Accepted for publication in *Parasitology*, 13/04/16

Interactions of warming and exposure affect susceptibility to parasite infection in a temperate fish species

Danny J. Sheath

Demetra Andreou

J. Robert Britton

Department of Life and Environmental Sciences, Bournemouth University, BH12 5BB, UK

Running title: Warming and parasite interactions

Corresponding author:

Danny Sheath: dsheath@bournemouth.ac.uk

22

Summary

Predicting how elevated temperatures from climate change alter host-parasite interactions requires understandings of how warming affects host susceptibility and parasite virulence. Here, the effect of elevated water temperature and parasite exposure level was tested on parasite prevalence, abundance and burden, and on fish growth, using *Pomphorhynchus laevis* and its fish host *Squalius cephalus*. At 60 days post-exposure, prevalence was higher at the elevated temperature (22 ^{o}C) than ambient temperature (18 ^{o}C), with infections achieved at considerably lower levels of exposure. Whilst parasite number was significantly higher in infected fish at 22 ^{o}C, both mean parasite weight and parasite burden was significantly higher at 18 ^{o}C. There were, however, no significant relationships between fish growth rate and temperature, parasite exposure, and the infection parameters. Thus, whilst elevated temperature significantly influenced parasite infection rates, it also impacted parasite development rates, suggesting warming could have complex implications for parasite dynamics and host resistance.

Key words: climate change, *Pomphorhynchus laevis*, *Squalius cephalus*, parasite prevalence, parasite abundance

Key Findings

- The effects of warming and parasite exposure were experimentally assessed on a fish host.
- Fish were exposed to known numbers of intermediate hosts and then held at two temperatures.
- Parasite infections developed from lower exposure levels at elevated temperatures.
- Parasite prevalences and abundances were higher at elevated temperature but mean weight and burden was lower.
- No consequences for host growth and condition were recorded.

Introduction

Climate change is predicted to alter host-parasite relationships during this century, especially where warming combines with other anthropogenic disturbances (Rohr *et al.* 2011; Paull *et al.* 2012; Lõhmus and Björklund, 2015). In northern latitudes, where climatic factors are important regulators of host-parasite population dynamics and parasite occurrence, and transmission is regulated by seasonal temperature changes, shortened winter periods could alter host-parasite relationships via alterations in host susceptibility and parasite virulence (Hakalahti *et al.* 2006; Lõhmus and Björklund, 2015). Should growth rates of the hosts and parasites be altered by temperature changes then pathology and transmission rates could also be affected (Raffel *et al.* 2006; Lafferty, 2009). Consequently, predictions tend to be for warming to increase the prevalence of parasites at higher latitudes (e.g. Harvell *et al.* 2002; Marcogliese, 2001, 2008), although there is limited empirical evidence to support this at present (Ward and Lafferty, 2004; Bentley and Burgner, 2011; Lõhmus and Björklund, 2015).

An understanding of how host-parasite interactions will shift under the effects of warming, and the consequences for host populations and their communities, is thus an important aspect of environmental management (Lafferty, 2009; Macnab and Barber, 2012). Integral to this is developing understandings of how elevated temperatures affect host susceptibility to infection versus their effects on parasite virulence and life cycle completion rates (Harvell *et al.* 2002; Altizer *et al.* 2013). The susceptibility of hosts to infection could increase through, for example, thermal stress that leads to reduced immune-competency (Weyts *et al.* 1999; Nikokelainen *et al.* 2004) and enhanced consumption rates of prey that leads to increased parasite exposure via intermediate hosts (Toscano *et al.* 2014). Parasite fitness and

transmission rates could be enhanced by warming through positive effects on their metabolism, resulting in higher numbers of transmission stages being produced, with their rate of development and growth within hosts also accelerated (Paull and Johnson, 2011; Callaway *et al.* 2012). However, should warming result in the temperature optimum for the parasite being exceeded then their decreased prevalence in host populations might result, with suggestions that increased parasite prevalence due to warming will only occur for a proportion of fish pathogens (Karvonen *et al.* 2010). Consequently, there is an outstanding requirement to derive enhanced understandings of how warming will affect host-parasite dynamics, particularly the decoupling of the underlying mechanisms involved, i.e. the effects of warming on host susceptibility versus on parasite transmission and virulence.

