0.1 - 5]$	day^{-1}	[1]
β_v	Handling time of free-living stages	$0.05[0.01 - 0.10]$	–	[7, 11]
β_s	Handling time of intermediate hosts	$0.05[0.01 - 0.10]$	–	[14]
$k(T)$	Temperature dependent parasite egg production	$10^5 \times (-0.7(\alpha) \times T^2 + 34(\beta) \times T - 240)^{0.5} \begin{bmatrix} \alpha = 0.5 - 0.9 \\ \beta = 10 - 50 \end{bmatrix}$	–	[15-16, 19, 21]
p_h	Proportion of parasite eggs hatching	$0.35[0.25 - 0.45]$	day^{-1}	[13, 18]
ε_v	Infectivity of free-living parasites	$0.13[0.01 - 0.25]$	–	[15, 20-21]
ε_s	Infectivity of plerocercoids parasites	$0.75[0.60 - 0.80]$	–	[8]
C	Density of intermediate hosts	$10[5 - 50]$	m^{-2}	[2, 6, 10]
B	Density of definitive, bird hosts	$0.01[0.003 - 0.03]$	m^{-2}	[27]

[1] Cooper (1918); [2] Elgmork (1955); [3] Dubinina (1980); [4] Allen and Wootton (1982); [5] Sakanari and Moser (1985); [6] Verreth (1990); [7] Ibrahim and Huntingford (1992); [8] Tierney and Crompton (1992); [9] Reed and McIntyre (1995); [10] Shires (1995); [11] Wickham (1995); [12] Elliott and Leggett (1997); [13] Christen et al. (2002); [14] Gils, Piersma, Dekinga, and Dietz (2003); [15] Dörücü et al. (2007); [16] Macnab and Barber (2012); [17] Hovel et al. (2015); [18] Simmonds and Barber (2016); [19] Franke et al. (2017); [10] Ismail (2018); [21] Franke et al. (2019a); [22] Schulhof, Shurin, Declerck, and Van de Waal (2019); [23] Section 3.3.2.1; [24] Section 3.3.1; [25] Section 4.3.1; [26] Section 4.3.2.2; [27] Section 6.2.2.12;

6.2.2.2 $\mu_v(T)$: Mortality of intermediate stages

Mortality rate of *Cyclops strenuus* copepods is increased with infection (Wedekind, 1997) and temperature. Additionally, the impact of infection is more severe at warmer temperatures (Ismail, 2018). Mean survival time of infected copepods at 20 °C is 3 weeks, while at 10 °C it is approximately 5 weeks (Ismail, 2018). From this the temperature dependent survival per day (day^{-1}) of infected copepods can be estimated as:

$$\mu_v = 0.99 - 0.002 \times T. \tag{6.5}$$

For uninfected (Section 3.3.2.1) as well as infected (Franke et al., 2017, 2019a) sticklebacks, mortality rate is temperature dependent. Yet it is much lower than that of copepod hosts and will not be considered, as the main limiting factor for parasite survival appears to be copepod mortality. Only the slope will be varied from 0.001 to 0.010.

6.2.2.3 μ_s : Mortality of adult worms

Of plerocercoids that successfully establish inside the bird host, all parasites die after 7 to 14 days inside the gut of the bird (Dubinina, 1980; Tierney & Crompton, 1992). Mortality of worms appears to not affect the total output of eggs and is thus set as 1 day^{-1}, i.e. after each simulated day in the mathematical model all previous living adult worms die.

6.2.2.4 α_v : Free-living stage encounter rate

No effect of temperature on the maximum feeding rate of copepods was found (Section 4.3.1). Also, infection rate of copepods was not affected by temperature in the microcosms (Section 4.3.2.2) indicating that there is no direct effect of temperature on transmission of *Schistocephalus solidus* from eggs to the copepod first intermediate host.

However, there could be indirect effects. For example, it is expected that the relative density of coracidia in the water column will decrease with increasing temperatures for two reasons. First, hatching of parasite eggs happens over a larger time

window without the total number of eggs hatching changing (Section 3.3.1). Second, the growth rate of the populations of other planktonic organisms is expected to increase at warmer temperatures (Schulhof et al., 2019). Coracidia are thus expected to be present in lower absolute densities and to be diluted more. This would explain the observed lower rate of infections at warmer temperatures in another cestode species (Sakanari & Moser, 1985). Based on Section 3.3.1 the relationship between temperature and encounter rate was described as:

$$\alpha_s = 25 - 0.5 \times T \tag{6.6}$$

per day (day^{-1}). Both the slope (0.5) and the intercept (25) will be independently varied. Slope values from 0.1 to 1.0 and intercept values from 12 to 50 will be tested. The smallest intercept value is chosen to prevent the equation from becoming negative, which would indicate negative encounter rates which is nonsensical. For the same reason the maximum slope was taken to be 1.0.

Overall feeding rates of sticklebacks and thus potential exposure to parasites, increase with increasing temperature (Allen & Wootton, 1982; Hovel et al., 2015), given that food composition remains largely unchanged. However, at the upper extremity of the temperature range (~25 °C) fish experience high levels of stress and stop feeding (Hovel et al., 2015). Although the exact threshold at which fish stop feeding is likely to be population dependent (Franke et al., 2019a). Additionally, warmer temperatures make sticklebacks more likely to feed on larger prey individuals (Elliott & Leggett, 1997). A shift in temperature from 3 °C to 15 °C can shift the preference for smaller prey (~4 mm) to larger prey items (> 5 mm). However, all these effects are expected to be smaller than any temperature effects on copepod feeding, as well as a lower overall feeding rates than that of copepods. Thus the model will be parameterised using copepod feeding rates.

6.2.2.5 α_s : Intermediate stage encounter rate

Stickleback hosts are consumed by a wide range of piscivorous birds (Cooper, 1918), but on the other hand is seems likely that many of these species have a wide diet breath. This makes it hard to estimate accurately how many infected sticklebacks are

likely to be consumed by definitive bird hosts per day. For this reason a wide range of values was tested. The fixed value was set to 1 day^{-1}, with a range from 0.1 to 5 day^{-1}.

6.2.2.6 β_v & β_s : Handling times

As handling times of prey for copepods (Wickham, 1995), sticklebacks (Ibrahim & Huntingford, 1992) and birds (Gils et al., 2003) are short (i.e. in the order of seconds to minutes) compared to the time scale under investigation (days), and not temperature dependent, both β's are set to 0.05 for the fixed value, with a range from 0.01 to 0.1.

6.2.2.7 *k(T)* : Number of parasite eggs

The number of eggs a mature plerocercoid produces was estimated from Dörücü et al. (2007). The number of eggs produced depends on plerocercoid size at the time of infection of the bird host, and whether sticklebacks carry one or multiple infections (Dörücü et al., 2007). Most sticklebacks that are naturally infected with *S. solidus* harbour more than one infection (Arme & Owen, 1967) therefore only values for multiple infections are used. At warmer temperatures *S. solidus* parasites grow faster in the stickleback host (Franke et al., 2017, 2019a; Macnab & Barber, 2012), so at warmer temperatures parasites are likely to have obtained larger sizes when infecting the fish host. Thus, the number of eggs produced was made temperature dependent in the model.

For plerocercoids from multiply infected fish the number of eggs produced by mature worms shows a slight negative dependence on worm size:

$$log_{10}(k) = 4.66 - 0.472 \times log_{10}(W_p) \tag{6.7}$$

(Dörücü et al., 2007). Here W_p is the weight of the plerocercoid at time of the infection of the bird.

The size of plerocercoids at the time of infection was estimated in Chapter 5, based on the assumption that the length of the period of infection remains largely unchanged, Equation 5.4 states:

$$W_p = -0.67 \times T^2 + 21.2 \times T - 0.15 \times W_f + 0.019 \times T \times W_f - 137.$$

This can be simplified to:

$$W_p = -0.67 \times T^2 + 34.2 \times T - 239, \tag{6.8}$$

if the mean weight of fish (W_f) at time of infection of 683 mg is taken (Section 5.3.2). Substituting Equation 6.8 into Equation 6.7 gives:

$$log_{10}(k) = 4.66 - 0.472 \times log_{10}(-0.67 \times T^2 + 34.2 \times T - 239), \tag{6.9}$$

which can be rewritten and simplified as:

$$k = 10^5 \times (-0.7 \times T^2 + 34 \times T - 240)^{0.5}, \tag{6.10}$$

describing the temperature dependence of the number of eggs produced by each mature parasite. This equation always produces positive values within the range of temperatures from 10 to 20 °C, but can become negative at lower values and is thus not appropriate for lower values.

