Solo *Schistocephalus solidus* tapeworms are nasty

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SUMMARY

Trophically transmitted parasites must trade-off own growth on one hand and energy drain from the intermediate host on the other hand, since killing the host before transmission to the next host is a dead end for both parasites and hosts. This challenge becomes especially intriguing when multiple parasites find themselves within the same individual host. The tapeworm *Schistocephalus solidus* may gain more than 98% of its final body mass within few months infecting its three-spined stickleback (*Gasterosteus aculeatus*) intermediate host. During these months the tapeworms may achieve a mass even larger than its host. We studied virulence of single and multiple infections of *S. solidus*, by comparing body condition of wild stickleback hosts in two perennial stickleback populations located at high latitudes, and each population was studied in two different years. Our results demonstrated multiple compared with single infections to be a highly significant predictor of the condition of stickleback hosts, with multiple-infected hosts having relatively higher body condition. However, this applied only after adjusting for parasite mass, which was another significant predictor for host condition. Thus, our results suggested that, at a given parasite mass, *S. solidus* was more harmful towards their host's body condition in single compared with multiple infections.

Key words: *Schistocephalus solidus*, *Gasterosteus aculeatus*, stickleback, multiple infections, parasite–host interactions, parasite cooperation, virulence.

INTRODUCTION

Trophically transmitted parasites face a number of challenges (Poulin, 1998). After escaping the host's immune response, additional challenges include modifying the present host to ensure transition to the next host (e.g. Milinski, 1985; Poulin and Thomas, 1999; Poulin, 2010). However, this manipulation must not occur before the parasite has reached a developmental stage where it is capable of infecting its next host (e.g. Koella *et al*. 2002; Hammerschmidt *et al*. 2009). Moreover, in host-specific parasites such host manipulation by parasites must not increase the present host's susceptibility to other predators than the next specific host (Levri, 1998; Parker *et al*. 2009), since host-specific parasites usually die when transferred to wrong host-species (e.g. Bråten, 1966). In addition, parasites must trade-off own growth and the host's and its own survival. Some populations of trophically transmitted parasites even experience time periods when transmission to the next host is temporarily inhibited, for example in lakes covered by ice which prevents transmission of parasites from intermediate aquatic hosts to terrestrial hosts for up to 6–7 months (Heins *et al*. 1999).

Trophically transmitted parasites face an additional challenge when two or more conspecific parasites infect the same host individual (Frank, 1996; Parker *et al*. 2003). On one hand, by cooperatively slowing down own growth, multiple parasites may exploit their present host more prudently. Hence, growth and survival of the present host may be ensured until the parasites are ready to infect the next host according to the life-history-strategy model suggested by Parker *et al*. (2003). Alternatively, the multiple parasites may selfishly (over-) exploit the host's resources before the other parasites do the same, known as the 'tragedy of the commons' (Hardin, 1968). These cooperative and selfish parasite strategies may both lead to reduced parasite fitness depending on whether the other conspecific parasites act selfishly or cooperatively (Frank, 1996; Christen and Milinski, 2003; Parker *et al*. 2003).

The cestode *Schistocephalus solidus* is a trophically transmitted parasite with a complex life cycle and is well suited for studies on effects of parasites on hosts (Jäger and Schjørring, 2006; Barber and Scharsack, 2010; Hafer and Milinski, 2016). The tapeworm has four consecutive stages and three hosts. It enters the water body as an egg which hatches into a free living coracidia which again turns into the procercoid stage when preyed upon by its first intermediate copepod host. The parasite enters the plerocercoid stage in its second obligatory and specific intermediate host which is a three-spined stickleback (*Gasterosteus aculeatus*, L.). The final host is usually a bird where the tapeworm matures sexually within 36–48 h (Hopkins and McCaig, 1963; Smyth, 1994) and releases its eggs with the host's faeces into the water body again (e.g. Smyth, 1946; Wootton, 1976). Infections of its secondary intermediate host, sticklebacks, has been described to occur in the spring (Meakins and Walkey, 1973;