The aim of this study was thus to test how elevated temperature affected host susceptibility to infection under different parasite exposure levels and how this affected parasite prevalence and intensity. Objectives were to quantify how temperature and parasite challenges affected: (i) infection outcomes (as parasite prevalence), (ii) host infection parameters (as parasite abundance, mean individual weight and burden); and (iii) host growth rates. Outcomes were assessed in relation to the effects of temperature elevation on the host-parasite relationship and the potential mechanisms involved. The model parasite was *Pomphorhynchus laevis* (Müller), an acanthocephalan with a complex lifecycle whose final hosts are a wide range of fishes (Neveda *et al.* 2003). The model final host was chub *Squalius cephalus* (Linnaeus), a preferred freshwater fish host of *P. laevis* (Hine and Kennedy, 1974). This parasite uses the freshwater shrimp *Gammarus pulex* (Linnaeus) as its intermediate host. It is also a conspicuous orange-yellow parasite that is visible through the transparent cuticle of *G. pulex* (Bakker *et al*. 1997). This enables individual *G. pulex* to be identified for both their parasite status (infected/ uninfected) and the number of parasites it is infected by. Transmission to fish

hosts is via consumption of infected *G. pulex*, with some evidence that the parasite manipulates the behaviour of *G. pulex* to increase their probability of being predated upon and so enabling the parasite to be transmitted to its final host (e.g. Franceschi *et al.* 2008; Dianne *et al.* 2011; Labaude *et al.* 2015).

Materials and methods

Ethical approval

All experimental procedures used in the study were approved by the Ethical Review Committee of Bournemouth University and completed under UK Government Home Office project licence 30/3094. Where fish were euthanized, the procedure followed the UK Government Home Office Schedule 1 regulations.

Experimental design and pre-experiment data collection

The *S. cephalus* used in the experiment were all between 69 and 89 mm starting length (mean 80.8 ± 0.8 mm) and age 1+ years. They were sourced from an aquaculture site in Southern England in August 2014. Although they had not been exposed to the parasite during their lifetime, they were produced from broodstock that had originally been collected from a river where *P. laevis* was present naturally. On the aquaculture site, the fish were reared in outdoor ponds (approximate water temperatures at the time of collection: 15 to 19 ^{o}C), with some supplementary feeding with pelletized fishmeal. On arrival to the laboratory, the fish were tagged with passive integrated transponder tags (PIT tags) so that individual fish could be tracked through the experiment. Concomitantly, they were measured (fork length, nearest mm) and weighed (*W*, nearest 0.1g). They were then allowed to recover and acclimate to laboratory conditions by being held in tanks held at 18 ^{o}C for 14 days on a 16:8 hour light:

dark cycle. In addition, a sub-sample of 5 fish were removed from the sample on arrival to the laboratory. These were euthanized and dissected to check for the presence of *P. laevis*. None of these fish were infected. Infections of other parasites were very light and considered part of the natural parasite fauna of the fish in Southern England and were recorded at levels that were not considered high enough to cause clinical pathology (Hoole *et al.* 2001).

Parasite exposure

The *S. cephalus* were challenged by *P. laevis* through exposing individuals to known numbers of infected *G. pulex*. These were collected from a local river, the Hampshire Avon (latitude: 50.8865, longitude: -1.7883), when water temperatures were approximately 18 ^{o}C. These were then held in laboratory conditions at 18 ^{o}C for 96 hours, with infectious individuals then identified visually (Bakker *et al*. 1997; Bauer and Rigaud, 2015), with a subset confirmed by dissection. As multiple infections were identifiable in the *G. pulex* (Bakker *et al*. 1997), then individuals were only used here that were host to one parasite. Exposure of the fish to the parasite was done individually, with the fish transferred to 10 L tanks containing dechlorinated water with supplementary oxygenation provided via an air stone and pump, and at a water temperature of 18 ^{o}C. Prior to parasite exposure, the fish were held in the tanks for 24 hours with no feeding to ensure standardised levels of hunger.

Each individual fish was then exposed to a specific number of infected *G. pulex* from the following options: 0 (as a control), 5, 10, 20, 40 and 60. There were 10 fish used at each level of exposure. After 24 hours, the fish were removed from the tanks, with confirmation that all the *G. pulex* had been consumed. For each exposure level, the fish were then split randomly into 2 groups of 5 and transferred into 45 L tank aquaria at either 18 or 22 ^{o}C. These tank aquaria were arranged on a flow-through system using recirculated water (originally

dechlorinated tap water), with a different system used for each temperature. Across the two flow-through systems used, the tanks were identical in dimensions, the water was taken from the same original source, and the tanks contained identical environmental enrichment for the fish in terms of refugia (lengths of plastic pipe of 65 mm diameter) and cover (artificial macrophytes).

