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# Virulence in the three-spined stickleback specific parasite *Schistocephalus solidus* is inherited additively

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## HIGHLIGHTS

- Hybrids of high and low virulence *S. solidus* are of intermediate virulence.
- Stickleback body condition is not related to the level of *S. solidus* virulence.
- The immune response to infection does not correspond to the level of virulence.

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## ABSTRACT

Parasite virulence is a key trait in host-parasite interactions and plays a crucial role in infection dynamics. Our study system offers the rare opportunity to study the virulence of an individual macroparasite (*Schistocephalus solidus*) in its vertebrate fish host (*Gasterosteus aculeatus*). The size of the tapeworm in the fish can be regarded as a good proxy for individual parasite virulence, as parasite size correlates negatively with fitness traits of the stickleback host (i.e. the bigger the parasite, the lower the host's reproductive success) as well as directly with the number of parasite offspring to be expected.

To investigate how virulence is inherited, laboratory bred, parasite-naïve stickleback were infected with a cross of two *S. solidus* populations of either high or low virulence, as well as one hybrid cross between the two. The relative weight of the parasite as expressed in the parasite index served as a measure of virulence. Furthermore, we measured several condition and immune related traits in the fish host to assess parasite impact on the stickleback. We hypothesized that parasite virulence is to a large extent genetically determined and correlated with several fitness traits in the stickleback host.

We found that virulence is inherited additively in *S. solidus,* with hybrids of high and low virulence parasites displaying intermediate levels. However, contrary to expectation, infection rate of *S. solidus* in three-spined stickleback is not related to virulence. Even though the presence of the parasite caused differences in host condition, these were indistinguishable between the different levels of virulence in this experiment. Fish immune traits also showed a response to infection but had no correlation with level of parasite virulence.

With this experiment we have taken the first step towards understanding how virulence is inherited and how it is driven in the *Schistocephalus*-stickleback system, even though virulence, as measured here, does not directly translate into cost for the host. A better understanding of the costs inflicted on the host by *S. solidus* infection is needed to understand this interaction in greater detail.



## 1. Introduction

Virulence is the measure of detrimental host exploitation by a parasite, and is crucial for understanding and predicting host-parasite dynamics, infection success, and pathogen evolution. However, the description and measurement of virulence - the costs of a parasitic infection - are not straightforward.

Even though the term virulence is widely used, its definition depends on the context of the study and the system using it. In a general sense, the virulence of a parasite is usually measured and described as a relative measure of the traits that contribute to negative effects in the host. The definition of virulence can include

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host mortality or the general harmfulness of the parasite to its host (Ebert and Herre, 1996; Ewald, 1995).

Other approaches include the measurement of host immune response caused by parasite exploitation (Day et al., 2007; Scharsack et al., 2007) or the associated costs inflicted on the host's reproduction (McPhail and Peacock, 1983; Herre, 1993; Kobasa et al., 2004; Schwanz, 2008; Gooderham and Schulte-Hostedde, 2011). To stay neutral in light of the constant struggle regarding the definition of parasite virulence, in this paper we will define it as anything that causes damage to the host, directly or indirectly.

From an energetic point of view, all organisms have finite quantities of resources available so any amount taken up by a parasite is deducted from the budget of the host (Roff, 2002). This fixed budget of resources forces the constant need for trade-offs between several traits involved in survival, growth and reproduction. These trade-offs are aggravated to the hosts disadvantage by parasitic infection (Thomas and Guégan, 2009). We can assume that the level of virulence correlates with the amount of resources taken up directly from the host's energy budget. Following this assumption and the definition by Herre (1995), describing virulence as any influence with negative consequences, we consider the size of an individual worm, after a specified time, a good proxy for its level of virulence (Kalbe et al., 2016). For example, high virulence, which equates to high energy demand, might threaten host survival and divert more resources away from less immediate demands like host reproduction. Alternatively, infected hosts may redirect the majority of resources from survival to reproductive effort (i.e., terminal investment) (Minchella and LoVerde, 1981).

The mode of virulence inheritance has been studied in several microparasites (Beverley and Turco, 1998; Mackinnon and Read, 1999; Taylor et al., 2006; Ben-Ami et al., 2008; Bouzid et al., 2013; Masri et al., 2015) as well as in macroparasites (Davies et al., 2001). This research has identified specific genes, or virulence factors, which can influence the severity of a parasitic infection. The presence or absence of virulence factors alone should not determine the fundamental capability of the parasite to establish a successful infection (Casadevall and Pirofski, 2001). They should only influence the level of virulence or severity of infection (Dubremetz and Lebrun, 2012).

Single or multiple genes may determine the level of virulence and the mode of determination can include other effects (Agrawal and Lively, 2002a). In complex cases, virulence might be influenced by multiple factors and involve multiple different mechanisms (Okhuysen and Chappell, 2002). The exact nature of the genes causing differences in virulence has been studied to some extent in microparasites but not as much in the more complex macroparasites.

In this study we focus on the costs inflicted on immune and condition traits of the host and investigate the mode of virulence inheritance for the diphyllobothriidean cestode *Schistocephalus solidus*. This tapeworm has a complex life cycle involving three different hosts, being highly specific only for its second intermediate host, the three-spined stickleback (*Gasterosteus aculeatus*) (Bråten, 1966; Orr and Hopkins, 1969; Henrich et al., 2013). The first stage of *S. solidus*, the procercoid, develops in a cyclopoid copepod. After successful transmission to the next host, a three-spined stickleback, the parasite develops into a plerocercoid in the body cavity of the fish. The life cycle is completed after the infected fish is eaten by a bird. Growth only takes place in the two intermediate hosts, and reproduction only in the final host, the fish eating bird (Hopkins and Smyth, 1951; Clarke, 1954; Dubinina, 1980).

The *Schistocephalus*-stickleback system has been used as a model for host-parasite interaction for more than half a century now (Barber and Scharsack, 2010). Contrary to many experimental infection systems using microparasites our system offers the rare opportunity to study the virulence of an individual macroparasite in its vertebrate host.

The virulence of *S. solidus* can be quantified by measuring the relative weight of the individual parasite, following a defined time post exposure. Relative parasite weight is not only linked to host reproduction, e.g. ovum mass (Heins and Baker, 2003), but also directly correlates with parasite fitness, i.e. the number of offspring produced by the parasite (Tierney and Crompton, 1992). This way we can assess the impact of different virulence levels even without detailed knowledge of the mechanisms involved (Heins and Baker, 2003; Scharsack et al., 2016).