6.2.2.8 p_h : Proportion of *Schistocephalus solidus* egg hatching

Under typical laboratory conditions between 10 and 50% of *S. solidus* eggs originating from outcrossed worms hatch successfully into coracidia (Christen et al., 2002; Simmonds & Barber, 2016) depending on the individual. The median number is between 30 and 40% (Section 3.3.1) thus, in the model, the number of eggs that is produced per worm is multiplied by 0.35 (day^{-1}) to correct for non-hatching of eggs. The range used is between 0.25 and 0.45, as no field data are available.

6.2.2.9 ε_v : Infectivity to intermediate hosts

The number of coracidia that successfully establish in copepods is not affected by temperature as shown in Ismail (2018). The observed establishment success is approximately 40% (47/120 individual exposures).

Similarly, the infectivity of procercoids with a cercomer in fish was independent of temperature as shown in Franke et al. (2019a) with an overall infectivity of 33% (139/422 exposed sticklebacks). The same was found in Macnab and Barber (2012) where approximately 50% (13/25) of exposed fish became infected, regardless of temperature.

Multiplying the infectivities gives a probability of between 13% and 20% for an individual coracidium successfully infecting a stickleback host after first infecting a copepod host. As the 13% estimate is based on a much larger sample size, this will be taken as a fixed value. The higher success rate of 20% will be included in the range of values tested; 5% to 25% to estimate the impact of infectivity on infection numbers.

6.2.2.10 ε_s : Infectivity to definitive hosts

Limited information on the establishment success of *S. solidus* in the definitive bird host is available. In chickens (*Gallus gallus*) the establishment of worms larger than 50 mg is approximately 75% (Tierney & Crompton, 1992). Additionally, a range from 60% to 80% will be tested, as that is the range that infectivity for most parasites sizes fall within.

6.2.2.11 C : Intermediate hosts densities

Copepod densities in a pond can reach about 89 individuals per litre (Verreth, 1990). This means that per square metre pond densities could reach about 9000 individuals, assuming that most copepods occupy the top layers of the water column. This estimate corresponds to earlier observations that found that the maximum number of *C. strenuus* individuals per square metre was 12000 (Elgmork, 1955).

Shires (1995) estimated the number of three-spined sticklebacks in an urban pond in Inverleith Park, Edinburgh. They first established the likelihood of sticklebacks being caught in traps in the lab and then used the same trapping method to catch fish in the pond. From these numbers they were able to estimate the total number of sticklebacks in the pond. The examined part of the pond was approximately 1680 m^2, and they estimated that area contained 84,821 sticklebacks. This means that per

square metre there are approximately 50 sticklebacks. However, densities in this pond are exceptionally high (Barber, I, *personal communication*).

Population densities of fish tend to be lower in ponds in urban areas, like the pond in Inverleith Park, than in rural areas (Fitzpatrick, Harris, Arnold, & Richards, 2004). This is likely to be true for sticklebacks as well because urban ponds are more likely to be eutrophic than rural ponds (Waajen, Faassen, & Lurling, 2014) and sticklebacks perform worse under eutrophic conditions (Gagnon, Grafnings, & Bostrom, 2017). However, rural ponds are also more likely to harbour fish predators (Fitzpatrick et al., 2004) of sticklebacks and thus reduce stickleback numbers. For this reason, a wide range of stickleback densities is assessed in the initial models.

As the life stages in the copepod and stickleback are combined in the model these values are combined here as well. Using only the stickleback, as there are fewer sticklebacks than copepods, the value will be varied between 5 and 50 in the analyses of the models, using a fixed value of 10 intermediate hosts.

6.2.2.12 B : Definitive host densities

Many different piscivorous bird species feed on three-spined sticklebacks (e.g. Giles, 1981). However, looking at the densities of some of the most abundant of these birds shows that the average number of birds per m^2 is likely to be low. Based on data from the Grebe (local birdwatchers newsletter, list compiled by Brian Moore) , Rutland water, a water body comprising approximately 9 km^2, counts between 1500 and 2500 individuals of the most abundant bird species (i.e. Widgeons, Coots and Tufted Ducks). This would give a density of 0.0003 birds per square metre of water. As multiple species of birds live alongside each other a range of bird host densities is chosen from 0.003 - 0.03, with 0.01 as the fixed value.

6.3 Model simulation

To get insight into the behaviour of the models and to estimate population densities of the different parasite life stages for the fixed values of the parameters, models were

simulated with a Runge-Kutta fourth-order method (Tan & Chen, 2012). This method gives a numerical solution to ordinary differential equations. Using the equations:

$$y_{i+1} = y_i + \frac{1}{6}(k_1 + 2k_2 + 2k_3 + k_4)h, \tag{6.11}$$

$$k_1 = f(x_i, y_i), \tag{6.12a}$$
$$k_2 = f(x_i + \frac{1}{2}h, y_i + \frac{1}{2}k_1 h), \tag{6.12b}$$
$$k_3 = f(x_i + \frac{1}{2}h, y_i + \frac{1}{2}k_2 h), \tag{6.12c}$$
$$k_4 = f(x_i + h, y_i + k_3 h), \tag{6.12d}$$

the numerical solution can be found. After 50 simulation days at 15 °C these simulations show that an equilibrium state is approached (Figure 6.2). The step size (h) was set to 0.00005 based on simulations with larger and smaller step sizes. The step size was a factor ten smaller then the step size at which step size stopped affecting simulation output. The simulations gave densities of 2.2×10^4 parasite eggs, 9.8 parasite immature stages and an average of 0.05 adult worms per square metre.

However, it is possible that different starting values could result in different equilibrium values being reached. Therefore the next step was to analytically approach all potential equilibrium states.

6.4 Model equilibria and their stability

By identifying the model equilibria, the values towards which the system tends can be found. This can inform us about the likely states that the system may occupy. Additionally, it is important to assess which equilibria are stable and which unstable. Stable equilibria are states that the system tends towards, whilst unstable equilibria are those the system can occupy (i.e. no net change in numbers) but, once disturbed, from which the system will diverge (Kot, 2001).

When the rate of change in all three equations is zero the system has reached an equilibrium state. To find the equilibrium states, first the change in the whole system

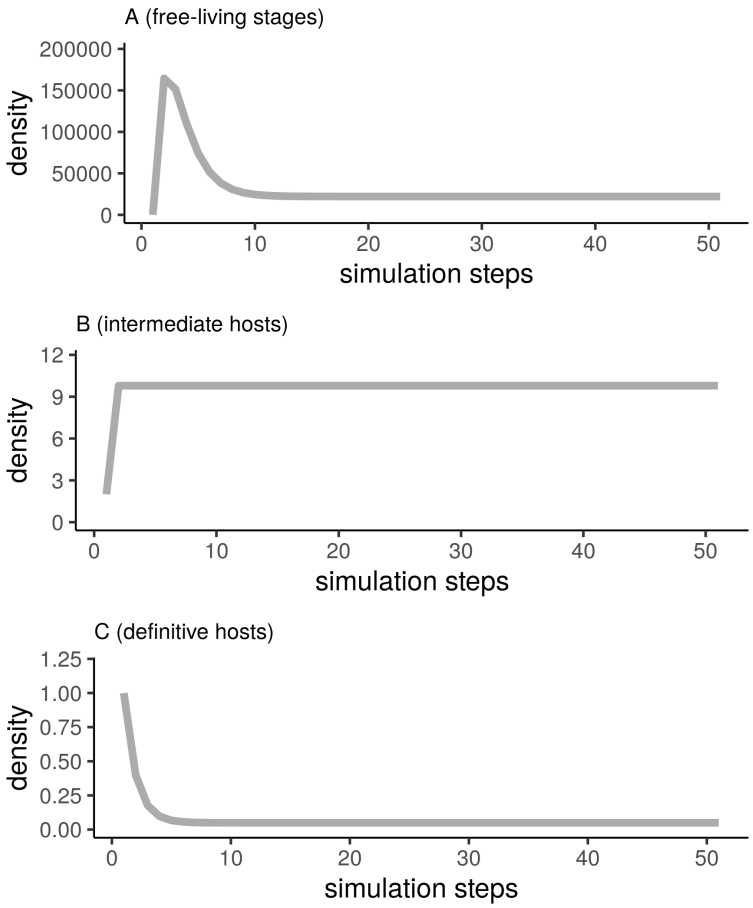


FIGURE 6.2: Output for each parasite stage of Runge-Kutta simulation for 50 days with step size = 0.00005. Starting values used are $u = 5$ (A), $v = 2$ (B), and $s = 1$ (C) at temperature 15 °C. After 50 simulation steps values are approximately $u = 22000$ (A), $v = 9.8$ (B), and $s = 0.049$. Values are densities per square metre.

was set equal to zero and the change in v (intermediate stages) was expressed as a function of u (free-living stages). Then finding the values for u where the change in v was equal to zero gave the equilibrium values. Finally, stability of equilibrium states was assessed by investigating the eigenvalues. If the real parts (as opposed to the imaginary parts) of the eigenvalues are all negative, this means that the systems moves towards the given equilibrium. Therefore the equilibrium is stable (Kot, 2001).

