Experimental parasite community ecology: intraspecific variation in a large tapeworm affects community assembly

Daniel P. Benesh1,2* and Martin Kalbe1

1Max Planck Institute for Evolutionary Biology, August-Thienemann-Str. 2, 24306 Plön, Germany; and 2Marine Science Institute, University of California, Santa Barbara, CA 93106-6150, USA

Summary

1. Non-random species associations occur in naturally sampled parasite communities. The processes resulting in predictable community structure (e.g. particular host behaviours, cross-immunity, interspecific competition) could be affected by traits that vary within a parasite species, like growth or antigenicity.

2. We experimentally infected three-spined sticklebacks with a large tapeworm (Schistoscephalus solidus) that impacts the energy needs, foraging behaviour and immune reactions of its host. The tapeworms came from two populations, characterized by high or low growth in sticklebacks. Our goal was to evaluate how this parasite, and variation in its growth, affects the acquisition of other parasites.

3. Fish infected with S. solidus were placed into cages in a lake to expose them to the natural parasite community. We also performed a laboratory experiment in which infected fish were exposed to a fixed dose of a common trematode parasite.

4. In the field experiment, infection with S. solidus affected the abundance of four parasite species, relative to controls. For two of the four species, changes occurred only in fish harbouring the high-growth S. solidus; one species increased in abundance and the other decreased. These changes did not appear to be directly linked to S. solidus growth though. The parasite exhibiting elevated abundance was the same trematode used in the laboratory infection. In that experiment, we found a similar infection pattern, suggesting that S. solidus affects the physiological susceptibility of fish to this trematode.

5. Associations between S. solidus and other parasites occur and vary in direction. However, some of these associations were contingent on the S. solidus population, suggesting that intraspecific variability can affect the assembly of parasite communities.

Key-words: Apatemon, Diplostomum, exclusion, facilitation, Gasterosteus aculeatus, indirect effects, life history strategy, Proteocephalus, spatial effects, Sphaerostomum

Introduction

In a sample of hosts, some parasite species may co-occur more or less frequently than expected by chance (Stock & Holmes 1988; Moore & Simberloff 1990; Kuris & Lafferty 1994; Poulin & Guéan 2000; Poulin & Luque 2003; Lello et al. 2004; Behnke 2008; Telfer et al. 2010). However, associations between parasite species can change over time or they may be detected in some locations but not others (Janovy & Hardin 1988; Poulin & Valtonen 2002; Vidal-Martínez & Poulin 2003; Calvete et al. 2004; Norton, Lewis & Rollinson 2004; Behnke et al. 2005). And many parasite communities appear to be assembled randomly, with species co-occurring in proportion to their overall prevalence in the host population (Gotelli & Rohde 2002; Muñoz, Mouillot & Poulin 2006; Kennedy 2009). Determining when and why a parasite community exhibits predictable structure requires identifying the various mechanisms producing non-random species associations (Pedersen & Fenton 2007; Poulin 2007). Broadly, these associations arise in two ways. First, the probabilities that different parasites encounter the host may not be independent (Lotz, Bush & Font 1995). For example, Bush & Holmes (1986) found that the helminths inhabiting the
gut of scaup ducks formed two suites of positively associated species. Each suite was transmitted by a different amphipod species, so the structure in the parasite community reflects a host’s preference to feed on one or the other amphipod. The second way that species associations arise is through interactions with and within the host (Rynkiewicz, Pedersen & Fenton 2015). Experimental coinfections have clearly established that parasite species can negatively or positively affect one another (Holmes 1961; Holland 1987). Species may compete for a shared resource (Smith & Holt 1996; Graham 2008; Griffiths et al. 2014), or one species can alter the suitability of the host for other species (Stock & Holmes 1987; Dobson & Barnes 1995). Parasites can also interact indirectly via the immune system, as immune responses to one species can either facilitate or impair infection by other species (e.g. Drulhe, Tall & Sokhna 2005; Cattadori, Albert & Boag 2007). Disentangling the multiple processes that structure parasite communities may require combining observational and experimental approaches (Johnson & Buller 2011).