Post-experiment data collection and analysis

Following their exposure to *P. laevis*, the fish were held in their tanks for 60 days under a 16:8 hour light: dark regime, with feeding daily using crushed pelletized fishmeal (approximately 2 % starting body mass/ day). At the end of this period, the fish were removed from their tanks, euthanized, scanned for their PIT tag, re-measured and weighed. They were then dissected, with intestinal examinations to identify individuals in which infections by *P. laevis* had developed. For infected fish, parasites were removed, counted and weighed (mg).

These data enabled parasite prevalence to be assessed as the proportion of infected fish per temperature/ exposure treatment. The effects of temperature (T) and parasite exposure (PE; as the number of consumed intermediate hosts) on prevalence were then tested using a probability of infection (PoI) model using binary logistic regression and the equation $\text{PoI} = e^{(a+b\text{T}+c\text{PE})} / 1+ e^{(a+b\text{T}+c\text{PE})}$, where a, b and c were binary logistic regression coefficients. This also provided the significance of both variables on parasite prevalence. As the tank conditions were identical across the individual fish, with only water temperature and levels of exposure to the parasite via intermediate hosts being different, then the model did not take account of the fish being within different tanks per temperature treatment. Thus, the individual fish were being treated as the replicate unit in the model.

The following infection parameters were then calculated from the data of the infected fish. Parasite abundance was determined as the total number of parasites per host and the total mass of parasites per host, and enabled calculation of the mean parasite weight per host. Parasite burden was calculated as the proportion of the body weight of each host comprising *P. laevis* (Pegg *et al*. 2015). Differences in these infection parameters, plus parasite prevalence, between temperatures were tested using generalized linear models (GLM), with parasite exposure level as the covariate. In all models, data on uninfected fish were not included as their inclusion in the models would introduce a bias in outputs, given the higher numbers of uninfected fish at the lower temperature/ levels of parasite exposure. For parasite number, a Poisson log-linear model was used as the data represented parasite counts. As with the binary logistic regression model, in these models, the data for individual fish were used as the replicate units due to the identical conditions the fish were in, i.e. this was not considered as artificially inflating the number of degrees of freedom in the models that would otherwise result in pseudo-replication. The reported model outputs then included the mean value of the infection parameters per temperature treatment (as estimated marginal means, with the effects of parasite exposure as the covariate controlled in the model) and their standard error. To identify if differences between these mean values were significant, linearly independent pairwise comparisons were used with Bonferroni adjustment for multiple comparisons. Differences in infection parameters were then tested between the exposure levels using the same process, except temperature was used as the covariate in these models.

Finally, to determine if infection influenced the growth rate of the fish, specific growth rate (*SGR*) was calculated as the change in body mass of the fish over the experimental period, from $[\ln W_{t+1} - \ln W_t] / t] \times 100$, where W_t = starting weight, W_{t+1}= finishing weight, and t = number of days between W_t and W_{t+1}. Differences in specific growth rates of fish between

temperatures and parasite exposure levels were then tested using GLMs as described above, with multiple linear regression analysis then used to test the influence of the infection parameters, temperature and parasite exposure on SGR. This provided the significance of the predictor variables and their standardized beta coefficients (β). Variables with the highest β value had the strongest singular contribution to the model.

Results

Probability of infection

At the conclusion of the 60 days after parasite exposure, there were considerable differences in infection levels apparent between temperatures and exposure levels (Fig. 1). The logistic regression model revealed both temperature and exposure level had significant effects on parasite prevalence (Fig. 1; Table 1). At 18 ^{o}C, infection required higher parasite exposure levels compared with 22 ^{o}C, with 50 % prevalence requiring exposure to 6 intermediate hosts at 22 ^{o}C, but 26 at 18 ^{o}C (Fig. 1).

Infection parameters

The GLM testing the effect of temperature on the parasite abundance of the infected fish revealed that there were significant differences in the mean numbers of parasites between the two treatments (Wald $\lambda^2 = 4.23$, $P = 0.04$), with mean parasite number significantly higher at 22 ^{o}C than 18 ^{o}C ($P < 0.01$; Fig. 2a). The effect of exposure on parasite abundance also revealed significant differences in mean number (Wald $\lambda^2 = 20.46$, $P < 0.01$), with significantly higher numbers of parasites per infected fish at exposure to 40 intermediate hosts (mean number: 7.80 ± 0.98) than at all than other exposure levels (mean numbers: 2.42