6.4.1 Derivation of equilibrium states

First, the system was rewritten from three algebraic equations for the equilibria to one equation. The net change in adult parasites (s; Equation 6.3) is set equal to zero and

the function rewritten as a function of s. For clarity, the temperature dependency is ignored in the notation.

$$\epsilon_s \frac{\alpha_s v}{1+\beta_s v} B - \mu_s s = 0, \tag{6.13a}$$

$$s = \frac{\epsilon_s}{\mu_s}\left(\frac{\alpha_s v B}{1+\beta_s v}\right). \tag{6.13b}$$

Now, s can be substituted into Equation 6.1 for the free-living parasites. Then also setting this equal to zero and simplifying gives:

$$hks - \frac{\alpha_v u}{1+\beta_v u} C - \mu_u u = 0, \tag{6.14a}$$

$$\frac{hk\epsilon_s}{\mu_s}\frac{\alpha_s v B}{1+\beta_s v} - \frac{\alpha_v u}{1+\beta_v u} C - \mu_u u = 0 \tag{6.14b}$$

$$\frac{\alpha_s v B}{1+\beta_s v} = \left(\frac{\alpha_v u}{1+\beta_v u} C + \mu_u u\right)\frac{\mu_s}{hk\epsilon_s} \tag{6.14c}$$

Then, setting the change in v (parasites in intermediate hosts; Equation 6.2) equal to 0 and rewriting the functions, this can be set equal to Equation 6.14c (Equation 6.15d):

$$\epsilon_v \frac{\alpha_v u}{1+\beta_v u}(C - v) - \frac{\alpha_s v B}{1+\beta_s v} - \mu_v v = 0, \tag{6.15a}$$

$$\frac{\alpha_s v B}{1+\beta_s v} = \epsilon_v \frac{\alpha_v u}{1+\beta_v u}(C - v) - \mu_v v, \tag{6.15b}$$

$$\left(\frac{\alpha_v u}{1+\beta_v u} C + \mu_u u\right)\frac{\mu_s}{hk\epsilon_s} = \frac{\alpha_s v B}{1+\beta_s v} = \epsilon_v \frac{\alpha_v u}{1+\beta_v u}(C - v) - \mu_v v, \tag{6.15c}$$

$$\left(\frac{\alpha_v u}{1+\beta_v u} C + \mu_u u\right)\frac{\mu_s}{hk\epsilon_s} = \epsilon_v \frac{\alpha_v u}{1+\beta_v u}(C - v) - \mu_v v. \tag{6.15d}$$

For clarity $\frac{\alpha_v u}{1+\beta_v u}$ is written as $\varphi(u)$. Then rewriting the equation as a function of v results in:

$$(\phi(u)C + \mu_u u)\frac{\mu_s}{hk\epsilon_s} = \epsilon_v \phi(u)(C - v) - \mu_v v, \tag{6.16a}$$

$$\epsilon_v \phi(u)C - \epsilon_v \phi(u)v - \mu_v v = (\phi(u)C + \mu_u u)\frac{\mu_s}{hk\epsilon_s}, \tag{6.16b}$$

$$v(-\epsilon_v \phi(u) - \mu_v) + \epsilon_v \phi(u)C = (\phi(u)C + \mu_u u)\frac{\mu_s}{hk\epsilon_s}, \tag{6.16c}$$

$$v = \frac{(\phi(u)C + \mu_u u)\frac{\mu_s}{\epsilon_s hk} - \epsilon_v \phi(u)C}{(-\epsilon_v \phi(u) - \mu_v)}. \tag{6.16d}$$

This function expresses v (the number of parasites in intermediate hosts) as a function of u (the number of parasite eggs) and can be substituted into Equation 6.2. This function will be called $g(u)$ and can be visually explored to find the location of equilibria states. Only one equilibrium state is found. Increasing temperature moves the equilibrium point to higher values of free-living parasite stages (the point where the curves cross zero on the y-axis in Figure 6.3).

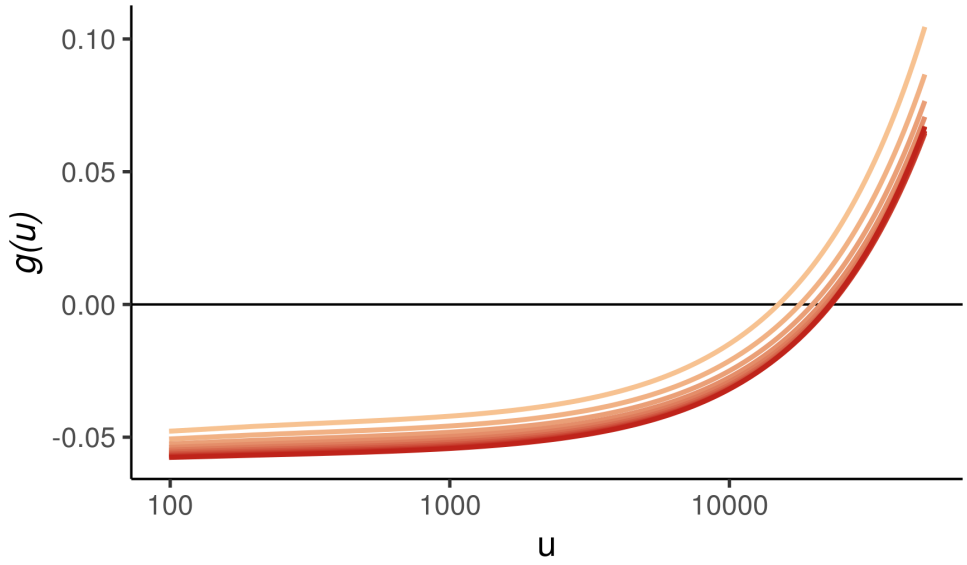


FIGURE 6.3: Visual exploration of the equilibrium states using Equation 6.16d (expressed as $g(u)$) for the temperature range from 10 °C (yellow) to 20 (dark red) °C. Temperature changes by 1 °C increments. The x-axis — the density of parasite eggs — is on a $\log_{10}$-scale.

Next, it is possible to approach the equilibrium values for the different life stages

numerically. This can be done using a simple bisection function on the graph. Thus, exact values were calculated for the equilibrium point at 15 °C.

$$u = 2.3 \times 10^4,$$

$$v = 9.8,$$

$$s = 0.049.$$

These values correspond to Figure 6.2, although not exactly, indicating that for the simulations the equilibrium had not been reached.

6.4.2 Stability of the equilibrium

To determine whether an equilibrium is stable, i.e. whether the system tends to move toward it or away from it, the eigenvalues of its Jacobian matrix should be considered. If the real part of all eigenvalues is negative the equilibrium is stable, however if at least one eigenvalue is positive the equilibrium is unstable and the system tends to move away from it (Kot, 2001).

If we consider:

$$f_1 = hk(T)s - \frac{\alpha_v u}{1+\beta_v u}C - \mu_u u, \tag{6.17a}$$

$$f_2 = \epsilon_v \frac{\alpha_v u}{1+\beta_v u}(C-v) - \frac{\alpha_s v}{1+\beta_s v}B - \mu_v v, \tag{6.17b}$$

$$f_3 = \epsilon_s \frac{\alpha_s v}{1+\beta_s v}B - \mu_s s, \tag{6.17c}$$

for $T = 15$, and

$$J = \begin{bmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{bmatrix}$$

where

$$
\begin{aligned}
a_{11} &= \frac{\partial f_1}{\partial x_1} = \frac{\partial f_1}{\partial u} = -\frac{\alpha_v C}{(1+\beta_v u^*)^2} - \mu_u, \\
a_{21} &= \frac{\partial f_2}{\partial x_1} = \frac{\partial f_2}{\partial u} = \frac{\epsilon_v \alpha_v (C - v^*)}{(1+\beta_v u^*)^2}, \\
a_{31} &= \frac{\partial f_3}{\partial x_1} = \frac{\partial f_3}{\partial u} = 0, \\
a_{12} &= \frac{\partial f_1}{\partial x_2} = \frac{\partial f_1}{\partial v} = 0, \\
a_{22} &= \frac{\partial f_2}{\partial x_2} = \frac{\partial f_2}{\partial v} = \frac{(\epsilon_v \alpha_v u^*)(1+\beta_v u^*)}{(1+\beta_v u^*)^2} - \frac{\alpha_s B}{(1+\beta_s v^*)^2} - \mu_v, \\
a_{32} &= \frac{\partial f_3}{\partial x_2} = \frac{\partial f_3}{\partial v} = \frac{\epsilon_s \alpha_s B}{(1+\beta_s v^*)^2}, \\
a_{13} &= \frac{\partial f_1}{\partial x_3} = \frac{\partial f_1}{\partial s} = k, \\
a_{23} &= \frac{\partial f_2}{\partial x_3} = \frac{\partial f_2}{\partial s} = 0, \\
a_{33} &= \frac{\partial f_3}{\partial x_3} = \frac{\partial f_3}{\partial s} - \mu_s.
\end{aligned}
$$