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Wedekind and Milinski, 1996; Christen and Milinski, 2005), summer (Pennycuick, 1971) or as one major wave in the autumn (Tierney *et al*. 1996), after which the plerocercoid finds its way to the body cavity of the fish. *Schistocephalus solidus* drains a lot of energy from its stickleback host (Walkey and Meakins, 1970). More than 98% of the growth of the *S*. *solidus* occurs within the stickleback body cavity according to Orr and Hopkins (1969) and Christen and Milinski (2003), and the total mass of *S*. *solidus* becomes relatively large and may even exceed the stickleback host mass (Arme and Owen, 1967). Net body mass at a given length is lower in sticklebacks infected by *S*. *solidus* as compared with uninfected fish (Tierney *et al*. 1996), and infected sticklebacks die sooner when starved (Walkey and Meakins, 1970). Acquiring resources and growing to a large body mass is important for *S*. *solidus* since larger parasites have higher reproductive success in the final host (Scharer and Wedekind, 1999). In a German stickleback population, small specimens at a length around 2 cm eat the small copepods (the first intermediate host of *S*. *solidus*) mainly during spring and summer, whereas sticklebacks larger than about 3·8 cm seem to consider the small copepods as sub-optimal prey items not worth consuming (Christen and Milinski, 2005). The tapeworm ends up in the narrow body cavity of a sticklebacks host and, in order to grow in size, the parasite must allow the stickleback to grow larger as well (Christen and Milinski, 2005). After about 3 months at 19° C as a plerocercoids in sticklebacks, *S*. *solidus* were found to be fully infective to their final host (ducklings) in an experiment by Orr and Hopkins (1969; see also Hopkins and McCaig, 1963). Infected sticklebacks have an elevated respiratory burst activity from day 47 after infection, and the cost of this burst is reflected in the hepatosomatic index which is reduced in infected relative to control fish (Scharsack *et al*. 2007; see also Hammerschmidt & Kurtz, 2005). Moreover, this increase in respiratory burst activity happens shortly after the *S*. *solidus* reaches 0·05 g (Scharsack *et al*. 2007). Interestingly, threshold parasite mass for being able to be infective in the final host (a bird) is about 0·05 g according to Orr and Hopkins (1969) and Tierney and Crompton (1992) although some plerocercoids smaller than 0·05 g may also be infective (Hopkins and McCaig, 1963; Meakins and Walkey, 1973). For simplicity, plerocercoids ⩽0·05 g and >0·05 g are hereafter referred to as 'non-infective' and 'infective', respectively. More details about the life cycle of *S*. *solidus* can be found in e.g. Wootton (1976) and Christen and Milinski (2005).

Non-infective and infective *S*. *solidus* are expected to have different interests. A non-infective *S*. *solidus* will die if transferred to the next host too early, whereas the infective will eventually die without reproducing if not transferred at all or to a non-host species, e.g. a predatory fish. Hence, non-infective tapeworms are expected to reduce or at least not increase the present host's susceptibility to the consecutive host, whereas the infective tapeworm should increase the present host's chances of being preyed upon by the next host (Hammerschmidt *et al*. 2009). A direct conflict arises when non-infective and infective *S*. *solidus* infect the same individual host. In a controlled experiment by Hafer and Milinski (2015), the procercoid *S*. *solidus* which was infective to the next host was able to increase the activity of the host copepod to make the copepod more vulnerable to predation. The non-infective tapeworm had no effect on the copepod host. The host's escape behaviour was the response variable in this experiment (Hafer and Milinski, 2015). The same authors reported increased energy drain, as opposed to active manipulation of host behaviour, in another controlled experiment with sequential infection of stickleback by plerocercoids *S*. *solidus* having a conflict of interest over the direction of host manipulation (Hafer and Milinski, 2016).

The relative effect of single and multiple *S*. *solidus* on its intermediate hosts has been examined in two more studies so far. Michaud *et al*. (2006) reported *S*. *solidus* procercoids growth rate and asymptotic total volume to be larger in multiple compared with single infections after experimentally infecting copepods by 1, 2 or 3 parasites. This suggests that the parasite can respond to signals about the presence of their competing conspecifics and respond by adjusting growth due to the number of competitors present (Michaud *et al*. 2006). In another controlled experiment, where Christen and Milinski (2003) infected sticklebacks by either one or several plerocercoid *S*. *solidus*, the condition factor of the stickleback host decreased significantly in single but not in multiple-infected fish. Thus, this study indicates that multiple plerocercoids are somehow able to avoid overexploiting their host (Christen and Milinski, 2003).

This present field study aims to test the hypothesis whether multiple as compared with single-infected plerocercoid *S*. *solidus* exploit their stickleback host to different extents as suggested by Christen and Milinski (2003). We examined this by comparing the body condition of single and multiple-infected hosts using datasets from two wild stickleback populations each sampled during two years.