228 to 3.46; $P < 0.01$ in all cases; Fig. 2b). In both GLMs, the effect of the covariate was also
229 significant ($P < 0.05$).
230
231 Temperature was not a significant predictor of parasite abundance when it was measured as
232 the total parasite mass in the infected fish (Wald $\lambda^2 = 0.01$, $P = 0.92$; Fig. 2c), but parasite
233 exposure was (Wald $\lambda^2 = 13.10$, $P = 0.01$). Mean total parasite mass was higher at 40
234 intermediate hosts (mean parasite mass: 24.23 ± 3.06 mg) than all other exposure levels
235 (mean parasite mass range: 9.02 to 17.12 mg), although the differences were only significant
236 between 40 and 60 hosts (difference 15.20 ± 4.35 mg; $P < 0.01$) (Fig. 2d).
237
238 The mean weight of individual parasites in the infected fish was significantly influenced by
239 temperature (Wald $\lambda^2 = 9.48$, $P < 0.01$), being higher at 18 than 22 ^{o}C ($P < 0.01$; Fig. 2e). The
240 effect of parasite exposure on the mean weight of individual parasites was also significant
241 (Wald $\lambda^2 = 13.29$, $P < 0.01$), with higher means at lower exposure levels (Fig. 2f). The effect
242 of temperature on parasite burden was significant (Wald $\lambda^2 = 15.37$, $P < 0.01$), with
243 significantly higher burdens at 18 (0.23 ± 0.03 %) than 22 ^{o}C (0.06 ± 0.03 %) ($P < 0.01$). The
244 effect of exposure on parasite burden was, however, not significant (Wald $\lambda^2 = 7.63$, $P =$
245 0.11).
246
247 *Fish growth*
248 Mean fish weight at the start of the experiment was 5.20 ± 0.16 g and at the end was 7.89 ±
249 0.31 g. The effect of temperature and parasite exposure on fish growth (as SGR) was not
250 significant in either GLM (Wald $\lambda^2 = 0.01$, $P = 0.91$; Wald $\lambda^2 = 5.01$, $P = 0.28$ respectively).
251 Multiple regression revealed the effects on SGR of all infection parameters, exposure and

252 temperature were not significant (R^2 = 0.11; $F_{5,23}$ = 0.77, P = 0.56), with no significant
253 predictors (all P > 0.05).
254
255 **Discussion**
256
257 Elevated water temperature had a significant and positive effect on parasite prevalence, with
258 parasite infections developing from exposure to lower numbers of intermediate hosts in the
259 warmer water. Despite these clear differences in prevalences, the effects of temperature and
260 parasite exposure on the infection parameters of the individual hosts were relatively complex.
261 Although elevated temperature resulted in increased parasite number in hosts, this involved a
262 trade-off with their mass, with significantly smaller parasites present in hosts held at higher
263 temperatures and resulting in significantly lower parasite burdens. These outputs on the
264 infection parameters are a contrast to Macnab and Barber (2012), who revealed that elevated
265 temperature increased the growth rates of the parasite *Schistocephalus solidus* (Müller) in
266 three-spined stickleback *Gasterosteus aculeatus* Linnaeus.
267
268 A major challenge in understanding how warming will affect host-parasite interactions is
269 decoupling the individual effects of warming on the susceptibility of hosts to infection from
270 the effects on parasite virulence. Here, the collection and holding of the parasite intermediate
271 hosts, and the holding of the fish and their exposure to the parasite, was all completed at 18
272 ^{o}C, an ambient temperature representative of temperate freshwaters in the late summer period
273 (Britton, 2007). The exposed fish were then held at this ambient temperature and an elevated
274 temperature (+4 ^{o}C) for the experimental period. With the initial parasite exposure all being
275 completed at ambient temperature, it is suggested that the effect of the sudden temperature
276 elevation in the treatment altered the susceptibility of the fish hosts to infection (Hakalahti *et*

al. 2006), rather than it affecting the parasite virulence (Lõhmus and Björklund, 2015). The sudden increase in temperature for this fish meant it was not possible to decouple the effect of the temperature effect on susceptibility *per se* from the specific effect of the rapid temperature increase. Nevertheless, that the net effect of the elevated temperature increased host susceptibility to infection was supported by evidence from other studies that suggest it often results in substantial negative consequences for fish immuno-competence (Dittmar *et al.* 2014), as it potentially shifts energy allocation from immunological processes (Poisot *et al.* 2009) and/ or acts as an additional stressor that compromises the immune response (Cramp *et al.* 2014).