Polyparasitism, that is sharing the host with other parasites, likely affects the evolutionary advantages of particular parasite strategies (Rigaud, Perrot-Minnot & Brown 2010; Alizon, de Roode & Michalakis 2013; Johnson et al. 2013). For instance, it is beneficial to avoid host immune defences, but a parasite that suppresses immunity potentially opens the host up to infection with competitor parasites or parasites with conflicting life histories (e.g. Ezenwa et al. 2010). Here, a trait (immune suppression) affects the assembly of the parasite community, which in turn affects the favourability of the trait. Parasite growth may be a trait subject to such dynamics. A rapidly growing parasite can impact host energy needs and foraging behaviour, and thus the host’s exposure to other parasites, or it may necessarily compete with other parasites utilizing a shared resource (Lagrué & Poulin 2008). Aggressive parasite growth could also require avoiding, tolerating or suppressing host immunity (e.g. Wilkes et al. 2004). An altered parasite community might be costly for a rapidly growing parasite if it exacerbates the risk of host mortality. Parasite growth or replication rates often vary within parasite species (e.g. Mackinnon & Read 1999; Davies et al. 2001), but how this variation affects parasite community assembly and how the parasite community in turn impacts selection on traits within parasite species is poorly explored.

We report the results of two experiments in which we experimentally infected fish (sticklebacks) with a large tapeworm (*Schistocephalus solidus*). The tapeworm *S. solidus* affects the energy budget (Walkey & Meakins 1970; Lester 1971), foraging behaviour (Milinski 1984; Godin & Sproul 1988; Cunningham, Tierney & Huntingford 1994; Barber & Huntingford 1995) and immune status (Scharsack et al. 2004, 2013; Scharsack, Koch & Hammerschmidt 2007) of sticklebacks, so it undoubtedly has the potential to modify a fish’s probability of encountering and becoming infected with other parasites (e.g. Rokicki, Rolbiecki & Jane 2001). In a laboratory experiment, we tested whether infection with *S. solidus* affects susceptibility to a common trematode (*Diplodistomum pseudospathaceum*). We also performed a field experiment in which fish infected with *S. solidus* were exposed to the natural parasite community. Our experimental approach excludes correlated exposure as the cause of associations between our focal tapeworm and other parasites. Additionally, we used two populations of *S. solidus* in our experiments that differ in growth in fish. Growth is beneficial, because larger *S. solidus* produce more eggs (Schärer et al. 2001), but excessive growth is thought to come at the cost of increased fish mortality (Barber & Scharsack 2010). We hypothesized that rapid *S. solidus* growth could be detrimental to the parasite if it predisposes host fish to infection with other parasites. By manipulating the presence of *S. solidus*, as well as its growth, we hoped to better understand how a particularly aggressive parasite can impact parasite community assembly as well as how this worm’s life history may affect, and be affected by, the parasite community.

Materials and methods

**STUDY SYSTEM**

Three-spined sticklebacks (*Gasterosteus aculeatus*) have well-studied parasite communities (Poulin et al. 2011). The tapeworm *S. solidus* has a three-host life cycle, infecting freshwater copepods, then three-spined sticklebacks, before finally reproducing in fish-eating birds (Clarke 1954). The relationship between *S. solidus* and sticklebacks is very specific; three-spined sticklebacks are the only suitable fish host for *S. solidus* (Braten 1966; Henrich, Benesh & Kalbe 2013). The size of *S. solidus* in sticklebacks is massive (i.e. over an order of magnitude larger than tapeworm species with comparable life cycles; Benesh, Chubb & Parker 2013), which impacts fish morbidity and mortality (reviewed by Barber & Scharsack 2010). Sticklebacks mount an innate immune response (monocyte proliferation) to early *S. solidus* infections (Scharsack, Koch & Hammerschmidt 2007), and the allelic diversity of major histocompatibility genes affects worm growth (Kurtz et al. 2004). Host immune responses are also modulated by *S. solidus*. For instance, leucocytes from infected fish respond to an unspecific stimulus but not to *S. solidus* antigens in vitro (Scharsack et al. 2004), an effect presumably mediated by parasite excretory products (Scharsack et al. 2013).

We worked with two *S. solidus* populations that are genetically differentiated at neutral markers (Samonte-Padilla et al., submitted manuscript) and that differ markedly in their growth in sticklebacks. Kalbe et al. (2016) performed a reciprocal cross-infection experiment with sticklebacks and *S. solidus* collected from two locations, Lake Skogseidvatnet, Norway (60° 13′ N, 5° 53′ E), and the Neustädter Binnenwasser, Germany (54° 6′ N, 10° 47′ E). After 3 months, the Norwegian tapeworms had grown larger than the German ones in both stickleback populations, indicating a genetically based growth difference between the two worm populations. Kalbe et al. measured the asymptotic size
attained by the worms in fish, but subsequent experiments (including the laboratory experiment we report) found the growth rate, not just the final size, to differ between the two populations. To ease recognition, we refer to the Norwegian and German populations as ‘high growth’ (HG) and ‘low growth’ (LG) throughout the manuscript. Although we denominate our *S. solidus* populations on the basis of a conspicuous phenotypic difference (i.e. growth), we acknowledge that differences between fish infected with the two populations need not be directly linked to tapeworm growth. For each population, two families of full-siblings were bred *in vitro* for our experiments; detailed descriptions of the breeding protocol can be found elsewhere (Smyth 1946; Wedekind, Strahm & Schärer 1998). Eggs were stored at 4 °C until needed.