MATERIALS AND METHODS

The three-spined sticklebacks and their *S*. *solidus* from two landlocked freshwater lakes in Northern Norway, were studied. Lake Nedre Vollvatn is approximately 45 m wide and 190 m long and located in Bodø at 67°17′N, 14°25′E at an altitude of 125 m. These sticklebacks are perennial and dominated in numbers by the three youngest age-groups

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Table 1. Number and prevalence of infection of three-spined sticklebacks sampled at two lakes. The sticklebacks are divided into three categories: non-infected sticklebacks, and sticklebacks infected by one, or more than one *Schistocephalus solidus*

	Number of *Schistocephalus solidus* infecting each stickleback				
Date of sampling	0	1	⩾2	Sum	Prevalence (%)
Lake Nedre Vollvatn					
29 August–9 September 1996	77	36	38	151	49·0
8–17 September 1997	124	17	7	149	16·1
Lake Storvatnet					
12 July 2012	60	3	13	76	21·1
2–4 July 2014	44	19	42	105	58·1
Total	305	75	100	481	36·4

(J.T. Nordeide, unpublished results). The fish become sexual mature at an age of 2 years. Spawning starts around 25 May and lasts the subsequent 5–6 weeks, and the spawning stock is dominated in number by 2- and 3-year-old fish but a few 4- and 5-year-old fish also participate (J.T. Nordeide, unpublished results). Lake Nedre Vollvatn is covered by ice and snow usually from November to April or early May. The other lake, Lake Storvatnet, is approximately 200 m wide and 600 m long, and located near Brønnøysund at 65° 43′N, 12°11′E at an altitude of 3 m. This lake is covered by ice for a variable number of weeks during the winter months. We had no information about the biology of the sticklebacks or parasites in Lake Storvatnet prior to this study.

The animals in each of the two lakes were sampled in different years (Table 1). In Lake Nedre Vollvatn the fish were sampled in late August to mid-September, which was about three months after the termination of the spawning period. Sampling in Lake Storevatnet was carried out in the first half of July (Table 1), and several males had reddish throat and some females still contained mature eggs indicating that the spawning season was not completely over yet. The fish were caught by traps with no bait, spread along the shoreline from 0·5 to 3·0 m from land and from 0·3 to 1·5 m depth. The traps were made by cutting 1·5 L soda bottles into two parts, turning the upper one-third part upside down and assembling the two parts by twine. The traps caught mainly sticklebacks with a body length larger than about 3·0 cm, although a few smaller specimens were caught as well. Sex of each fish specimen was determined by inspecting the gonads, and total length was measured to the nearest mm. *Schistocephalus solidus* in each fish were counted and wet weight of each parasite was measured to the nearest 0·001 g immediately after removal from the host. Body dry mass of each fish was measured to the nearest 0·001 g after carefully removing the stomach, intestine, potential *S. solidus*, and remains of eggs of mature females (from the July samples), and drying the fish at 105 °C for 10 h (until no further weight loss). Dry weight of the fish was used in the calculations of condition (see below) since starving fish may compensate the loss of muscle weight by increasing the water content in the remaining muscle (Love, 1980). To further substantiate our choice of presenting the parasite index estimated from dry (and not wet) weight of the fish, we calculated the ratio of wet weight/dry weight of non-infected fish as $4{\cdot}78 \pm 1{\cdot}015$ (mean ± S.D.) and infected fish as $5{\cdot}35 \pm 0{\cdot}959$. The difference was significant ($t = 6{\cdot}20$, $P < 0{\cdot}001$, d.f. = 472, Student's t-test) and suggests that infected fish have higher water content in their remaining flesh.

A 'Parasite-index' was calculated for each fish as:

$$\text{Parasite-index} = \text{wet weight of all } S.\ solidus / (\text{wet weight of all } S.\ solidus + \text{body dry weight of the fish})$$

where both the weight measurements were in grams.

Statistics were carried out using SPSS version 20·0 (SPSS Inc. Chicago, IL, USA). Body 'condition' of each fish was first estimated as the residual from a linear regression of ($x^{0{\cdot}25}$ transformed, see below) dry weight over length, as recommended by Jakob *et al*. (1996). Before the linear regression was carried out the dry body weight of each stickleback was $x^{0{\cdot}25}$-transformed in order to achieve linearity when plotted against length (see Supplementary information S1). The final analyses were carried out as General Linear Models (GLM) module after checking that the dataset conformed to the assumptions of homoscedasticity of variance, linearity, normality of errors, collinearity and independence (Grafen and Hails, 2002). Stickleback body 'condition', as defined above, was used as response variable and 'single-multiple infection', whether the fish was parasitized by one or more than one *S. solidus*, was added as fixed factors. 'Year' of sampling was added as a fixed factor to block for different years of sampling. 'Year' blocks for difference between lakes as well since the two lakes were

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sampled in different years (see Table 1). 'Parasite-index' (as defined above) was added as a covariate. Non-parasitized fish were not included in the GLMs. The reason for this is that non-infected stickleback hosts have a 'Parasite-index' of zero by definition, and it makes no sense to run the GLMs with zero values of the covariate 'Parasite-index' of the non-parasitized hosts.