The complex effects of both temperature and parasite exposure on the infection parameters within the hosts were related to either temperature impacting the development rate of parasites or the increased parasite number in hosts at elevated temperatures resulting in marked density-dependent effects, resulting in relatively high densities of parasites with relatively small body sizes (Luong *et al.* 2011). It is suggested that the latter explanation was more consistent with the outcomes of the experiment, given that these revealed fish exposed to high numbers of intermediate hosts at the ambient temperature resulted in low parasite numbers compared with the elevated temperature, but with these parasites being substantially larger, resulting in significantly higher parasite burdens.

Notwithstanding, as elevated temperatures can have both marked effects on the development rates of parasites in temperate regions (Tinsley *et al.* 2011) and on fish immune function, disease resistance and fitness (Cramp *et al.* 2014), then it is remains difficult to definitively decouple the effects of warming on these aspects of the infection dynamics from these data. It is thus recommended that these outputs serve as an initial assessment of the effects of

warming temperatures and parasite exposure levels on these host-parasite dynamics, enabling the design of subsequent experiments of greater complexity that should enable, for example, greater assessment of how warming affects the development rate the parasite within hosts, such as their maturity (e.g. Altizer *et al.* 2013), how temperature affects the immune response of hosts (e.g. Nikokelainen *et al.* 2004), and how parasite virulence is affected by the interactions of warming with other environmental variables, and the influence of this on selection (e.g. Wolinska and King, 2009). Given the ease at which fish final hosts, such as *S. cephalus*, can be infected experimentally with known numbers of *P. laevis* via *G. pulex* intermediate hosts, then this host-parasite model would provide a strong model host-parasite system to answer these questions in both controlled and semi-controlled conditions. For example, to decouple the effects of host susceptibility from parasite virulence across different temperatures could utilise experiments where the fish and intermediate hosts are held at the different temperatures prior to exposure (unlike here, where they were all initially held at 18 ^{o}C) and then used in the experimental design used here. Parasites from these initial experiments could then be harvested and used to produce laboratory grown parasites in *G. pulex* that are raised across the different temperatures. Their subsequent exposure to the fish would then be completed in a fully-factorial experimental design that enables quantification of differences in virulence and hosts susceptibility across the different generations and rearing temperatures of both *G. pulex* and the host fish.

Despite the strong effect of temperature on parasite prevalence and development, there were no measureable consequences for the hosts, with no differences in the specific growth rates of the fish between the controls, temperature and exposure treatments. Studies have suggested that *P. laevis* is a relatively benign parasite in temperate European fluvial fishes (Hine and Kennedy, 1974), with the effects of ancanthocephalan parasites generally being more related

to the consequences of their pathology rather than their loading (Latham and Poulin, 2002). Thus, it is suggested that the effect of elevated temperature on this host-parasite system was primarily in relation to altering host susceptibility to infection, with this then influencing parasite development and dynamics via density-dependent mechanisms within hosts. Consequently, the importance of these findings are that they indicate that warming could result in substantial shifts in disease progression via altered host susceptibility, but potentially with concomitant changes in parasite infectivity and development.

Acknowledgments

The study was supported by the Natural Environment Research Council (NERC research grant reference number NE/J016675/1).

References

Altizer, S., Ostfield, R. S., Johnson, P. T. J., Kutz. S. and Harvell, C. D. (2013). Climate change, infectious diseases: from evidence to a predictive framework. *Science* **341**, 514-519.

Bakker, T.C., Mazzi, D. and Zala, S. (1997). Parasite-induced changes in behavior and color make Gammarus pulex more prone to fish predation. *Ecology* **78**: 1098-1104.

Bauer, A. and Rigaud, T. (2015). Identifying a key host in an acanthocephalan-amphipod system. *Parasitology*. doi:10.1017/S0031182015001067

Bentley, K. T. and Burgner, R. L. (2011). An assessment of parasite infestation rates of juvenile sockeye salmon after 50 years of climate warming in southwest Alaska. *Environmental Biology of Fishes* **92**, 267-273.

Britton, J. R. (2007). Reference data for evaluating the growth of common riverine fishes in the UK. *Journal of Applied Ichthyology* **23**, 555-560.

Callaway, R., Shinn, A. P., Grenfell, S. E., Bron, J. E., Burnell, G., Cook, E. J. and Shields, R. J. (2012). Review of climate change impacts on marine aquaculture in the UK and Ireland. *Aquatic Conservation Marine and Freshwater Ecosystems* **22**, 389-421.

Cramp, R. L., Reid, S., Seebacher, F. and Franklin, C. E. (2014). Synergistic interaction between UVB radiation, temperature increases susceptibility to parasitic infection in a fish. *Biology Letters* **10**, 20140449.