If we then substitute the default values into the Jacobian matrix we get:

$$
J = \begin{bmatrix} -0.82 & 0 & 4.4 \times 10^5 \\ 0.0004 & 38.01 & 0 \\ 0 & 0.0034 & -1 \end{bmatrix}.
$$

From this Jacobian the eigenvalues can be calculated, by then setting the determinant of (J - λ) to zero, resulting in:

$$
\begin{bmatrix} -0.82 \\ -1.00 \\ -39.92 \end{bmatrix}.
$$

Since all the real parts of the eigenvalues are negative the equilibrium is stable.

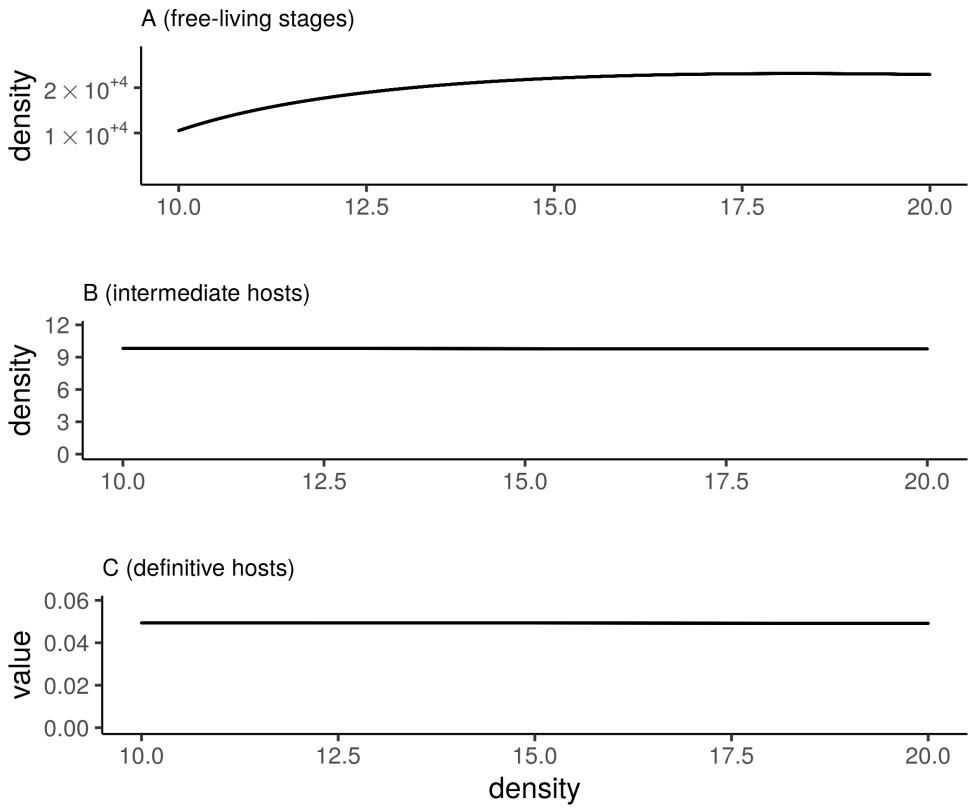


FIGURE 6.4: Temperature dependence of equilibrium values of the model for the full scale of temperatures. Each line is based on 15000 individual points (simulations).

6.5 Analysis of the model for key parameters

All parameters in the model were varied individually for a range of temperatures (i.e. 10 - 20 °C) to assess the impact of changing their value on the model outcomes. Both temperature and the parameter under investigation was tested at 100 values, resulting in 10000 simulations (100 ×100) for each combination. In total five parameters were selected, based on their behaviour, to be plotted in density plots here. All other parameters were tested as well and showed similar behaviour or did not impact model simulation outcomes. For optimal representation of the data, all densities of free-living parasite stages are plotted on a $\log_{10}$-scale. Also, the viridis colour scheme was used for density plots as this allows for the most accurate interpretation (Borkin et al., 2011).

6.5.1 The impact of the base ingestion of free-living stages (α_v - *intercept*)

The densities of the different stages of the *Schistocephalus solidus* life cycle responded to a change in the base ingestion and temperature in opposite directions. At low temperatures and high consumption rates the density of free-living stages was low, while at high temperatures and low consumption rates it was highest (Figure 6.5A). The response in the other two life stages, i.e. intermediate and adult stages, was the opposite, low densities at high temperatures and low consumption rates (Figure 6.5B & C).

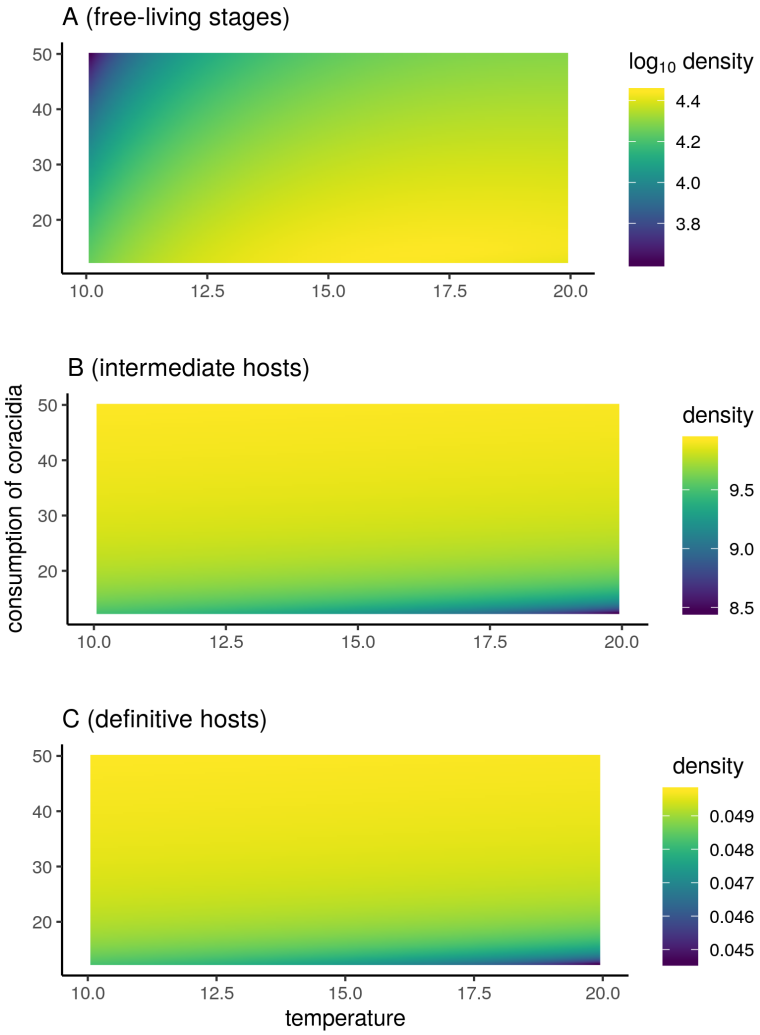


FIGURE 6.5: Densities per m^2 of different parasite stages of the life cycle (A. free-living, B. in intermediate host, C. adult parasites) for different temperatures and consumption rates (temperature independent) of free-living stages — coracidia — by intermediate hosts (α_v - *intercept*).

6.5.2 The impact of the temperature dependent ingestion of free-living stages (α_v - *slope*)

Changing the temperature dependence of the ingestion of free-living stages by intermediate hosts shows that at high values for both temperature and temperature dependence there is a decrease in the density of intermediate and adult parasite stages (Figure 6.6B & C). In the free-living stages, the reverse pattern in visible; at low temperatures densities decrease and at high temperatures, especially at high temperature dependent feeding, density increases (Figure 6.6A). This is as expected as temperature has a negative effect on consumption rate of copepods (see Table 6.1).