Although *S. solidus* growth in its secondary host is largely controlled by the hosts adaptive immune system (Kurtz et al., 2004), different parasite populations show massive differences in intrinsic virulence (Kalbe et al., 2016; Scharsack et al., 2016). We expect virulence in *S. solidus* to be a complex trait with multiple influencing factors. Differences in intrinsic virulence are recorded for multiple naturally occurring populations of this parasite (Scharsack et al., 2016). We postulate that the costs of being infected are directly linked to these different levels of intrinsic virulence in the parasite populations and we aim to investigate several immune and condition related traits of the stickleback host.

To identify the mode of virulence inheritance in *S. solidus* we combined a highly virulent parasite, from a population with high prevalence, with another parasite from a population with low virulence and prevalence, which showed consistent levels of virulence throughout several experiments (Kalbe et al., 2016; Scharsack et al., 2016).

To unravel the genetic base of virulence we bred F1 crosses of two *S. solidus* populations of either high or low virulence, as well as a hybrid cross between these two populations and exposed parasite-naïve laboratory bred sticklebacks to them (Fig. 1). The relative weight of the parasite after a given time, as expressed in the parasite index (PI) served as a measure of virulence (Herre, 1995; Kalbe et al., 2016). Our crossbreeding experiment enabled us to examine the mode of inheritance in the spread of phenotypic variance within the F1 individuals.

## 2. Material and methods

### 2.1. Experimental fish

All stickleback families used in this experiment were lab-bred offspring from fish caught in the lake “Groβer Plöner See” in

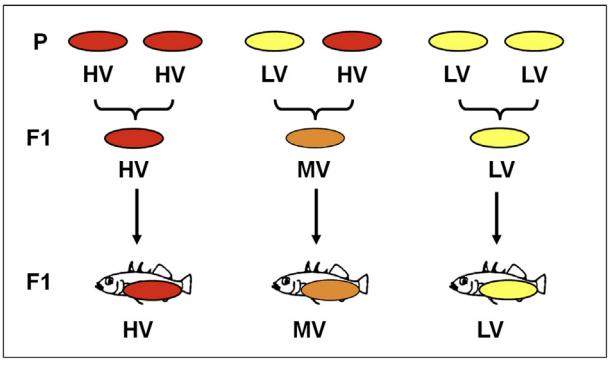


**Fig. 1. Parasite breeding and experimental design**. The parental generation of *Schistocephalus solidus* tapeworms were used to breed high virulence (HV), low virulence (LV) or mixed virulence (MV) F1 offspring. Three-spined sticklebacks (*Gasterosteus aculeatus*) were then exposed to one parasite each.

Germany (54°08′48″ N, 10°24′30″ E). The fish were 4–5 months old at the time of exposure to *S. solidus* and were kept under 16 h of light per day and in constant 18 °C water and room temperature. Fish were fed a diet of frozen copepods, daphnids and chironomid larvae *ad libitum* three times per week.

### 2.2. Experimental parasites

The two *S. solidus* populations of different known virulence levels were caught in two separate locations, in Norway and Germany. The Norwegian population was caught in lake "Skogseidvatnet" (60° 14′ 44″ N, 5° 55′ 03″ E) and the German population at "Neustädter Binnenwasser" (54° 06′ 40″N, 10° 48′ 50″ E). Using the breeding system by Wedekind et al. (1998), modified from Smyth (1946), plerocercoids were bred with another plerocercoid from the same population, as well as with individuals from the other population. Using this method, we obtained offspring from within-population and between-population mating (Fig. 1). Breeding pairs were weight matched to maximize outcrossing rates (Lüscher and Milinski, 2003). Based on previous results indicating the stability of virulence inheritance within lines (Kalbe et al., 2016), the F1 *S. solidus* were given conceptual names. The Norwegian derived *S. solidus* were designated the high virulence group (HV), and the worms bred from the German population were designated the low virulence group (LV). *S. solidus* bred from a combination of the two populations were designated the mixed virulence group (MV). After successful breeding, *S. solidus* eggs were hatched following Dubinina (1980). The first intermediate host *Macrocyclops albidus* (Dubinina, 1980), was subsequently exposed to them. The copepods used here were taken from "Neustädter Binnenwasser" and kept in laboratory culture at a water temperature of 18 °C and 16 h of light per day. Nine days after exposure to *S. solidus*, all copepods were checked with a microscope for successful infection and sticklebacks subsequently exposed to the singly infected copepods. Copepods with a single procercoid present in the body cavity were scored as infected. We then calculated the infection rate for each *S. solidus* virulence group, by dividing the number of infected by the number of exposed copepods.

### 2.3. Experimental infections

Experimental exposure of sticklebacks to *S. solidus* infected copepods was performed at three days for three consecutive weeks. Each week fish were exposed to copepods infected with *S. solidus* from each virulence group in equal numbers. A control group of unexposed fish was included in every week as well. One day before exposure, fish were isolated in 2 L tanks, starved for 24 h and exposed to infected copepods. Exposure was performed by placing a small Petri dish with one infected copepod in each tank for the fish of the treatment group. Fish in the control group fish were sham exposed to an uninfected copepod under the same conditions. In total 920 fish of four fish families were exposed to copepods infected with *S. solidus* from 15 different worm sibships. Three *S. solidus* sibships were of the HV group, eight of MV and four of the LV group. 60 fish were sham exposed to non-infected copepods. 48 h post exposure fish were transferred to larger tanks, each holding 10 individuals.

### 2.4. Dissection and index calculation

Dissections of sticklebacks were performed in three rounds after a growth period of 8 weeks post-exposure. Fish were euthanized with an overdose of MS222, weighed (to the nearest 0.1 mg) and measured (to nearest mm). All fish were sexed by inspection of the reproductive organs and the body cavity was screened for possible *S. solidus* infections. If present, plerocercoids were weighed (to the nearest 0.1 mg) and a parasite index (PI) was calculated as 100 × tapeworm weight/fish weight (Kurtz et al., 2004). We recorded all fish with a single present plerocercoid in the body cavity as infected. All fish without a present plerocercoid were scored as uninfected. We then calculated the infection rate for each *S. solidus* virulence group, by dividing the number of infected by the number of exposed hosts.

To describe condition-related traits in the host, the condition factor (Frischknecht, 1993) was calculated as CF = 100 × fish weight/fish length$^b$ (b = 2.439 as determined by regression analysis). Liver weight was recorded (to the closest 0.1 mg) and the hepatosomatic index as a measure for the nutritional status of the host (Chellappa et al., 1995) was calculated as HSI = 100 × liver weight/fish weight.