Three separate GLMs were carried out and these three models differed only concerning which sticklebacks were included in the model. In the first model, all parasitized fish from both lakes and from all 4 years were included. Due to potential different virulence of non-infective and infective *S. solidus* (see the Introduction section), only part of the dataset was included in the second and third GLMs. In the second GLM we examined potential effect of infective tapeworms only. We included only stickleback hosts infected by one or more *S. solidus* with infective (>0·05 g) tapeworms, regardless of whether or not they were infected by non-infective ones. The third GLM included only stickleback hosts with non-infective *S. solidus* (⩽0·05 g). *P*-values were two-tailed and $P < 0{\cdot}05$ was considered significant.

To test for potential differences in host body condition between parasitized and non-parasitized sticklebacks (as non-parasitized hosts could not be included in the GLMs, see above), potential difference in body condition between non-parasitized and parasitized sticklebacks was carried out without adjusting for 'Parasite-index'. Mean (±S.D.) was calculated for non-infected and infected hosts separately from estimated residuals of $x^{0{\cdot}25}$-transformed dry weight of the fish over length.

This study was carried out in accordance with ethical guidelines stated by the Norwegian Ministry of Agriculture through the Animal Welfare Act. The number of infected sticklebacks used in this study was determined based on the criteria of: (*i*) including animals from two different populations, each in two different years, in order to make the study reasonable general for stickleback populations of this region. (*ii*) At the same time our intention was not to sacrifice more fish than necessary to be able to detect reasonable effect sizes.

RESULTS

A total of 481 stickleback hosts were examined (Table 1). The prevalence of tapeworms varied between the two lakes and years from 16·1 in Lake Nedre Vollvatn in 1997, to 58·1% in Lake Storvatnet in 2014 (Table 1). Uninfected sticklebacks and sticklebacks infected by one or more than one tapeworms were 305 (63·4%), 75 (15·6%) and 100 (20·8%), respectively. The maximum number of plerocercoids in one host was 24, from a stickleback caught in Lake Storvatnet in 2012, and they were all small (⩽0·0341 g). Maximum number of plerocercoids in one fish from Lake Nedre Vollvatn was 9. The parasite index (with fish body weight measured in dry weight as defined above, see the Materials and methods section) varied from 6·7 to 84·7 as shown in Fig. 1a (an alternative parasite index calculated using wet weight of the fish as opposed to dry weight of the fish and otherwise equal, varied from 0·01 to 0·68, see Supplementary information S3b). In stickleback hosts infected by a single *S. solidus* ('single infected') in Lake Nedre Vollvatn, 39·6% (21 of 53 individuals) of the parasites were non-infective (Fig. 2a). The corresponding numbers for *S. solidus* from multiple-infected fish were 51·6% (65 of 126) non-infective (Fig. 2a), and number of non-infective and infective did not differ between *S. solidus* found as the sole (single) and multiple *S. solidus* ($\chi^2 = 2{\cdot}14$, $P = 0{\cdot}288$, d.f. = 1, χ^2 test). In Lake Storvatnet, 31·8% (7 of 22) of the single-infected *S. solidus* were non-infective, whereas 78·3% (296 of 378) of the multiple-infected ones were non-infective (Fig. 2b), and this difference was significant ($\chi^2 = 24{\cdot}45$, $P < 0{\cdot}001$, d.f. = 1, χ^2 test). Mean (±S.D.) total mass of all parasites pooled in each host (excluding fish without *S. solidus*) was 0·228 (±0·0236) g in Lake Storvatnet and 0·177 (±0·0124) g in Lake Nedre Vollvatn, and the distribution of total mass did not differ significantly between the lakes ($Z = 1{\cdot}045$, $P = 0{\cdot}225$, Kolmogorov–Smirnov test). The number of sticklebacks infected by a single or by multiple *S. solidus* were 75 and 100, respectively, when fish from both lakes and years were pooled (Table 1). Mean (±S.D.) total mass of *S. solidus* in each stickleback, of single and multiple-infected sticklebacks, was 0·116 g (±0·0906) and 0·243 g (±0·1564), respectively, in Lake Nedre Vollvatn, and this difference was significant ($U = 565{\cdot}5$, $P < 0{\cdot}001$, Mann–Whitney *U*-test, $N_1 = 53$, $N_2 = 45$). Corresponding numbers for Lake Storvatnet was 0·110 g (±0·0781) and 0·275 g (±0·2230), and this difference was significant as well ($U = 264{\cdot}5$, $P < 0{\cdot}001$, Mann–Whitney *U*-test, $N_1 = 22$, $N_2 = 55$). The mean (±S.D.) body length of sticklebacks infected by single and multiple *S. solidus* was 4·2 (±0·12) cm and 4·6 (±0·08), respectively, when all data were pooled. This difference was significant ($P = 0{\cdot}017$, see Supplementary information S2). Of the 100 multiple-infected sticklebacks, 35, 19, 11 and 35 were infected by 2, 3, 4 and ⩾5 parasites, respectively.