6.5.3 The impact of the infectivity of free-living stages (ε_v)

The infectivity of free-living stages to intermediate hosts was initially tested at values from 0.01 (1%) to 1.0 (100% of parasites managing to establish in the host). Inspection of the plots showed that infectivity levels higher than approximately 25% did not affect the outcome of the simulations so only values between 0.01 and 0.25 are shown for more convenient interpretation (Figure 6.7).

Only at very low values (<5%) of infectivity of free-living stages to intermediate hosts were the densities of parasite stages in the system affected. At low levels of infectivity, densities of all life stages drop (Figure 6.7), but especially in the intermediate and the definitive hosts. The free-living stages on the other hand showed a stronger temperature dependence, with low densities at low temperatures (Figure 6.7A).

6.5.4 The impact of the number of intermediate hosts (C)

The number of available intermediate hosts per square metre of pond surface was varied from 5 to 50 copepod individuals. Densities of free-living stages were low at low intermediate host densities at all temperatures, but increased at higher host densities except for low temperatures where high intermediate host densities were associated with a drop in free-living stages. At high temperatures, intermediate host densities had a positive effect on the density of free-living stages (Figure 6.8A). The number

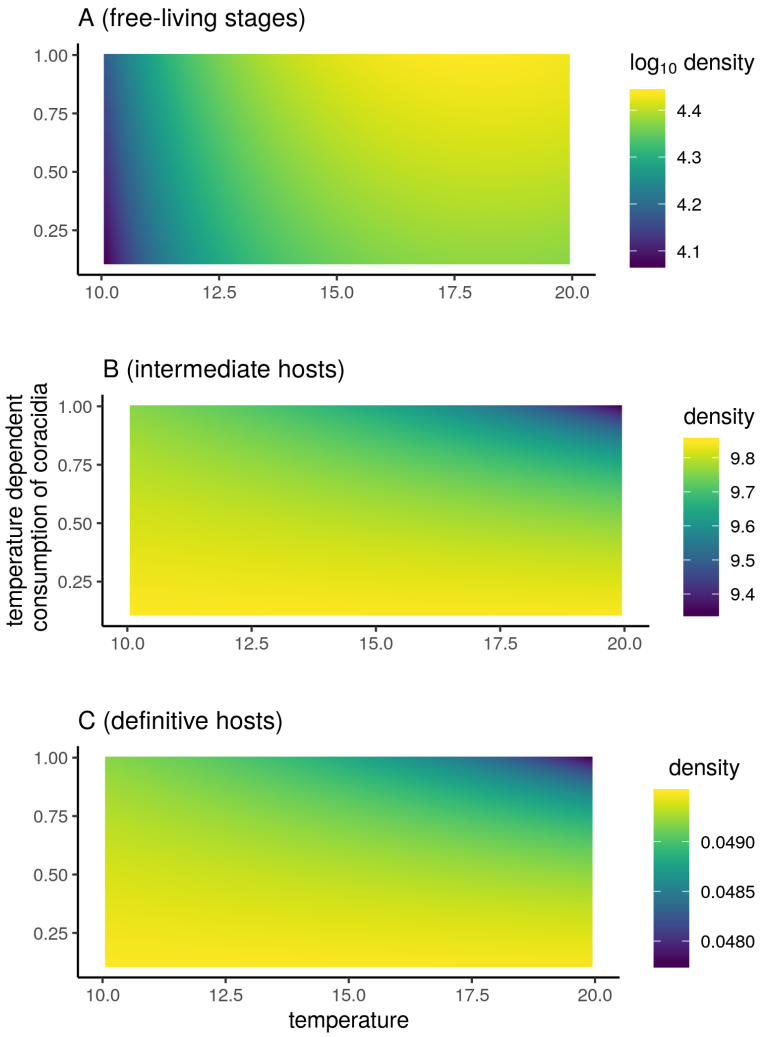


FIGURE 6.6: Impact on the density of the life stages of the parasite (A. free-living, B. in intermediate host, C. adult parasites) of varying the temperature dependent consumption rate of free-living stages — coracidia — by intermediate hosts (α_v - *slope*) and temperature. Note that consumption is temperature dependent. Densities are per m^2.

of infections in the intermediate hosts increased linearly with increasing intermediate host numbers, and was not temperature dependent (Figure 6.8B). The density of adult parasites showed a convex response to increasing copepod numbers, so following the increase in intermediate hosts (and infections therein) the number of infections in definitive hosts increased, but with a delay. Infection rate in the intermediate hosts was always close to saturation.

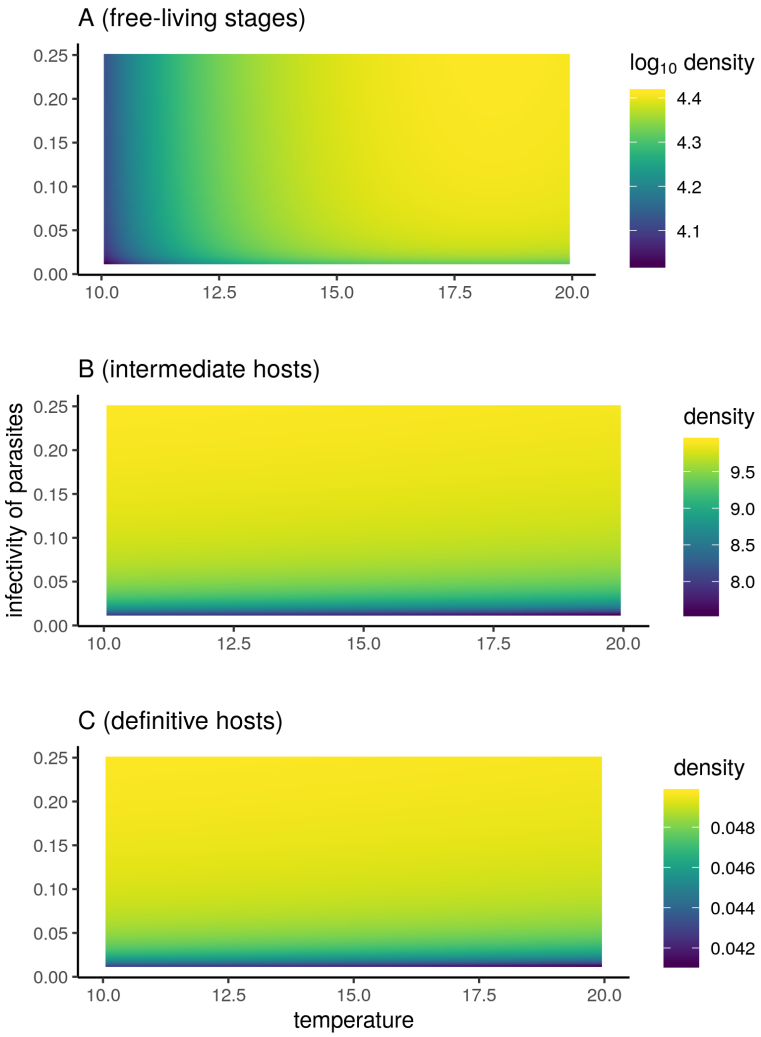


FIGURE 6.7: Impact of varying infectivity of free-living parasite stages to the intermediate hosts (ε_v) on densities per m^2 of the parasite life stages (A. free-living, B. in intermediate host, C. adult parasites).

6.5.5 The impact of the number of definitive hosts (*B*)

The density of bird definitive hosts was varied between 0.003 and 0.03 birds per square metre of pond. The number of parasites inside the bird hosts increases linearly with the number of bird hosts available and no effect of temperature is seen (Figure 6.9C). This results in an increase in the density of free-living stages at high bird densities and warmer temperatures, while at low temperature and low bird host densities their densities drop (Figure 6.9A). The latter, in particular, is reflected in the intermediate