As proxies for immunological traits we extracted the spleen and head kidney, weighed them (to the closest 0.1 mg) and calculated a splenosomatic (SSI) and head kidney (HKI) index calculated as 100 × organ weight/fish weight (Bolger and Connolly, 1989; Kurtz et al., 2004) to estimate immunological activation. Head kidney cells were then analysed by fluorescence-activated cell sorting (FACS) to calculate the ratio of granulocytes to lymphocytes (GLratio). The GLratio is an estimate of the relative number and activation of granulocytes in the head kidneys during the innate immune response against macroparasites (Scharsack et al., 2004). To determine the respiratory burst activity of head kidney granulocytes (RLU per granulocyte), a lucigenin enhanced chemiluminescence assay (Kurtz et al., 2004) modified after Scott and Klesius (1981) was used.

### 2.5. Data analysis

All statistics and plots were performed in R v3.3.0 (R Development Core Team, 2016). *S. solidus* of all three virulence levels were tested for significant differences in infection success in copepods and fish by performing a $\chi 2$ test using the *chisq.test* function. We tested for differences in fish mortality by comparing death rates of treatment and control group fish using the *prop.test* function of the "stats" package.

The influence of the virulence level on the variation in parasite index (PI), condition factor (CF), hepatosomatic index (HSI), splenosomatic index (SSI), head kidney index (HKI), granulocyte/lymphocyte ratio (GLratio) and respiratory burst activity of head kidney granulocytes (RLU per granulocyte) was analysed using the *lmer* function for linear mixed effect models (LMM) in the "lme4" package (Bates et al., 2015). For evaluation each of these indices was added as the dependent variable in our LMMs. P-values for the fixed effects of the models were obtained using the *lmer* function from the "lmerTest" package (Kuznetsova et al., 2016).

To account for variation caused by the separation of our experiment into rounds, we added the experiment round as a random term. Because fish sex and, worm sibship, as well as fish family are influencing factors, we included those three variables as random terms in our analysis as well. We did not test for specific effects of our random terms but included them in our models as a full set to avoid the analysis of each index with a separate set of random terms. Using this approach we accounted for the same amount of random variation in all the different variables datasets and avoided a possible biasing effect of the random term selection process. Tukey's honest significant differences, calculated with the *glht* function from the "multcomp" package (Hothorn et al., 2008), were used as post-hoc tests.

## 3. Results

A total of 980 fish were initially used in the experiment, 920 of which were exposed to *S. solidus* while 60 were used as an uninfected control group. From the exposed fish, 11 had to be excluded from the experiment due to accidental double infection and 92 fish died during the 8 weeks after exposure to *S. solidus*. The survival of fish was not significantly different between treatment and control group (HV = 86%, MV = 88%, LV = 86%, CTRL = 90%, $\chi^2_{3,\ 1.3887}$, $P = 0.7082$). We were unable to obtain a sufficient number of head kidney cells from 20 fish, which excluded them from the immune assays. One liver and one spleen sample were lost during dissection.

### 3.1. Infection rates

Infection rates of copepods were significantly different between virulence groups (HV 55.31%, MV 49.65%, LV 40.89%, $\chi^2_{2,\ 27.593}$, $P = <0.001$). Even though significantly different, the infection rates were of a similar order of magnitude in all three virulence groups and resulted in similar levels of fish infection between high and low virulence levels. Infection success in fish was observed to be significantly different between virulence levels (HV 27.67%, MV = 37.26%, LV = 27.09%, $\chi^2_{2,\ 8.7994}$, $P = 0.013$), with intermediate virulence (MV) having the highest success.

### 3.2. Parasite Index (PI)

The parasite index (PI), which is used as a proxy for virulence of the plerocercoid in the stickleback, indicates an additive mode of inheritance for this trait. While offspring of the HV and LV groups had respectively highest and lowest average PI, MV plerocercoids had intermediate PI (mean PI% ± SE, HV = 25.47% ± 1.510, MV = 21.09% ± 1.783, LV = 12.31 ± 1.472, $F_{(2,\ 262)} = 29.339$, $P = <0.001$, Fig. 2). A plot showing sibship specific PI is available in the supplementary material (supplementary material, Fig. 1).

### 3.3. Condition and immune related traits

Virulence level in *S. solidus* has no significant effect on host condition or immune related traits in the host. A general effect of infection by *S. solidus* could, however, be identified in various traits. The condition factor (CF) showed a cost of infection, resulting in lower average CF for HV and MV infected fish compared to control fish (CF; $F_{(3,316)} = 5.6835$, P = <0.001; HV-CTRL Z = −3.166 P = <0.001; MV-CTRL Z = −3.328 P = <0.001, Fig. 3A).

The HSI in all three treatment groups differed significantly from control fish (HSI; $F_{(3,315)} = 7.991$, P = <0.001; HV-CTRL Z = −4.397, P = <0.001, MV-CTRL Z = −4.266 P = <0.001; LV-CTRL, Z = −3.607, P = <0.001, Fig. 3B).

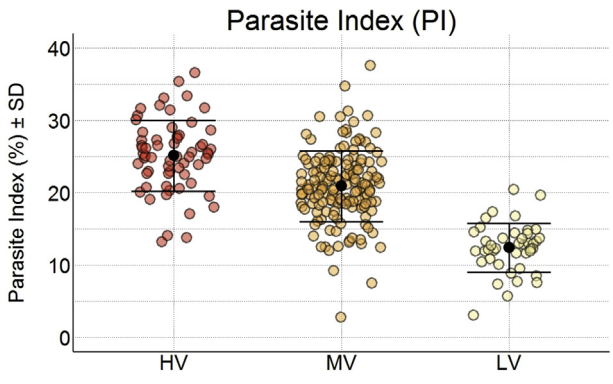


**Fig. 2.** Parasite index (PI) for groups infected with *S. solidus* of different virulence levels.

In immune related traits only the splenosomatic index (SSI) showed a significant response to the hosts infection status for two virulence levels (SSI; $F_{(3,315)} = 3.6843$, $P = 0.012$; HV-CTRL $Z = 2.967$, $P = 0.015$; MV-CTRL $Z = 2.977$, $P = 0.014$, Fig. 3C). As in the condition related traits, the response of the host in this immune-related trait was most prominent in the HV and MV infected groups.