The three GLMs presented below differ with regard to which sticklebacks were included in the analyses. In the first GLM, all sticklebacks with one or more *S. solidus* were included regardless of parasite mass and infection stage. Both predictors 'parasite index' (relative mass of the parasites), and 'single-multiple infection' (whether the stickleback was infected by one or more parasites) were significant (Table 2). The 'parasite index' was negatively associated with the sticklebacks' 'condition' (Table 1a) demonstrating that sticklebacks with large parasite

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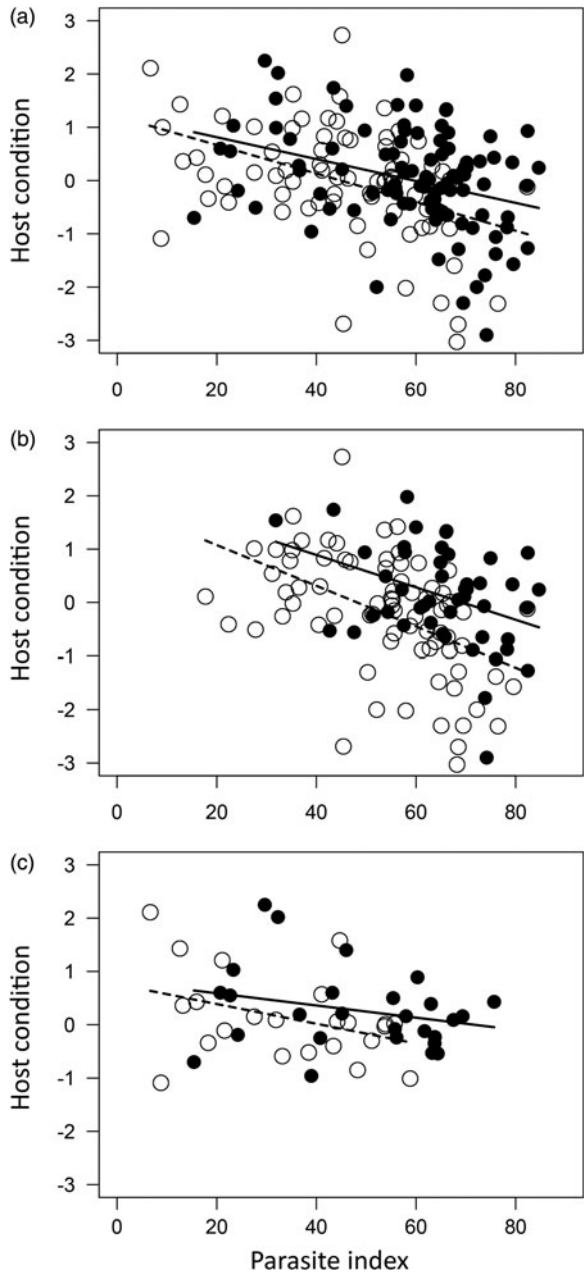

Fig. 1. Scatter-plots of stickleback host condition versus parasite index from Lake Nedre Vollvatn 1996 and 1997 and Lake Storvatnet 2012 and 2014. All infected hosts were included in (a). In (b) only hosts infected by *Schistocephalus solidus* with a mass >0·05 g were included, and in (c) only hosts infected by *S. solidus* with a mass ⩽0·05 g (and no parasites >0·05 g) were included. Hosts infected by 1 and ⩾2 tapeworm(s) are shown as open and filled circles, respectively. Dashed and full lines show linear regression lines for hosts infected by 1 and ⩽2 tapeworm(s), respectively. The *y*-axis shows residuals of $x^{0·25}$-transformed dry weight adjusted for host body length. Parasite index is parasite wet weight/(parasite wet weight + host dry weight). Non-infected hosts are not included in these figures since their 'Parasite-index' is zero by definition.

mass (regardless of number of individual parasites) had lower body condition than sticklebacks infected with lower mass of parasites. Single-infected sticklebacks had a lower condition than multiple-infected fish, as demonstrated by the lower linear regression line of 'condition' plotted against 'parasite index' of single- compared to multiple-infected fish (Fig. 1a). This means that at a given parasite mass host condition was lower when infected by one compared with more than one parasites. This model explained 22·7% of the variation of the response variable. Running the same model again with wet (instead of dry) fish weight (both in the response variable and in the Parasite-index) gave similar results (see Supplementary information S3).