Dianne, L., Perrot-Minnot, M. J., Bauer, A., Gaillard, M., Léger, E., & Rigaud, T. (2011). Protection first then facilitation: a manipulative parasite modulates the vulnerability to predation of its intermediate host according to its own developmental stage. *Evolution* **65**, 2692-2698.

Dittmar, J., Janssen, H., Kuske, A., Kurtz, J., Scharsack, J. P. and Ardia, D. (2014). Heat, immunity: an experimental heat wave alters immune functions in three-spined sticklebacks (*Gasterosteus aculeatus*). *Journal of Animal Ecology* **83**, 744-757.

Franceschi, N., Bauer, A., Bollache, L. and Rigaud, T. (2008). The effects of parasite age and intensity on variability in acanthocephalan-induced behavioural manipulation. *International Journal for Parasitology* **38**, 1161-1170.

Hakalahti, T., Karvonen, A. and Valtonen, E. T. (2006). Climate warming, disease risks in temperate regions - *Argulus coregoni, Diplostomum spathaceum* as case studies. *Journal of Helminthology* **80**, 93-98.

Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S. and Samuel, M. D. (2002). Climate warming, disease risks for terrestrial, marine biota. *Science* **296**, 2158-2162.

Hine, P. M. and Kennedy, C. R. (1974) The population biology of the acanthocephalan *Pomphorhynchus laevis* (Müller) in the River Avon. *Journal of Fish Biology* **6**, 665-679.

Hoole, D., Bucke, D., Burgess, P. and Wellby, I. (2001) *Diseases of carp and other cyprinid fishes*. Fishing News Books, Oxford.

Karvonen, A., Rintamaki, P., Jokela, J. and Valtonen E. T. (2010). Increasing water temperature, disease risks in aquatic systems: climate change increases the risk of some, but not all, diseases. *International Journal of Parasitology* **40**, 1483–1488.

Labaude, S., Rigaud, T. and Cézilly, F. (2015). Host manipulation in the face of environmental changes: Ecological consequences. *International Journal for Parasitology: Parasites and Wildlife* **4**, 442-451.

Lafferty, K. D. (2009). Calling for an ecological approach to studying climate change, infectious diseases. *Ecology* **90**, 932-933.

Latham, A. and Poulin R. (2002). Field evidence of the impact of two acanthocephalan parasites on the mortality of three species of New Zeal, shore crabs (Brachyura). *Marine Biology* **141**, 1131-1139.

Luong, L. T., Vigliotti, B. A. and Hudson, P. J. (2011). Strong density-dependent competition, acquired immunity constrain parasite establishment: Implications for parasite aggregation. *International Journal of Parasitology* **41**, 505-511.

Macnab, V. and Barber, I. (2012). Some (worms) like it hot: fish parasites grow faster in warmer water and alter host thermal preferences. *Global Change Biology* **18**, 1540–1548.

Marcogliese, D.J. (2001). Implications of climate change for parasitism of animals in the aquatic environment. *Canadian Journal of Zoology* **79**, 1331-1352.

Marcogliese, D. J. (2008). The impact of climate change on the parasites, infectious diseases of aquatic animals. *Revue Scientifique et Technique* **27**, 467-484.

Nikoskelainen, S., Bylund, G. and Lilius, E. M. (2004). Effect of environmental temperature on rainbow trout (*Oncorhynchus mykiss*) innate immunity. *Developmental and Comparative Immunology* **28**, 581–592.

Okamura, B. and Feist, S.W. (2011). Emerging diseases in freshwater systems. *Freshwater Biology* **56**, 627-637.

Paull, S.H. and Johnson, P.T. (2011). High temperature enhances host pathology in a snail–trematode system: possible consequences of climate change for the emergence of disease. *Freshwater Biology* **56**, 767-778.

Pegg, J., Andreou, D., Williams, C.F. and Britton, J.R. (2015). Temporal changes in growth, condition and trophic niche in juvenile Cyprinus carpio infected with a non-native parasite. *Parasitology* **142**, 1579-1587.

Poisot, T., Šimková, A., Hyršl, P. and Mor, S. (2009). Interactions between immuno-competence, somatic condition, parasitism in the chub *Leuciscus cephalus* in early spring. *Journal of Fish Biology* **75**, 1667-1682.

Raffel, T. R., Rohr, J. R., Kiesecker, J. M. and Hudson, P. J. (2006). Negative effects of changing temperature on amphibian immunity under field conditions. *Functional Ecology* **20**, 819-828.