Our data showed no significant reaction to virulence level or to infection status for HKI (HKI, $F_{(3,316)} = 1.904$, $P = 0.159$, Fig. 3D).

The GLratio showed no significant differences between infection status or virulence level (GLratio; $F_{(3,316)} = 3.8468$, $P = 0.111$, Fig. 3E).

The respiratory burst activity of head kidney granulocytes, as indicated by RLU per granulocyte ratio, was significantly higher in infected fish in general (infected fish - CTRL, Z = 2.104, P = 0.035) while no virulence level specific differences were significant (RLU/granulocyte, $F_{(3,316)} = 1.5204$, $P = 0.209$, Fig. 3F). We added "Total RLU" as an additional measure for respiratory burst activity without relation to the number of granulocytes to the supplementary material (Fig. 2).

## 4. Discussion

In the present study, sticklebacks were exposed to a high virulent (HV), low virulent (LV) or hybrid of mixed virulence (MV) parasite. We quantified differences in infection rate of both hosts, the mode of virulence inheritance of *Schistocephalus solidus* as well as if different levels of parasite virulence account for differences in host condition and immune system. Overall, we found that parasites of mixed virulence were intermediate in their virulence in terms of relative parasite size but not in regard to host condition or immune traits.

### 4.1. Infection rates

Infection success of *S. solidus* was not related to the level of virulence. HV and LV *S. solidus* did not differ significantly in their infection success. The rate of successful infection seems to be influenced by the hosts' genotype and the ability of the host to successfully prevent or clear the early infection, rather than by the parasite's genotype (Kalbe et al., 2016). It is worth noting that *S. solidus* infection can only be cleared within a very short time frame of about two weeks post exposure. Once the parasite has successfully established within the body cavity, infection is usually maintained (Scharsack et al., 2007). At the time point of dissection only successfully established parasites should remain in the hosts. Surprisingly, the hybrid MV parasites had an overall higher infection success rate. This might be because these novel genotypes can possess characteristics that impede the ability of hosts to combat or detect early infections. (reviewed by King et al., 2015).

### 4.2. Virulence is a complex, probably polygenic trait

Virulence is a complex trait and multiple virulence factors usually form the sum of what we describe as virulence. Previous work on our model system already showed that the complex nature of the interaction is based on different immunological pathways and other means of interacting and influencing factors. These include immune evasion (Hammerschmidt and Kurtz, 2005; Franke et al., 2014) and immune modulation (Scharsack et al., 2007, 2013; Kutyrev et al., 2014).

The measured PI for the MV *S. solidus* plerocercoids covered the

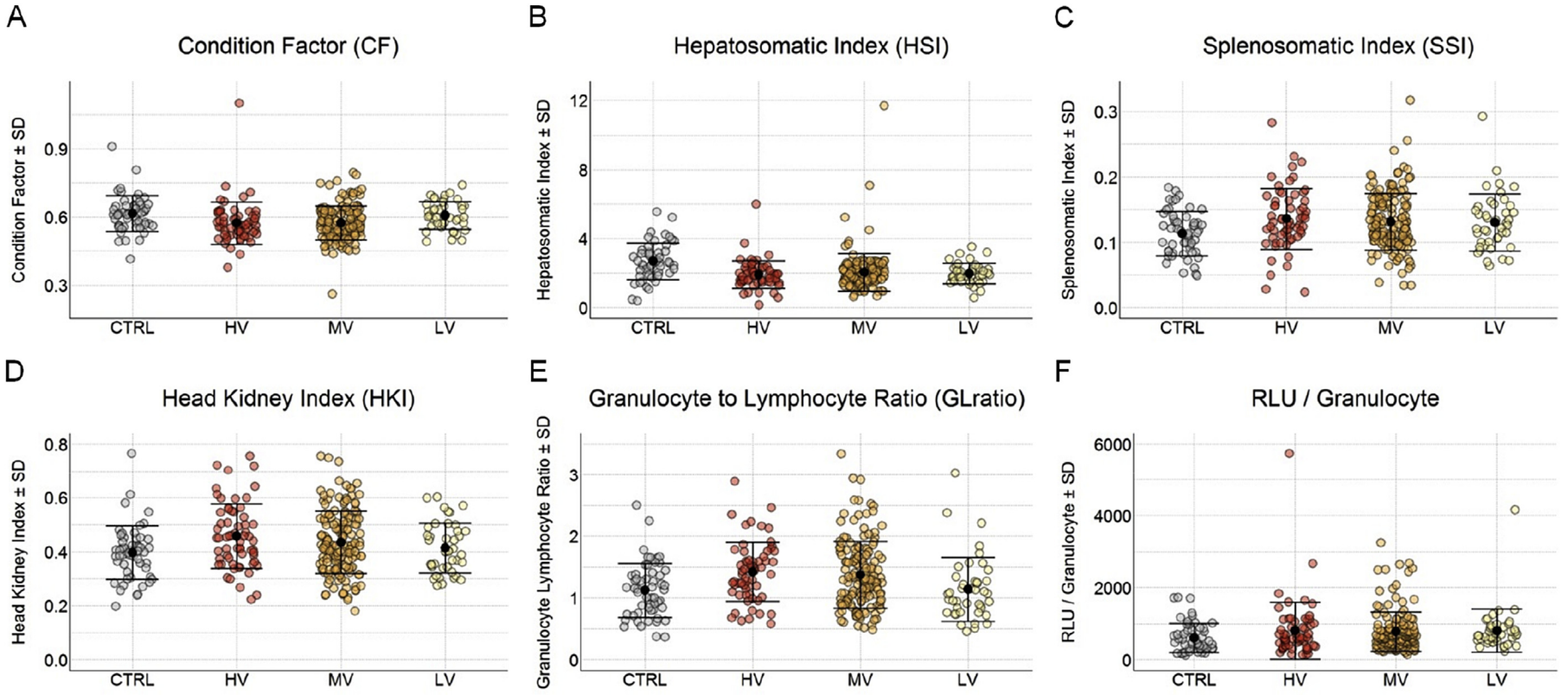


**Fig. 3.** Condition and immune related indices for treatment groups infected with *S. solidus* of different virulence levels.

whole range of the parental HV and LV indices, but was on average intermediate. This spread of individual PI shows the complex nature of virulence inheritance in *S. solidus*. Considering the number of known factors causing variations in virulence, the same PI can potentially be the result of various different genetic backgrounds. The involvement of several genes in an additive mode of inheritance, including additional effects of recombination, forms the likeliest explanation for the wide range of phenotypic spread described here. Due to the lack of sufficient genetic data, other explanations like single locus phenotype determination have to be considered as possible explanations. The phenotypic spread might as well be the result of environmental variation or co-dominance effects. Additional studies, focusing on the genetic architecture of the trait, are necessary to describe it in further detail. The inclusion of additional host-parasite populations with similar differences in contrasting levels of virulence should give additional insight about the potential complexity of virulence in other populations.