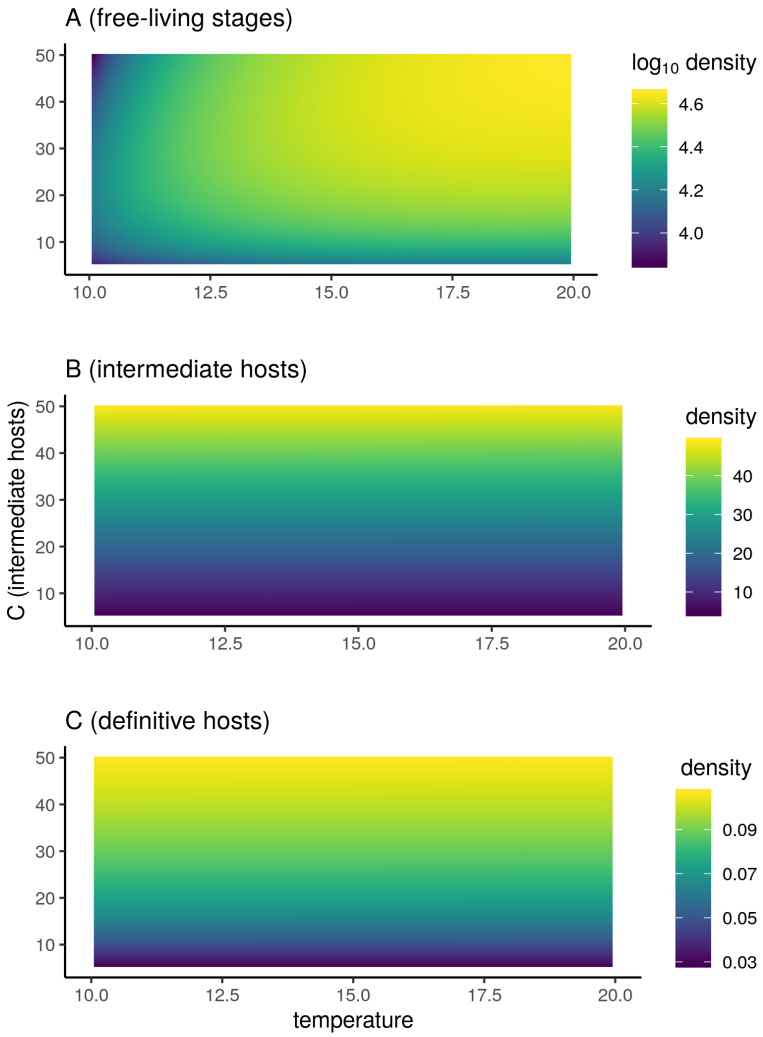


FIGURE 6.8: Density of different parasite life stages (A. free-living, B. in intermediate host, C. adult parasites) in response to densities of intermediate hosts (C) per m^2.

hosts where at very low temperatures and definitive host densities, densities of parasite stages drop (bottom-left of Figure 6.9B). Otherwise, the pattern of intermediate parasite stages is a mirror image of the pattern seen in free-living stages; high definitive host densities and high temperature decrease intermediate parasite stage densities. Low values of both parameters increase intermediate parasite densities, except at very low values (Figure 6.9B).

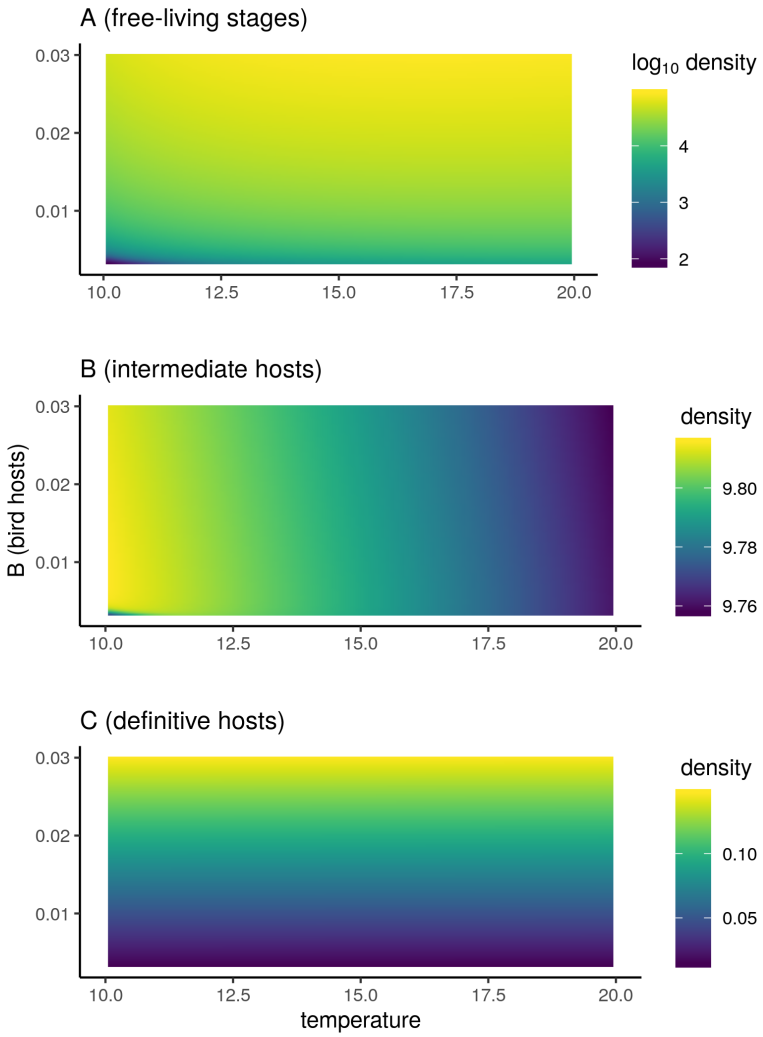


FIGURE 6.9: Response of different parasite life stages (A. free-living, B. in intermediate host, C. adult parasites) to varying density of bird hosts (B) and temperature.

6.6 Discussion

A mathematical model using coupled differential equations, was constructed of the simplified life cycle of *Schistocephalus solidus*. For each of the parameters a realistic range of values was established based on the literature. The model reaches equilibrium for the range of values used and this equilibrium is stable and temperature dependent. The availability of hosts and the consumption rate of infective stages appear to be the most important parameters affected by temperature. No bifurcations were found.

6.6.1 Consumption of infective stages (α_v)

The magnitude of the temperature dependent consumption of infective free-living stages (i.e. the slope in Equation 6.6) had a negative effect on the densities of intermediate and adult parasite stages (Figure 6.6B & C). This effect was strongest at warmer temperatures where the steepest gradient for parasite densities was seen for increasing the consumption rate of free-living stages by prospective intermediate hosts (Figure 6.6B & C). Estimates for the 'base consumption' (i.e. the intercept in Equation 6.6) had a positive effect on the densities of these stages in the life cycle (Figure 6.5).

This indicates, that with warming temperatures, the ingestion rate of free-living parasites by intermediate hosts has an increasing impact on the density of intermediate and adult parasites stages. This means that at warmer temperatures reliable estimates of encounter rates are more important although the current parameterisation suggests only small changes in densities.

6.6.2 Infectivity of infective stages

To the best of our knowledge, there is no direct effect of temperature on the infectivity of free-living stages (coracidia) or proceroids to their respective next hosts (Franke et al., 2019a; Ismail, 2018). This is reflected in the densities of intermediate and adult parasite stages in the current model; no temperature dependence is seen (Figure 6.7). However, at low levels of infectivity there is a clear drop in densities of all life stages, but, in particular, intermediate and adult stages. In the free-living stages an additional effect of temperature is seen, most likely caused by the temperature dependence of egg production in adult stages (see Section 6.2.2.7).

6.6.3 Host densities

The impact of changes in intermediate host densities appears to be temperature dependent in free-living stages at high intermediate host densities (Figure 6.8A). The other life stages of the parasite are not directly impacted by temperature but are by the density of intermediate hosts, with parasite densities closely following host densities (Figure 6.8B & C). Parasite densities in intermediate hosts tend be near saturation,

a consequence of high free-living stage densities and high encounter rates (ε_v) and the model's restriction on the number of parasites in intermediate hosts (C - v).

Varying the density of definitive (B; bird) host leads to the largest changes in absolute densities of the different parasite stages (Figure 6.9). While adult parasite densities reach the most extreme values — higher and lower than for varying any other parameter — free-living stages reach their lowest density at low bird densities combined with low temperatures. The latter results in lower average numbers of eggs produced by adult parasite worms as their growth rate is reduced (Chapter 5).

6.6.4 Number of infected fish in natural populations

Infection rates in the simulations were very high for intermediate stages (i.e. copepods infected with procercoids and sticklebacks infected with plerocercoids). To test the appropriateness of the model it is useful to compare these numbers to field observations of infection levels. Infection levels in naturally infected populations in England of three-spined sticklebacks with *Schistocephalus solidus* are generally high (Chappell, 1969). Infection rates are especially high during the second half of the year, after the young-of-the-year fish have been infected (Arme & Owen, 1967; McPhail & Peacock, 1983), although there is annual variation as well (Pennycuick, 1971c). On the other hand, there are other three-spined stickleback populations in the United Kingdom where *S. solidus* are completely absent, or incidence is very low (Rahn et al., 2016; Simmonds, 2015). This is more commonly seen in stickleback populations worldwide (Karvonen et al., 2013; MacColl, 2009). The definitive bird hosts have high mobility and it is expected that it could easily spread *S. solidus* between neighbouring lakes and ponds (Koprivnikar & Leung, 2015). However, this is not the case and it appears that either some local environmental variable is stopping the spread of the parasites or short a reproductive period prevents this. Overall, the infection levels observed in the model match those of naturally infected populations indicating that the model captures (this part of) the life cycle dynamic accurately.