Tinsley, R. C., York, J. E., Stott, L. C., Averard, A. L. E., Chapple, S. J. and Tinsley, M. C. (2011). Environmental constraints influencing survival of an African parasite in a north temperate habitat: effects of temperature on development within the host. *Parasitology* **138**, 1039-1052.

Toscano, B. J., Newsome, B. and Griffen, B. D. (2014). Parasite modification of predator functional response. *Oecologia* **175**, 345-352.

Weyts, F. A. A., Cohen, N., Flik, G. and Verburg-Van Kemenade, B. M. L. (1999). Interactions between the immune system, the hypothalamo-pituitary-interrenal axis in fish. *Fish and Shellfish Immunology* **9**, 1–20.

Figure captions

Figure 1. Parasite exposure versus (i) proportion (0-1) of infected fish at 18 ^{o}C (filled circles) and 22 ^{o}C (open circles), and (ii) probability of infection (0 to 1 scale) according to binary logistic regression (*cf.* Table 1, Equation 1) at 18 ^{o}C (solid line) and 22 ^{o}C (dashed line).

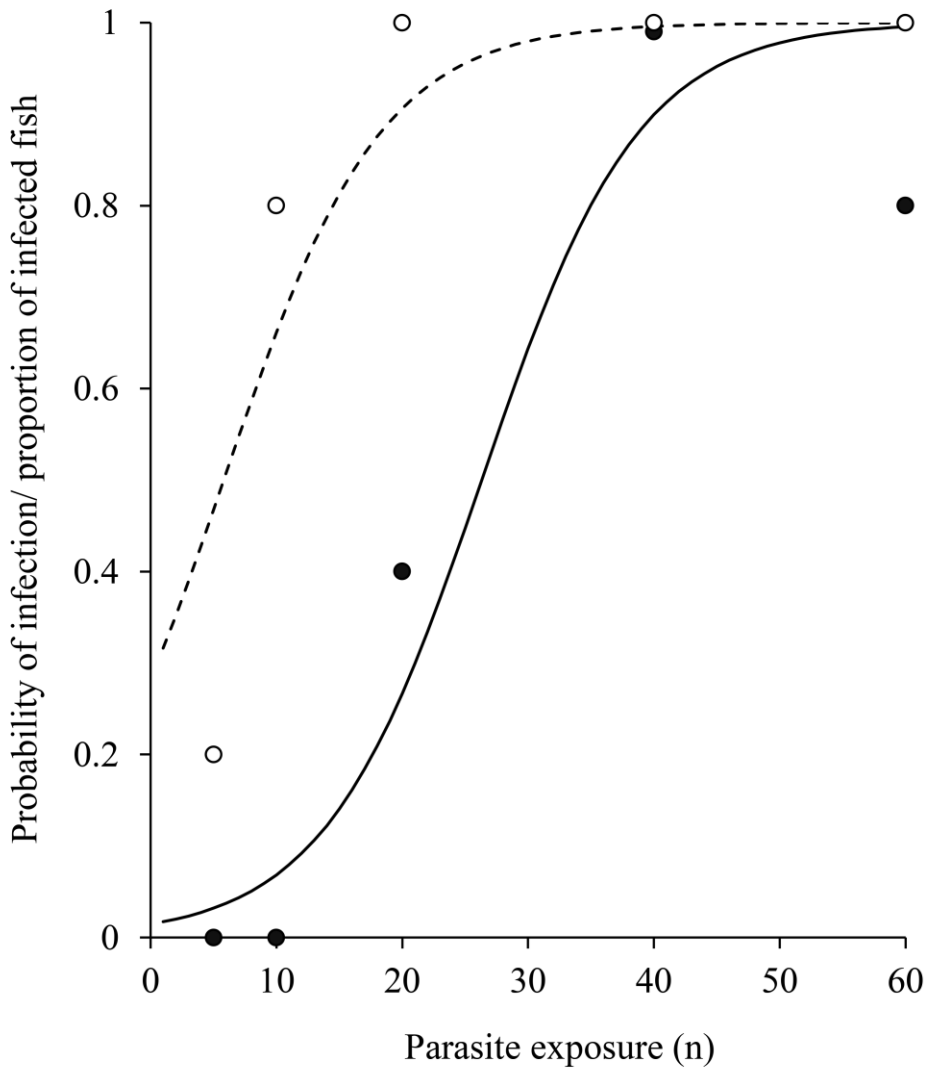
Figure 2. Mean adjusted parasite number and mass, and mean parasite weight per fish (from generalised linear models) according to temperature (a, c, e), where parasite exposure was the model covariate, and parasite exposure (b, d, f), where temperature was the covariate. Error bars represent standard error.