Since the observed spread of PI values was measured in an F1 cross experiment, a polygenic determination of virulence seems more likely than variation caused by variations of the same gene. Agrawal and Lively (2002b) illustrate, in a theoretical approach, how the pattern of trait inheritance in our study possibly may be explained. The described theoretical model, including recombination effects and gene for gene matching, has some strikingly similar features to the results of our cross breeding experiment. Without knowledge of the mechanistic background in our system, cause and effect relationships have to remain speculative for now.

Other host parasite systems, studied in greater detail, suggest a complex nature of interaction. *Leishmania* sp. for example, has a wide variety of different genes with equally varying degrees of virulence differences (Bifeld and Clos, 2015). A similar mode of inheritance involving many factors has been reported for *Cryptosporidium* (Bouzid et al., 2013), and *Toxoplasma* (Dubremetz and Lebrun, 2012).

### 4.3. Host condition is not correlated with parasite size

The lack of virulence level-specific detrimental effects in the measured condition related traits in this experiment was surprising. We can assume that differences in the PI are the product of different amounts of host resources consumed. Despite this, different virulence levels did not result in corresponding negative effects in their hosts (Fig. 2). This lack of detrimental effects could be at least partially explained by potential weight compensation of the fish by different tissue-water ratios in response to parasitic infection (Nordeide and Matos, 2016).

Another explanation is the possibility that fish were presented with an unnaturally high amount of food under experimental conditions, resulting in a lack of difference in the nutritional status of individual hosts. The equalising effect of this rich feeding regime might have concealed the difference between hosts infected with parasites of different virulence levels and weakened the difference between parasite-infected and control group fish (Candolin and Voigt, 2001):

### 4.4. Host immune system traits do not correlate with parasite size

Another striking finding was the lack of virulence level specific differences in immune related traits. Despite being used and published before, the methods employed in this study represent only a rather crude estimate of the fish's immune system activity. The measures of HKI and SSI only yielded rough estimates of the correlation between organ weights and immune activity. These indices potentially represent most of the whole spectrum of the stickleback's immune response, but unfortunately did not have the specificity to identify virulence-level specific effects. Yet, a general effect of infection was found to be significant for SSI.

### 4.5. Additional fitness costs of the host may be related to parasite size

Several studies have shown a wide range of different negative effects caused by *S. solidus* in its host. Physiological effects include increased oxygen consumption (Meakins and Walkey, 1975) and increased nutritional demand (Pascoe and Mattey, 1977). The detrimental effects of infection might also influence traits not measured in this experiment. The degree of mobility impairment of infected fish for example was not determined. Under natural conditions this loss of mobility would most likely lead to a potentially higher average predation rate of parasitized fish and lowered ability to compete for available resources.

Influences on behaviour (Milinski, 1985, Milinski and Bakker, 1990; Barber and Huntingford, 1995; Barber et al., 2004) and phenotypic manipulation (LoBue and Bell, 1993) increasing the hosts' risk of avian predation have been reported before. The potential costs regarding a loss in fecundity could also not be determined here.

Field studies have described cases of infected sticklebacks not reaching maturity, preventing successful reproduction (McPhail and Peacock, 1983; Tierney et al., 1996) even though there is evidence some stickleback populations with especially high *S. solidus* prevalence and virulence have evolved mechanisms for fecundity compensation (Heins et al., 1999; Heins and Baker, 2014). This negative influence of infection on host reproduction is important to consider, but due to the complicated methods of measuring it, is not often tested (Abbate et al., 2015).

The influence on several traits of the host's immune system caused by *S. solidus* also has the potential to change the infection success of other parasite species, leading to an increase of infection rates of said parasite species in the process. Benesh and Kalbe (2016) describe a quantitative effect of secondary parasite infections, linked to the virulence level of a preceding *S. solidus* infection. This example emphasizes that the impact of *S. solidus* virulence has to be regarded in context with the parasite species composition of the host. As not all parasite species seem to be able to take advantage of different levels of *S. solidus* virulence, the same level of virulence can cause a different sum of costs in different populations depending on the parasite species composition.

### 4.6. Parasite virulence evolves toward optimality

Our experiment shows that virulence of *S. solidus* can rapidly change when novel genotypes are combined. Given that this parasite uses a highly mobile final host (which can be any piscivorous bird species), the introduction of novel genotypes into a parasite population can rapidly alter the host-parasite dynamics and therefore the evolution of virulence.

Parasites, as any species, by whatever mode of resource acquisition, have to evolve towards optimized fitness (Alizon and Michalakis, 2015). Parasite fitness is tightly linked to virulence, showing the acquisition rate of resources in the system used here. The optimal, sustainable level of virulence depends on various factors. Examples include the trade-off of between transmission and virulence or the parasites influence on host survival. In contrast to the results of early modelling approaches, optimal virulence is not necessarily the maximal level obtainable by the parasite (O'Keefe and Antonovics, 2002). Instead, an intermediate level of virulence might be optimal in the sense of maximized lifetime reproductive success (Jensen et al., 2006).

This intermediate optimum was strikingly similar in at least two

populations of *S. solidus*, (Kalbe et al., 2016). The similar optimum might very well be connected to parasite prevalence in these populations. Host-parasite combinations with high parasite prevalence could have developed a higher level of intrinsic virulence in response to the increase in host defence. Low prevalence populations on the others hand could have decreased the necessity for fish to develop a strong immune response resulting in lower parasite virulence (Kalbe et al., 2016). The resulting level of virulence might seem similar in sympatric combinations, but the causing effects might be entirely different for each population.

### 4.7. Conclusion

We showed the additive inheritance of virulence in *Schistocephalus solidus* in the intermediate phenotype of mixed virulence plerocercoids. Interestingly, this did not translate into correlated costs for the host in the measured traits. This lack of correlation leaves doubt about the suitability of worm size as a proxy for parasite virulence in this system. Either the measure supposed to indicate virulence was not suited to do so, or the varying costs have been imposed on host traits not measured here. With this experiment we have taken the first step into understanding how virulence is inherited and how it is driven in the *Schistocephalus* - stickleback system, but we need a better understanding on the costs of parasite infection on the host.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.exppara.2017.02.016.