Due to the potential conflict of interests between infective and non-infective parasites as outlined in the Introduction, we ran a second and a third model. In the second GLM (see Table 3, Fig. 1b) we included only stickleback hosts with one or more *S. solidus* which were ready to infect the final host, regardless of whether or not they were infected by non-infective *S. solidus* (⩽0·05 g) as well. The percentage of the variation explained by this model increased to 51·2 (Table 3), and the model gave similar results as the first model concerning the significance level of the predictors and their association to the response variable (Table 3, Fig. 1b). The third GLM included only the data from sticklebacks infected by only non-infective *S. solidus*. Again this model explained a relatively large part of the variation (45·2%, Table 4), and the model gave similar results as the two other models concerning the significance level of the predictors and their association to the response variable (Table 4, Fig. 1c). The blocking factor 'year' explained a significant although relative small part of the variation in each of the GLM models (Tables 2, 3 and 4).

Mean (±S.D.) residuals ($x^{0·25}$-transformed dry mass over length) host condition estimated without correcting for 'Parasite-index' (see Materials and methods), was 0·245 (±0·947) for uninfected hosts, and –0·421 (±0·946) for infected hosts. The two means are significantly different ($t = 7·384$, $P < 0·001$, d.f. = 471, *t*-test).

DISCUSSION

The predictors 'parasite index' and 'single-multiple infections', each explained a significant part of the variance in the response variable 'condition' of the host. Parasite index was an important predictor in all three statistical models, and was negatively associated with condition of the host. More interestingly, at a given parasite index an infection by one individual *S. solidus* depleted the condition of its stickleback host more than multiple infections. Splitting the dataset into two parts, depending on whether the individual *S. solidus* had reached a mass where they were large enough to be infective (>0·05 g) or still to small (⩽0·05 g) to infect the consecutive host, approximately doubled the percentage of variation explained by the models.

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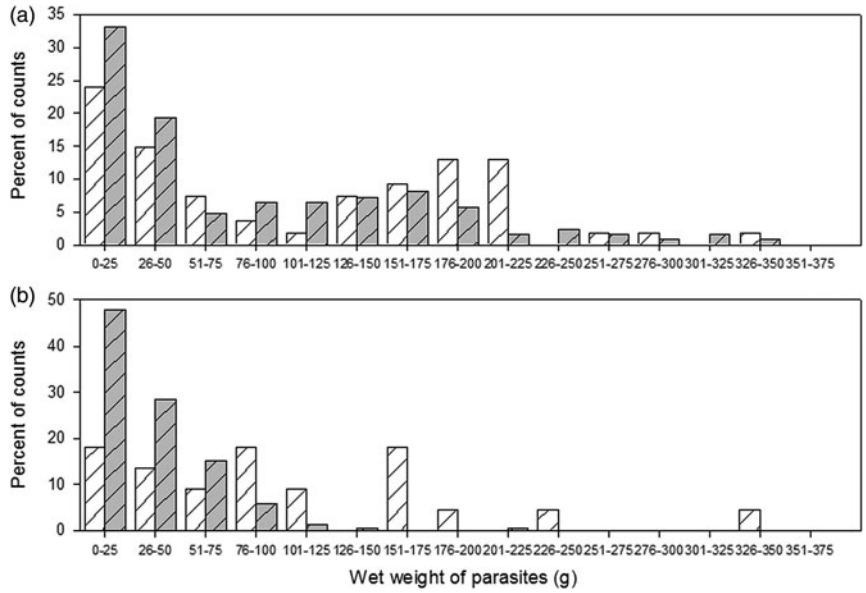

Fig. 2. Histograms of the distribution of wet mass of individual parasitic tapeworms (*Schistocephalus solidus*). The tapeworms and their hosts were sampled in (a) Lake Nedre Vollvatn in 1996 and 1997 (pooled) and (b) in Lake Storvatnet in 2012 and 2014 (pooled). White and grey bars show parasites which were the sole or one of multiple (⩾2) tapeworm(s) infecting one particular stickleback host, respectively. See Table 1 for information about *N*. The scale of the *y*-axis differs between the two figures.