6.6.5 Ecological impacts of changing temperatures

In the current model the densities of parasite life stages appear to be most strongly affected by the density of bird hosts. This means that changes in bird densities, and more specifically piscivorous bird feeding patterns, are likely to affect the distribution of *Schistocephalus solidus* densities as well as absence and presence in specific water bodies. Changes in mean temperature can affect the overall distributions of bird species (Hitch & Leberg, 2007; Jonzén et al., 2006), but is also likely to influence microhabitat selection (Brambilla et al., 2018) and consequently feeding patterns (MacDonald, Bridge, McMahon, & Jones, 2019). If feeding is shifted from water bodies in open landscapes to more sheltered areas with smaller or fewer waterbodies, this could cause a significant decrease in transmission of *S. solidus*. As a consequence local extinctions, or at least drops in population densities could occur. This could then have positive effects on stickleback densities, in two ways. First, directly through decreased predation by birds, especially omnivorous birds that can feed on alternative food sources. Second, local extinctions of *S. solidus* will reduce mortality due to infection and parasite induced transmission mortality (Pennycuick, 1971c).

It is also important to take into consideration other factors that might change as a result of environmental temperature variation when interpreting the modelling outputs. For example, the food availability for copepod or fish hosts could be affected, altering their feeding patterns and thus their exposure.

6.6.6 Limitations the model

The current model groups the procercoid and the pleroceroid stages of the *Schistocephalus solidus* life cycle into one stage. As these two life stages are likely to be exposed to similar temperature effects and because the current parameterisation is based on both, this is justified. However, splitting this combined intermediate stage into two separate equations could expose more subtle dynamics. This expansion could lead to further biological insights but could limit the possibilities of analytically finding equilibria and would thus rely more heavily on simulations.

To further assess the importance of each of the model parameters on equilibria sensitivity analyses should be carried out (Kot, 2001).

A final step in assessing the model would be to attempt to validate model predictions in the field. For this local densities of hosts and parasites could be measured in regions with different mean temperature regimes and compared to model predictions. This will provide valuable information about the validity of the model and the ability of the model to make predictions about more extreme temperatures and other regions. Ideally this would be done within a region in which other environmental conditions are similar.

All these things need to be considered carefully if any policy decisions are to be based on these predictions, as the devil is in the detail.

6.6.7 Conclusions

Using the mathematical model developed here (Objective 6.1a) with parameters derived from experiments and the wider literature (Objective 6.1b), temperature dependence of the life cycle of *S. solidus* was investigated (Objective 6.1c). Densities of intermediate and adult stages appeared largely unaffected by temperature, but the number of free-living stages (eggs and coracidia) showed a dome-shaped response to temperature. Densities of the host, specifically definitive hosts, were the most important parameter in the model in regulating *S. solidus* densities (Objective 6.1e). No bifurcations of the model equilibira were found (Objective 6.1d).

Chapter 7

General Discussion

7.1 Summary of findings

This thesis investigated the effects of temperature on different life stages of *Schistocephalus solidus* and its intermediate hosts, cyclopoid copepods and the three-spined sticklebacks. This gives a better understanding of the probable effects of climate change on *S. solidus* populations. First, I shall give an overview of the findings (summarised in Table 7.1), then some general remarks regarding the effects of changing environmental temperatures on the *S. solidus* life cycle, and finally some suggestions for future research.

7.1.1 *Schistocephalus solidus* eggs

Survival of *Schistocephalus solidus* eggs was not affected by temperature, as reflected by the unchanged overall proportion of the number of eggs that hatched at the different temperature treatments. The proportion of eggs hatching matched those found in other studies, confirming the validity and accuracy of the experiments. *Schistocephalus solidus* eggs, on average, hatch earlier at higher temperatures. Higher temperatures lead to earlier hatching over a longer time period. Despite this hatching for all temperatures ceases after 34 days and the total proportion hatching is unaffected by temperature (Chapter 3).

7.1.2 Copepod hosts

The overall feeding of the copepod hosts showed a dome-shaped response to temperature. Copepod hosts becoming infected with *Schistocephalus solidus* is feeding dependent and infection likelihood thus showed a dome-shaped response to temperature. Because copepod feeding is highest at intermediate temperatures exposure to *S. solidus* coracidia is likely to be highest at those temperatures. *Mesocyclops pehpeiensis* copepods feeding on algae with a similar dependence on temperature (Sarma et al., 2013). For copepods in general, the decrease in feeding at high temperatures is probably due to a complex interaction between metabolism, capture success (as modulated by prey activities), and copepod activity levels. High activity levels at warmer temperatures are possibly detrimental and could encourage copepods to switch to a sit-and-wait feeding strategy. This strategy could conserve energy in the short term, but might be detrimental if metabolism remains high and not enough prey is captured in the long term. Survival of copepods exposed to *S. solidus* coracidia was largely unaffected by temperature but showed a steep drop at high temperatures, which supports this proposed mechanism.

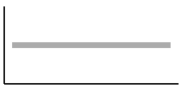
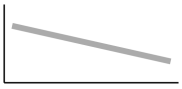
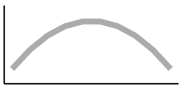
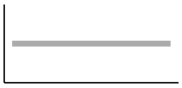
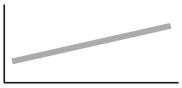
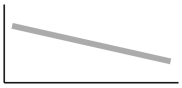
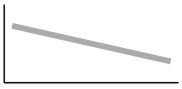
Temperature did not have an effect on the infection risk of copepods. This replicates previous work, but the small number of infections in the current work would only make it possible to detect relatively large effect sizes. It is important to note that *S. solidus* can use a wide range of copepod species as its first intermediate host and that these different copepod hosts could be differently affected by changes in thermal regimes.

The survival of copepod hosts in microcosms was negatively correlated with food densities at low temperatures and positively at high temperatures. This indicates a positive interaction between food availability and temperature (Chapter 4).

7.1.3 Stickleback hosts and *Schistocephalus solidus* plerocercoids

Three-spined sticklebacks (*Gasterosteus aculeatus*) grew faster at lower temperatures. The decrease in growth rate at warmer temperatures is most likely due to increased metabolic rates and increased stress response. Expenditure of resources on base metabolism and stress, leaves fewer for growth. The stress response of sticklebacks — as measured

TABLE 7.1: Graphical summary of responses of the different *Schistocephalus solidus* life stages and hosts to temperature. On the x-axes temperature is shown.

	survival	
S. solidus eggs	developmental time	
	variability in developmental time	
	feeding rate	
copepod host	survival	
	infection risk	
	feeding dependent survival	
	growth rate	
stickleback host	health	
	survival	
	growth rate	
S. solidus plerocercoids	host size dependent growth	

by the increased splenosomatic index — at higher temperatures, indicating a decrease in overall fish health. This may have led to the drop in survival of sticklebacks at the highest temperature, similar to that of copepods in microcosms (Chapter 3).

By contrast, the growth of *S. solidus* plerocercoids inside stickleback hosts was positively affected by temperature, and the growth of *S. solidus* plerocercoids was positively correlated with host size (i.e. plerocercoids grew faster in larger hosts). Additionally, there was a positive interaction between temperature and host size, leading to the fastest growth of plerocercoids in large fish at high temperatures (Chapter 5).

7.1.4 Model predictions

Data from the results chapters were combined with data from the literature to parameterise a mathematical model to describe the whole life cycle of *Schistocephalus solidus*. This model included both direct and indirect temperature effects on *S. solidus*. Model simulations predicted that mean densities of *S. solidus* parasites are dependent upon intermediate host densities and weakly dependent on any direct effects of temperature. However, it is possible that host densities are affected indirectly by changes in temperature not taken into account in the mathematical model, and will thus affect *S. solidus* densities and prevalence indirectly (Chapter 6).

7.2 Synthesis and implications

Overall effects of temperature increases on the *Schistocephalus solidus* life cycle seem positive. However, the different life stages show different responses. The responses to temperature increases of the intermediate hosts are similarly variable, but by contrast are generally negative (see Table 7.1). A modest increase in temperature with warming climates will likely benefit *S. solidus*. However, intermediate host densities could prevent large increases in parasite populations (see Chapter 6). Extreme temperatures — long or short term — could lead to increased mortality of intermediate hosts and an overall decrease in host health could also have a negative impact on *S. solidus* populations in the long term.