## References

Abbate, J.L., Kada, S., Lion, S., 2015. Beyond mortality: sterility as a neglected component of parasite virulence. PLoS Pathog. 11, 1–10. http://dx.doi.org/10.1371/journal.ppat.1005229.

Agrawal, A.F., Lively, C.M., 2002a. Modelling infection as a two-step process combining gene-for-gene and matching-allele genetics. Proc. R. Soc. B Biol. Sci. 270, 323–334. http://dx.doi.org/10.1098/rspb.2002.2193.

Agrawal, A.F., Lively, C.M., 2002b. Infection genetics: gene-for-gene versus matching-alleles models and all points in between. Evol. Ecol. Res. 4, 79–90.

Alizon, S., Michalakis, Y., 2015. Adaptive virulence evolution: the good old fitness-based approach. Trends Ecol. Evol. 30, 248–254. http://dx.doi.org/10.1016/j.tree.2015.02.009.

Barber, I., Scharsack, J.P., 2010. The three-spined stickleback-*Schistocephalus solidus* system: an experimental model for investigating host-parasite interactions in fish. Parasitology 137, 411–424. http://dx.doi.org/10.1017/S0031182009991466.

Barber, I., Huntingford, F.A., 1995. The effect of *Schistocephalus solidus* (Cestoda: Pseudophyllidea) on the foraging and shoaling behaviour of three-spined sticklebacks, *Gasterosteus aculeatus*. Behaviour 132, 1223–1240. http://dx.doi.org/10.1163/156853995X00540.

Barber, I., Walker, P., Svensson, P.A., 2004. Behavioural responses to simulated avian predation in female three spined sticklebacks: the effect of experimental Schistocephalus solidus infections. Behaviour 141, 1425–1440. http://dx.doi.org/10.1163/1568539042948231.

Bates, D., Mächler, M., Bolker, B.M., Walker, S., 2015. Fitting linear mixed-effects models using {lme4}. J. Stat. Softw. 67, 1–48. http://dx.doi.org/10.18637/jss.v067.i01.

Ben-Ami, F., Mouton, L., Ebert, D., 2008. The effects of multiple infections on the expression and evolution of virulence in a *Daphnia*-endoparasite system. Evolution 62, 1700–1711. http://dx.doi.org/10.1111/j.1558-5646.2008.00391.x.

Benesh, D.P., Kalbe, M., 2016. Experimental parasite community ecology: intraspecific variation in a large tapeworm affects community assembly. J. Anim. Ecol. 85, 1004–1013. http://dx.doi.org/10.1111/1365-2656.12527.

Beverley, S.M., Turco, S.J., 1998. Lipophosphoglycan (LPG) and the identification of virulence genes in the protozoan parasite *Leishmania*. Trends Microbiol. 6, 35–40. http://dx.doi.org/10.1016/S0966-842X(97)01180-3.

Bifeld, E., Clos, J., 2015. The genetics of *Leishmania* virulence. Med. Microbiol. Immunol. 204, 619–634. http://dx.doi.org/10.1007/s00430-015-0422-1.

Bolger, T., Connolly, P.L., 1989. The selection of suitable indices for the measurement and analysis of fish condition. J. Fish. Biol. 34, 171–182. http://dx.doi.org/10.1111/j.1095-8649.1989.tb03300.x.

Bouzid, M., Hunter, P.R., Chalmers, R.M., Tyler, K.M., 2013. *Cryptosporidium* pathogenicity and virulence. Clin. Microbiol. Rev. 26, 115–134. http://dx.doi.org/10.1128/CMR.00076-12.

Bråten, T., 1966. Host specificity in *Schistocephalus solidus*. Parasitology 56, 657–664. http://dx.doi.org/10.1017/S0031182000071687.

Candolin, U., Voigt, H.R., 2001. No effect of a parasite on reproduction in stickleback males: a laboratory artefact? Parasitology 122, 457–464. http://dx.doi.org/10.1017/S0031182001007600.

Casadevall, A., Pirofski, L., 2001. Host-pathogen interactions: the attributes of virulence. J. Infect. Dis. 184, 337–344. http://dx.doi.org/10.1086/322044.

Chellappa, S., Huntingford, F.A., Strang, R.H.C., Thomson, R.Y., 1995. Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. J. Fish. Biol. 47, 775–787. http://dx.doi.org/10.1111/j.1095-8649.1995.tb06002.x.

Clarke, A.S., 1954. Studies on the life cycle of the pseudophyllidean cestode *Schistocephalus solidus*. Proc. Zool. Soc. Lond. 124, 257–302. http://dx.doi.org/10.1111/j.1469-7998.1954.tb07782.x.

Davies, C.M., Webster, J.P., Woolhous, M.E., 2001. Trade-offs in the evolution of virulence in an indirectly transmitted macroparasite. Proc. Biol. Sci. 268, 251–257. http://dx.doi.org/10.1098/rspb.2000.1367.

Day, T., Graham, A.L., Read, A.F., Kl, C., 2007. Evolution of parasite virulence when host responses cause disease. Proc. Biol. Sci. 274, 2685–2692. http://dx.doi.org/10.1098/rspb.2007.0809.

Dubinina, M.N., 1980. Tapeworms (Cestoda, Ligulidae) of the Fauna of the USSR. Amerind Publishing Co., Pvt., New Dehli, India.

Dubremetz, J.F., Lebrun, M., 2012. Virulence factors of *Toxoplasma gondii*. Microbes Infect. 14, 1403–1410. http://dx.doi.org/10.1016/j.micinf.2012.09.005.

Ebert, D., Herre, E.A., 1996. The evolution of parasitic diseases. Parasitol. Today 12, 96–101. http://dx.doi.org/10.1016/0169-4758(96)80668-5.

Ewald, P.W., 1995. The evolution of virulence: a unifying link between parasitology and ecology. J. Parasitol. 81, 659–669. http://dx.doi.org/10.2307/3283951.

Franke, F., Rahn, A.K., Dittmar, J., Erin, N., Rieger, J.K., Haase, D., Samonte-Padilla, I.E., Lange, J., Jakobsen, P.J., Hermida, M., Fernández, C., Kurtz, J., Bakker, T.C.M., Reusch, T.B.H., Kalbe, M., Scharsack, J.P., 2014. Invitro leukocyte response of three-spined sticklebacks (*Gasterosteus aculeatus*) to helminth parasite antigens. Fish. Shellfish Immunol. 36, 130–140. http://dx.doi.org/10.1016/j.fsi.2013.10.019.