Table 2. Test statistics when including all stickleback hosts from both Lake Nedre Vollvatn in 1996 and 1997 and from Lake Storvatnet in 2012 and 2014

Source	SS	d.f.	*F*	*P*-value
Analysis of variance				
Single–multiple infection	0·136	1	4·900	0·028
Parasite index	0·522	1	18·765	<0·001
Year	0·598	3	7·169	<0·001
Error	4·698	169		
Total	27·982	175		
Term				
Coefficients	Coeff.	s.e. Coeff.	*t*-value	*P*-value
Constant	0·625	0·0507	12·34	<0·001
Single–multiple infection	−0·0654	0·0295	2·22	0·028
Parasite index	−0·00352	0·00081	−4·34	<0·001

'Condition' (residuals of power-transformed dry body weight adjusted for length) as response variable in a GLM Type III (adjusted) sums of squares (SS). The predictor 'single–multiple infection' is whether the stickleback host was parasitized by one or multiple tapeworms (*Schistocephalus solidus*) (non-parasitized hosts are excluded), and 'parasite index' is wet weight of all tapeworms, as percentage of the sum of fish dry body weight (after removing weight of parasites) plus wet weight of all tapeworms. The model explained 22·7% of the variation (adjusted $R^2 = 0·227$).

The lower virulence of multiple *S. solidus* compared to single ones, on stickleback host in this field study, concurs with results from controlled experimental infections using the same species and carried out by Christen and Milinski (2003), and with the theoretical life history strategy (LHS) model suggested by Parker *et al.* (2003). Christen and Milinski (2005) suggested that less virulence of multiple-infected plerocercoids can be explained as multiple plerocercoids need to allow the host to grow larger to enable not just one but multiple parasites to reach the threshold of 0·05 g body mass required to infect the consecutive host (see the Introduction section).

A second explanation to consider has to do with the temporal lack of future transmission possibilities from the stickleback body cavity to the final bird intestine for *S. solidus* during the long winters in North Norway. There are at least two contrasting strategies how *S. solidus* might prepare for the winter when lakes are covered by ice for months and *S. solidus* plerocercoids cannot be transferred to their final bird host. One strategy might be to drain energy from the stickleback host severely in order to grow large enough to become infective for the final host as early in the summer or autumn as possible. Becoming infective (>0·05 g) early

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Table 3. Test statistics including only stickleback hosts parasitized by *Schistocephalus solidus* which are infective (individual *S. solidus* mass >0·05 g) if transferred to the next host regardless of whether or not hosts were parasitized by smaller (⩽0·05 g) non-infective *S. solidus*

Source	SS	df	*F*	*P*-value
Analysis of variance				
Single–multiple infection	0·586	1	38·623	<0·001
Parasite index	1·182	1	77·839	<0·001
Year	0·253	3	5·565	0·001
Error	1·776	117		
Total	20·932	123		
Term				
Coefficients	Coeff.	S.E. Coeff.	*t*-value	*P*-value
Constant	0·948	0·0604	15·70	<0·001
Single–multiple infection	−0·1628	0·0262	−6·21	<0·001
Parasite index	−0·00767	0·00087	−8·82	<0·001

The data were collected in Lake Nedre Vollvatn in 1996 and 1997 and Lake Storvatnet from 2012 and 2014. 'Condition' (residuals of power-transformed dry body weight adjusted for length) as response variable in a GLM Type III (adjusted) sums of squares (SS). The predictor 'single–multiple infection' is whether the stickleback host was parasitized by one or multiple infective (*S. solidus*) (non-parasitized hosts were excluded), and 'parasite index' is wet weight of all tapeworms, as percentage of the sum of fish dry body weight (after removing weight of parasites) plus wet weight of all tapeworms. The model explained 51·2% of the variation (adjusted $R^2 = 0{\cdot}512$).

Table 4. Test statistics including only stickleback hosts which are both (i) parasitized by *Schistocephalus solidus* which are non-infective (individual *S. solidus* mass ⩽0·05 g) if transferred to the next host, and (ii) at the same time not parasitized by *S. solidus* which are infective (individual *S. solidus* mass >0·05 g) in the next host

Source	SS	df	*F*	*P*-value
Analysis of variance				
Single–multiple infection	0·147	1	6·056	<0·018
Parasite index	0·346	1	14·281	<0·001
Year	0·295	3	4·058	0·012
Error	1·092	45		
Total	6·729	51		
Term				
Coefficients	Coeff.	S.E. Coeff.	*T*-value	*P*-value
Constant	0·673	0·0691	9·74	<0·001
Single–multiple infection	−0·1358	0·0552	−0·22	0·018
Parasite index	−0·00535	0·00142	−3·77	<0·001

The data are from Lake Nedre Vollvatn in 1996 and 1997 and Lake Storvatnet from 2012 and 2014. 'Condition' (residuals of power-transformed dry body weight adjusted for length) as response variable in a GLM Type III (adjusted) sums of squares (SS). The predictor 'single–multiple infection' is whether the stickleback host was parasitized by one or multiple non-infective tapeworms (*S. solidus*) (non-parasitized hosts were excluded), and 'parasite index' is wet weight of all tapeworms, as percentage of the sum of fish dry body weight (after removing weight of parasites) plus wet weight of all tapeworms. The model explained 45·2% of the variation (adjusted $R^2 = 0{\cdot}452$).