It is difficult to predict the impact of changing thermal regimes on species, in particular, on parasites with complex life cycles. Parasites with complex life cycles have many different life stages and temperature is likely to affect each of the many interactions with other species (see Figure 1.1). Furthermore, indirect effects could be obscure (Barber et al., 2016) as, for example, host fecundity or host food availability could be affected by warming temperatures. This could lead to changes in host population densities, which, in turn, is likely to affect parasite densities. This thesis provides new experimental and modelling tools (Chapters 2 & 5) for addressing these questions from which new insights are gained (Chapter 3, 5 & 6). This novel approach confirms previous hypotheses (Chapter 4) regarding temperature effects on parasite life cycles. The mathematical framework provided here can be expanded and adjusted for other parasite species and, in turn, patterns observed in other species can be compared to the temperature responses observed.

Ectotherm animals are expected to be most severely affected by changes in environmental temperatures and as such ectotherm hosts have been studied most in host-parasite interaction studies. Endotherm hosts of parasites, such as the bird host of *S. solidus*, are also be affected by extreme changes in temperature. This is because only small changes in temperatures are expected to fall within the thermoneutral zone of endotherms (Buckley et al., 2012), but more extreme changes can reduce host metabolic rates. Consequently, at extreme temperatures host feeding could be reduced, decreasing the exposure to trophically transmitted parasites. Alternatively, reduced metabolism could result in increased energy available for immune responses and therefore diminished establishment of parasites at extreme temperatures (Morley & Lewis, 2014). This range of possibilities shows the importance of also studying endotherm hosts if large temperature changes are expected.

Changes in thermal regimes can affect animal phenology (Both et al., 2009). Previous work showed that temperature-induced synchronised release of parasites stages could increase mortality of intermediate hosts (Meissner & Bick, 1999; Mouritsen & Jensen, 1997), however the current experiments on *S. solidus* eggs show that warming temperature can also reduce overall densities of free-living infective stages. It seems that these responses differ between species and life stages. These differences prompt

the need for further investigations into which processes are accelerated by increasing temperatures, and which will show increased variation before general rules can be formulated. Changes in the phenology of host species could additionally cause mismatches between parasite emergence and host presence. For example, the *Cyclops strenuus* host of *S. solidus* is generally monocyclic (Maier, 1990), but may produce two generations under favourable conditions (Frisch, 2001). This has the potential to reduce the overlap in the presence of susceptible copepods when *S. solidus* eggs hatch.

Temperature changes also lead to changes in the spatiotemporal distributions on a macro-ecological scale of animals (Thomas & Lennon, 1999) and microhabitat selection of motile animals (Rickards & Boulding, 2015). Among hosts of freshwater parasites, birds are expected to show the greatest potential for changes in geographic distribution because of their great vagility (Criscione & Blouin, 2004). Changes in host spatiotemporal distributions of hosts are likely to negatively impact the completion of parasite life cycles, especially for parasites that are trophically transmitted or have high levels of host specificity (Marcogliese, 2001, 2002). As a result, hosts could experience a temporary parasite release (Goedknegt et al., 2016). Parasite release could provide increased scope for host species to adapt to new local conditions before parasites 'catch up' and re-establish in host populations. Altered distributions of hosts and parasites could also expose parasites to new potential host species and facilitate host-switching (Brooks & Hoberg, 2007). Some parasite species will be better pre-adapted to include new host species into their life cycle than others, and the abundance and prevalence of different parasite species could be altered as a result which could lead to local extinctions.

7.2.1 Temperature stochasticity and seasonality

Schistocephalus solidus and its hosts live in relatively stable thermal environments in the short term, as water buffers mean air temperature changes. However, in any habitat with temporal variations in temperature, such as seasonal variations, it is critical to consider this variability to understand the impacts of changing thermal regimes entirely. An increase in mean temperatures due to global warming, will increase the incidence of extreme temperatures and the stochasticity in thermal regimes (Meehl &

Tebaldi, 2004). To which the development of parasites in ectothermic hosts are particularly vulnearable (Saunders et al., 2002). For instance, at extreme temperatures the development of different life stages of sea lice (*Lepeophtheirus salmonis*) becomes more sensitive to environmental parameters (Groner et al., 2014). This means that the impact of parasites on their hosts at warmer temperatures could be more severe than predicted and should not be based solely on mean temperatures, but on temperature ranges. Additionally, seasonal variations in temperature and heatwaves can also play an essential role in regulating animal densities (Dittmar, Janssen, Kuske, Kurtz, & Scharsack, 2014; Hancock, Brackley, & Palmer, 2011) and could further modulate the interactions between hosts and parasites.

7.2.2 Temperature and other environmental changes

Temperature is likely to be one of the most pressing environmental changes on parasite life cycles. However, other changes in aquatic habitats are expected to coincide with changes in temperature, and thus it is important to consider these factors in concert. Other potentially important environmental factors known to affect ecological interactions include changes in acidity (MacLeod & Poulin, 2016b), heavy metal concentrations (Lefcort et al., 2002), salinity (Pietrock & Marcogliese, 2003) and underwater noise pollution (Edmonds, Firmin, Goldsmith, Faulkner, & Wood, 2016). These changes can act synergistically or antagonistically on parasite life stages (Marcogliese, 2016), and it is thus important to develop a concrete understanding of how the interaction of these factors affects parasites and their interactions with their hosts. Especially as some short-term reductions in growth can be off-setted later on in life if conditions are more favourable (Hakalahti, Bandilla, & Valtonen, 2005). The results presented here could be used as a starting point for inquiries into combined effects of temperature and environmental disturbances.

7.3 Suggestions and directions for future research

To further our understanding of changes in environmental temperatures future research should focus on five key areas;

First, a crucial factor in understanding how parasite populations will respond to future temperature changes on local scales is the variability between individuals in their response to environmental stressors. The time until hatching of *Schistocephalus solidus* eggs shows considerable variation between individuals. Although this might allow the eggs from the same parent to survive in a range of habitats, only heritable variation between individuals (i.e. genotypes) will allow for natural selection to take place. For example, there is heritable variation between sticklebacks from different populations in their ability to deal with extreme temperatures. This provides potential for adaptation to changing climates (Barrett et al., 2011). Large variability will, in theory, provide more potential for adaptation to future conditions in large populations. Future experiments should aim to quantify not only mean responses, but also variability within populations in response to environmental changes. This can help in addressing questions with regards to the adaptation of populations to environmental change.

Second, investigating the short-term impact of temperature change is necessary to understand short-term population dynamics, but to understand long-term dynamics, long-term experiments are needed. These should investigate 'carryover' effects. For example, these fish could be exposed to *S. solidus* infective stages at different thermal regimes and compare the infectivity of the parasites developing from the eggs. A more ambitious approach could aim to complete several *S. solidus* life cycles at slowly increasing mean temperatures for each life cycle and assess the fitness of each generation. This would allow investigating potential carryover effects as well as a more realistic acclimation of parasites and hosts to increasing temperatures.

Third, because variability in environmental temperatures is likely to increase, it is important to quantify the effects of temperature variability on the *S. solidus* life cycle at different mean temperatures. Potential 'hidden effects' of changes in mean temperature could be unmasked by adding temperature variability. These hidden effects could consist of small cumulative effects on the physiology or development of the animal that do not impact fitness until combined with other hidden effects. For example, an increase in mean temperature might not be detrimental for survival as energy

stores can be expended to deal with adverse effects of increases in temperature. Animals might not be able to also deal with (stochastic) variability in temperature and could show increases in mortality at high temperatures with high levels of variability in temperature.

Fourth, the same applies to changes in other environmental factors. As mentioned, changes in thermal regimes are not the only anthropogenic stressors on populations in natural environments. Each of the individual stressors may not have severe impacts on populations of aquatic animals, but combined, they could prove detrimental. It is thus key to design more multifactorial experiments.

Fifth, findings from this study and other, similar studies should be validated in non-model species. Model species help discover general patterns. Alas, model species do not represent all species, and the validity of experimental results need to be verified in non-model species as well. In particular, for host-parasite interactions under environmental change, there is still much work to be done in validating these responses.

7.4 Concluding remarks

With progressive anthropogenic environmental changes, more data will become available on the impacts of temperature on parasite life cycles. These data could be used to verify the applicability of experimental data, such as presented in this thesis, to the field. Combined these data can then be used to test potential local ameliorations to habitats to prevent local extinctions and retain biodiversity. However, these measures can only be successful if, at the same time, significant measures are taken to reduce global anthropogenic changes to habitats.

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