Frischknecht, M., 1993. The breeding colouration of male three-spined sticklebacks (*Gasterosteus aculeatus*) as an indicator of energy investment in vigour. Evol. Ecol. 7, 439–450. http://dx.doi.org/10.1007/BF01237640.

Gooderham, K., Schulte-Hostedde, A., 2011. Macroparasitism influences reproductive success in red squirrels (*Tamiasciurus hudsonicus*). Behav. Ecol. 22, 1195–1200. http://dx.doi.org/10.1093/beheco/arr112.

Hammerschmidt, K., Kurtz, J., 2005. Surface carbohydrate composition of a tapeworm in its consecutive intermediate hosts: individual variation and fitness consequences. Int. J. Parasitol. 35, 1499–1507. http://dx.doi.org/10.1016/j.ijpara.2005.08.011.

Heins, D.C., Baker, J.A., 2003. Reduction of egg size in natural populations of threespine stickleback infected with a cestode macroparasite. J. Parasitol. 89, 1–6. http://dx.doi.org/10.1645/0022-3395(2003)089 [0001:ROESIN]2.0.CO;2.

Heins, D.C., Baker, J.A., 2014. Fecundity compensation and fecundity reduction among populations of the three-spined stickleback infected by *Schistocephalus solidus* in Alaska. Parasitology 141, 1088–1096. http://dx.doi.org/10.1017/S0031182014000535.

Heins, D.C., Singer, S.S., Baker, J. a, 1999. Virulence of the cestode *Schistocephalus solidus* and reproduction in infected threespined stickleback, *Gasterosteus aculeatus*. Can. J. Zool. 77, 1967–1974. http://dx.doi.org/10.1139/z99-180.

Henrich, T., Benesh, D.P., Kalbe, M., 2013. Hybridization between two cestode species and its consequences for intermediate host range. Parasit. Vectors 6, 33. http://dx.doi.org/10.1186/1756-3305-6-33.

Herre, E.A., 1993. Population structure and the evolution of virulence in nematode parasites of fig wasps. Science 259, 1442–1445. http://dx.doi.org/10.1126/science.259.5100.1442.

Herre, E.A., 1995. Factors affecting the evolution of virulence: nematode parasites of fig wasps as a case study. Parasitology 111, 179–191.

Hopkins, C.A., Smyth, J.D., 1951. Notes on the morphology and life history of *Schistocephalus solidus* (Cestoda: Diphyllobothriidae). Parasitology 41, 283–291. http://dx.doi.org/10.1017/S0031182000084134.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biom. J. 50, 346–363. http://dx.doi.org/10.1002/bimj.200810425.

Jensen, K.H., Little, T., Skorping, A., Ebert, D., 2006. Empirical support for optimal