increases the time period available for infecting the final bird host before such transmission is impeded by the ice, and this will decrease the parasite's generation time and hence increase its fitness. It is reasonable to assume that a single-infected plerocercoid *S. solidus* has the potential to reach the infective mass of 0·05 g earlier in the summer or autumn compared with their multiple-infected conspecifics, since single-infected ones do not share resources. Hence, single infective *S. solidus* have a longer time period where they are able to infect a bird. An alternative strategy is to exploit the stickleback host prudently in order to allow the stickleback host, and the *S. solidus*, to survive the harsh winter months and go for transmission to the bird intestine during the next spring or summer. This strategy assumes a perennial stickleback population and a reasonable probability that infected stickleback hosts survive the harsh winter. Both these assumptions seem to apply in a stickleback population in Alaska infected by *S. solidus* (Heins *et al*. 1999). In the perennial stickleback population in Nedre Vollvatn the prevalence of *S. solidus* dropped from 49·0% in September 1996 (Table 1) to 6·2% (8 of 129) the following spring (J.T. Nordeide, 20–27 May 1997, unpublished results), suggesting substantial mortality of infected sticklebacks during the winter months. Our data do not allow us to

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distinguish between energy drainage from the parasites, selective predation of infected sticklebacks (see e.g. Jakobsen *et al*. 1988), or a combination of both, as the reason for this presumably high winter mortality. Several authors have reported relatively low prevalence of *S. solidus* plerocercoids in spring and early summer (Meakins, 1974; McPhail and Peacock, 1983), and increasing prevalence during early summer and autumn (Pennycuick, 1971; Meakins, 1974; McPhail and Peacock, 1983). However, despite this winter mortality it is likely that a larger ratio of multiple compared with single *S. solidus* go for the prudent host energy drainage strategy (see above). If so, this may contribute to explain the lower virulence of multiple relative to single-infected *S. solidus* demonstrated in the present study.

A possible third explanation for the relatively low virulence of multiple compared with single *S. solidus* may be natural selection for mutual cooperative behaviour to allow the host to grow for the two parasites' mutual benefit, for example as in a TIT-FOR-TAT strategy (Axelrod and Hamilton, 1981). This suggestion applies to the 35% of the multiple-infected sticklebacks which were infected by two *S. solidus*. Fourthly, in an experimental study Jäger and Schjørring (2006) found related compared with non-related *S. solidus* to be more successful in infecting stickleback hosts. Similarly, we cannot exclude the possibility that multiple parasites within each host in the present may theoretically co-operate to restrain exploitation of the host because they are related. However, we lack information about relatedness of *S. solidus* and are unable to confirm or disprove this hypothesis.

Our understanding of the proximate mechanisms causing the multiple compared with single *S. solidus* to be less virulent towards their stickleback host remains poor, leaving this question open for future exiting research. One potential mechanism may be related to acting relatively gently towards the host is a side effect of a fierce struggle between the multiple parasites allocating resources to fight each other by unknown mechanisms, at the expense of resources invested to exploit and harm the host (reviewed by Read and Taylor, 2001).

Although testing all prediction in the theoretical LHS model by Parker *et al*. (2003) was beyond the scope of this field study, we should mention that the results from our present field study seem to concur with another prediction from this model as well. Total mass of all *S. solidus* in a host was higher in multiple-infected *S. solidus* compared with single ones, as expected from the LHS model, and contrary to results by Christen and Milinski (2003).

A potential flaw in this field studies is our lack of information about when the hosts in these perennial populations became infected. The different *S. solidus* infected their hosts at different times and we cannot exclude the possibility that some of them might even have survived the winter as plerocercoids. The different *S. solidus* might therefore consequently have drained energy from their stickleback hosts during different time periods. On the other hand, the distribution of mass of individual in single- and multiple-infected *S. solidus* did not differ much in the Lake Nedre Vollvatn samples (see the Results section, Fig. 2a), indicating that the time of infection does not differ much between the two groups. Running the GLM on data from Lake Nedre Vollvatn only gave very similar results (Supplementary information S4) as the pooled results from both lakes (see the Results section). In addition, only by controlled experiments can we potentially rule out the possibility that single-infected sticklebacks had lower condition anyways, and that those with higher body condition were prone to multiple infections.

To conclude, this field study suggests that multiple infections by *S. solidus* lowers the body condition of their intermediate stickleback host less severely compared to single-infected *S. solidus*, at a given parasite mass.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at http://dx.doi.org/10.1017/S0031182016000676.

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