438 **Table 1.** Binary logistic regression coefficients (Equation 1), and their statistical significance,
439 for the probability of infection of *Squalius cephalus* by *Pomphorhynchus laevis* according to
440 temperature and parasite exposure.

Parameter	Symbol in equation 2	Coefficient	Standard error	P
Constant	a	-18.97	6.21	0.02
Temperature	b	0.82	0.28	<0.01
Parasite exposure	c	0.16	0.05	<0.01

441

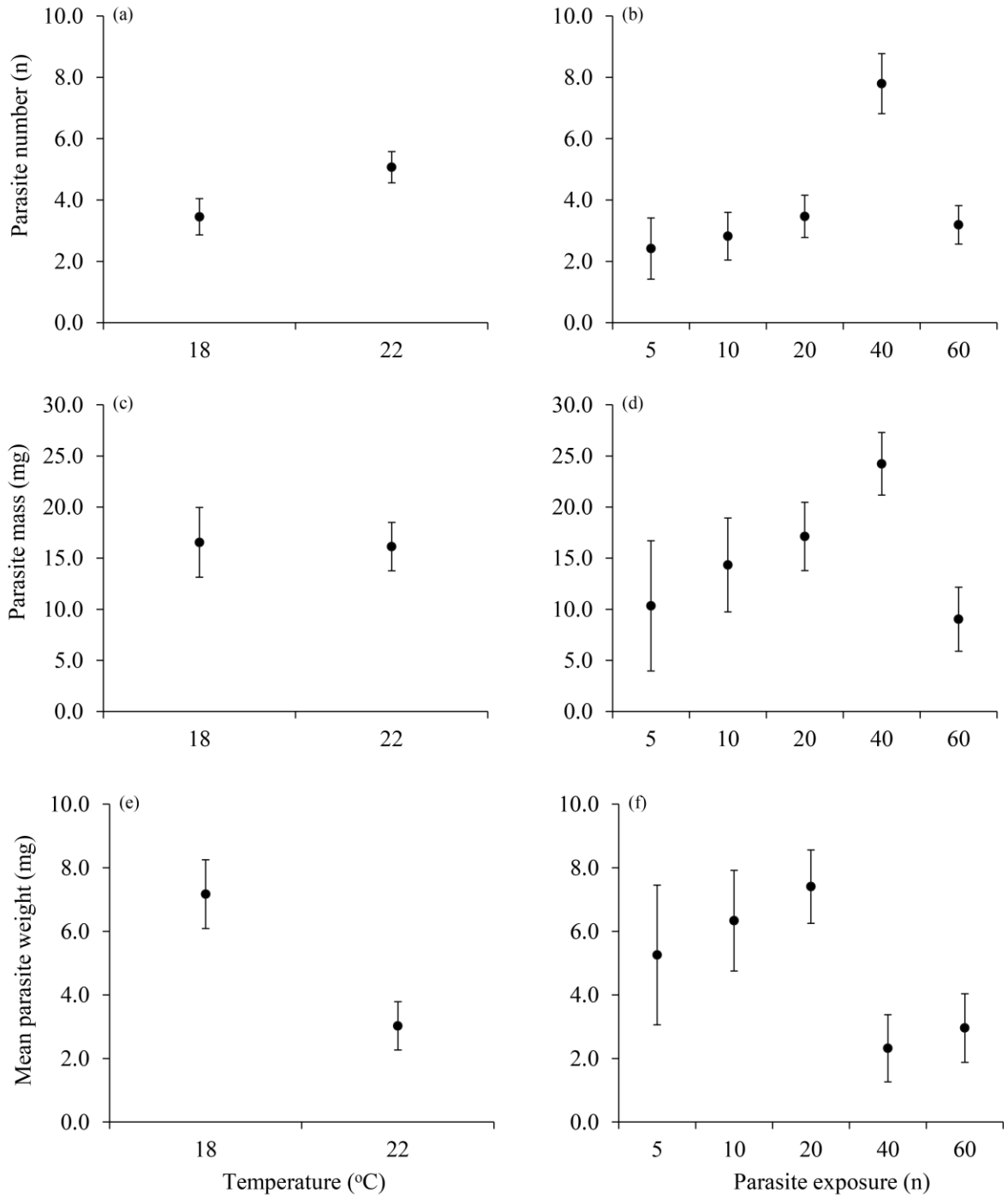
442



443

444 Figure 1.

445



446

447 Figure 2.