virulence in a castrating parasite. PLoS Biol. 4, 1265–1269. http://dx.doi.org/10.1371/journal.pbio.0040197.
Kalbe, M., Eizaguirre, C., Scharsack, J.P., Jakobsen, P.J., 2016. Reciprocal cross infection of sticklebacks with the diphyllobothriidean cestode *Schistocephalus solidus* reveals consistent population differences in parasite growth and host resistance. Parasit. Vectors 9, 130. http://dx.doi.org/10.1186/s13071-016-1419-3.
King, K.C., Stelkens, R.B., Webster, J.P., Smith, D.F., Brockhurst, M.A., 2015. Hybridization in parasites: consequences for adaptive evolution, pathogenesis, and public health in a changing world. PLoS Pathog. 11, 1–12. http://dx.doi.org/10.1371/journal.ppat.1005098.
Kobasa, D., Takada, A., Shinya, K., Hatta, M., Halfmann, P., Theriault, S., Suzuki, H., Nishimura, H., Mitamura, K., Sugaya, N., Usui, T., Murata, T., Maeda, Y., Watanabe, S., Suresh, M., Suzuki, T., Suzuki, Y., Feldmann, H., Kawaoka, Y., 2004. Enhanced virulence of influenza A viruses with the haemagglutinin of the 1918 pandemic virus. Nature 431, 703–707. http://dx.doi.org/10.1038/nature02951.
Kurtz, J., Kalbe, M., Aeschlimann, P.B., Häberli, M. a, Wegner, K.M., Reusch, T.B.H., Milinski, M., 2004. Major histocompatibility complex diversity influences parasite resistance and innate immunity in sticklebacks. Proc. Biol. Sci. 271, 197–204. http://dx.doi.org/10.1098/rspb.2003.2567.
Kutyrev, I.A., Franke, F., Büscher, J., Kurtz, J., Scharsack, J.P., 2014. Invitro effects of prostaglandin E2 on leucocytes from sticklebacks (*Gasterosteus aculeatus*) infected and not infected with the cestode *Schistocephalus solidus*. Fish. Shellfish Immunol. 41, 473–481. http://dx.doi.org/10.1016/j.fsi.2014.09.031.
Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2016. lmerTest: Tests in Linear Mixed Effects Models. R package version 2.0-32. https://CRAN.Rproject.org/package=lmerTest.
LoBue, C.P., Bell, M.A., 1993. Phenotypic manipulation by the cestode parasite *Schistocephalus solidus* of its intermediate host, *Gasterosteus aculeatus*, the threespine stickleback. Am. Nat. 142, 725–735. http://dx.doi.org/10.1086/285568.
Lüscher, A., Milinski, M., 2003. Simultaneous hermaphrodites reproducing in pairs self-fertilize some of their eggs: an experimental test of predictions of mixed-mating and Hermaphrodite's Dilemma theory. J. Evol. Biol. 16, 1030–1037. http://dx.doi.org/10.1046/j.1420-9101.2003.00552.x.
Mackinnon, M.J., Read, A.F., 1999. Selection for high and low virulence in the malaria parasite *Plasmodium chabaudi*. Proc. Biol. Sci. 266, 741–748. http://dx.doi.org/10.1098/rspb.1999.0699.
Masri, L., Branca, A., Sheppard, A.E., Papkou, A., Laehnemann, D., Guenther, P.S., Prahl, S., Saebelfeld, M., Hollensteiner, J., Liesegang, H., Brzuszkiewicz, E., Daniel, R., Michiels, N.K., Schulte, R.D., Kurtz, J., Rosenstiel, P., Telschow, A., Bornberg-Bauer, E., Schulenburg, H., 2015. Host–pathogen coevolution: the selective advantage of *Bacillus thuringiensis* virulence and its cry toxin genes. PLoS Biol. 13, 1–30. http://dx.doi.org/10.1371/journal.pbio.1002169.
McPhail, J.D., Peacock, S.D., 1983. Some effects of the cestode (*Schistocephalus solidus*) on reproduction in the threespine stickleback (*Gasterosteus aculeatus*): evolutionary aspects of a host–parasite interaction. Can. J. Zool. 61, 901–908. http://dx.doi.org/10.1139/z83-118.
Meakins, R.H., Walkey, M., 1975. The effects of parasitism by the plerocercoid of *Schistocephalus solidus* Muller 1776 (Pseudophyllidea) on the respiration of the three-spined stickleback *Gasterosteus aculeatus* L. J. Fish. Biol. 7, 817–824. http://dx.doi.org/10.1111/j.1095-8649.1975.tb04652.x.
Milinski, M., 1985. Risk of predation of parasitized sticklebacks (*Gasterosteus Aculeatus* L.) under competition for food. Behaviour 93, 203–216. http://dx.doi.org/10.1163/156853986X00883.
Milinski, M., Bakker, T.C.M., 1990. Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. Nature 344, 330–333. http://dx.doi.org/10.1038/344330a0.
Minchella, D.J., LoVerde, P.T., 1981. A cost of increased early reproductive effort in the snail *Biomphalaria glabrata*. Am. Nat. 118, 876–881. http://dx.doi.org/10.1086/283879.
Nordeide, J.T., Matos, F., 2016. Solo *Schistocephalus solidus* tapeworms are nasty. Parasitology 143, 1–9. http://dx.doi.org/10.1017/S0031182016000676.
O'Keefe, K.J., Antonovics, J., 2002. Playing by different rules: the evolution of virulence in sterilizing pathogens. Am. Nat. 159, 597–605. http://dx.doi.org/10.1086/339990.
Okhuysen, P.C., Chappell, C.L., 2002. *Cryptosporidium* virulence determinants - are we there yet? Int. J. Parasitol. 32, 517–525. http://dx.doi.org/10.1016/S0020-7519(01)00356-3.
Orr, T.S.C., Hopkins, C.A., 1969. Maintenance of *Schistocephalus solidus* in the laboratory with observations on rate of growth of, and proglottid formation in, the plerocercoid. J. Fish. Res. Board Can. 26, 741–752. http://dx.doi.org/10.1139/f69-073.
Pascoe, D., Mattey, D., 1977. Dietary stress in parasitized and non-parasitized *Gasterosteus aculeatus* L. Z. für Parasitenkd 51, 179–186. http://dx.doi.org/10.1007/BF00500957.
R Development Core Team, 2016. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.R-project.org/.
Roff, D., 2002. Life history, evolution of. In: Encyclopedia of Biodiversity. Elsevier. http://dx.doi.org/10.1016/B978-0-12-384719-5.00087-3.
Scharsack, J.P., Kalbe, M., Derner, R., Kurtz, J., Milinski, M., 2004. Modulation of granulocyte responses in three-spined sticklebacks *Gasterosteus aculeatus* infected with the tapeworm *Schistocephalus solidus*. Dis. Aquat. Organ 59, 141–150. http://dx.doi.org/10.3354/dao059141.
Scharsack, J.P., Franke, F., Erin, N.I., Kuske, A., Büscher, J., Stolz, H., Samonte, I.E., Kurtz, J., Kalbe, M., 2016. Effects of environmental variation on host–parasite interaction in three-spined sticklebacks (*Gasterosteus aculeatus*). Zoology 119, 375–383. http://dx.doi.org/10.1016/j.zool.2016.05.008.
Scharsack, J.P., Gossens, A., Franke, F., Kurtz, J., 2013. Excretory products of the cestode, *Schistocephalus solidus*, modulate in vitro responses of leukocytes from its specific host, the three-spined stickleback (*Gasterosteus aculeatus*). Fish. Shellfish Immunol. 35, 1779–1787. http://dx.doi.org/10.1016/j.fsi.2013.08.029.
Scharsack, J.P., Koch, K., Hammerschmidt, K., 2007. Who is in control of the stickleback immune system: interactions between *Schistocephalus solidus* and its specific vertebrate host. Proc. Biol. Sci. 274, 3151–3158. http://dx.doi.org/10.1098/rspb.2007.1148.
Schwanz, L.E., 2008. Chronic parasitic infection alters reproductive output in deer mice. Behav. Ecol. Sociobiol. 62, 1351–1358. http://dx.doi.org/10.1007/s00265-008-0563-y.
Scott, A.L., Klesius, P.H., 1981. Chemiluminescence: a novel analysis of phagocytosis in fish. Dev. Biol. Stand 49, 243–254.
Smyth, J.D., 1946. Studies on tapeworm physiology, the cultivation of *Schistocephalus solidus* in vitro. J. Exp. Biol. 23, 47–70. http://dx.doi.org/10.1017/S0031182000071936.
Taylor, S., Barragan, A., Su, C., Fux, B., Fentress, S.J., Tang, K., Beatty, W.L., Hajj, H., El, Jerome, M., Behnke, M.S., White, M., Wootton, J.C., Sibley, L.D., 2006. A secreted serine-threonine kinase determines virulence in the eukaryotic pathogen *Toxoplasma gondii*. Science 314, 1776–1780. http://dx.doi.org/10.1126/science.1133643.
Thomas, F., Guégan, J.F., 2009. Ecology and evolution of parasitism. Oxf. Biol. 103, 1355–1363. http://dx.doi.org/10.1093/aob/mcp122.
Tierney, J.F., Huntingford, F. a, Crompton, D.W.T., 1996. Body condition and reproductive status in sticklebacks exposed to a single wave of *Schistocephalus solidus* infection. J. Fish. Biol. 49, 483–493. http://dx.doi.org/10.1111/j.1095-8649.1996.tb00043.x.
Tierney, J.F., Crompton, D.W.T., 1992. Infectivity of plerocercoids of *Schistocephalus solidus* (Cestoda: Ligulidae) and fecundity of the adults in an experimental definitive host, *Gallus gallus*. J. Parasitol. 78, 1049–1054. http://dx.doi.org/10.2307/3283228.
Wedekind, C., Strahm, D., Schärer, L., 1998. Evidence for strategic egg production in a hermaphroditic cestode. Parasitology 117, 373–382. http://dx.doi.org/10.1017/S0031182098003114.