**GENERAL INFECTION PROTOCOL FOR COPEPODS AND FISH**

Eggs developed at 18 °C for 3 weeks. Hatching was induced by exposing eggs to light for approximately 4 h the day before use. Copepods (*Macrocyclops albidus*) for infection were taken from laboratory cultures, isolated in 24-well plates, and then, after 1 day of starvation, exposed to a single tapeworm coracidium. Copepods were maintained at 18 °C with a 16:8-h light:dark cycle and were fed every other day with either three *Artemia salina* nauplii or ~100 paramecia. Food items alternated between feeding days (i.e. *artemia, paramecium, artemia*, etc.).

Naïve sticklebacks from six laboratory-bred, full-sib families were used for infection. These fish originated from the Lake Gesser Ploner See, Germany (54° 9’ N, 10° 25’ E), which is also where our field experiment took place. The fish used in our two experiments were handled at the same time. Fish were individually isolated in small tanks (18 x 13 x 11 cm), and each exposed fish was given one infected copepod that harboured a single, 14-day-old worm. Control fish were treated in the same way but were not given a copepod. About a week after exposure, fish were weighed and measured, and a dorsal spine was clipped to provide DNA. A tail clip for DNA was also collected during fish dissection, and intermediate hosts. In each cage, we placed 10 unexposed controls, 22 fish exposed to *S. solidus* and 22 fish exposed to *HG S. solidus*. All fish in a cage belonged to the same full-sib family. Fish were released in late spring (12–20 May, 2009) and dissected after having been exposed to the local parasite community for about 3 months (10–14 Aug, 2009). All surviving fish were killed with MS222, weighed, measured and dissected. The skin, gills, eyes, musculature and all inner organs were screened for parasites with standard methods (Kalbe, Wegner & Reusch 2002).

**FIELD EXPERIMENT – THE EFFECT OF *S. solidus* ON PARASITE COMMUNITY ASSEMBLY**

We placed laboratory-exposed fish into six cages in the littoral zone of the Grosser Ploner See. The LG *S. solidus* population is found ~25 km from this lake, so these are nearly sympatric populations. *Schistocephalus solidus* occurs in this lake, but it is extremely rare (<1% prevalence), so we are confident that all *S. solidus* infections originated in the laboratory. Cages (1.0 × 0.5 × 0.5 m in dimension) had a mesh size (5 mm) that was small enough to keep out predators and non-experimental sticklebacks, but large enough to allow in parasite propagules and intermediate hosts. In each cage, we placed 10 unexposed controls, 22 fish exposed to *LG S. solidus* and 22 fish exposed to *HG S. solidus*. All fish in a cage belonged to the same full-sib family. Fish were released in late spring (12–20 May, 2009) and dissected after having been exposed to the local parasite community for about 3 months (10–14 Aug, 2009). All surviving fish were killed with MS222, weighed, measured and dissected. The skin, gills, eyes, musculature and all inner organs were screened for parasites with standard methods (Kalbe, Wegner & Reusch 2002).

**DATA ANALYSES**

We first tested whether worms from the two populations differed in their growth, as in other experiments (Kalbe et al. 2016), by performing ANCOVAs with worm population as a factor and initial fish weight as a covariate. To examine the effect of *S. solidus* on host condition, we tested whether fish organ weights differed between groups using MANOVA. A number of fish in our field experiment died. To assess if this mortality was associated with *S. solidus*, we took the infection rates from the laboratory experiment, where there was no mortality, as expectations for the field experiment. For each parasite population, the observed and expected prevalence was compared with a chi-square test.

The two *S. solidus* populations clearly differed in their growth (see Results), and we defined the following four treatment groups for our main analyses: (i) unexposed controls, (ii) exposed but uninfected fish, (iii) fish infected with a LG *S. solidus* and (iv) fish infected with a HG *S. solidus*. For the laboratory experiment, the number of *D. pseudospathaceum* found in the eye lenses were compared between these four groups using a generalized linear model with a log link and negative binomial errors (Wilson & Grenfell 1997). In addition to treatment, we evaluated the effects of initial fish weight, fish sex and fish family.

For the field experiment, we compared the parasite communities of the four treatment groups using a method based on a multivariate extension of generalized linear models (Warton, Wright & Wang 2012). Generalized linear models can explicitly account for the overdispersion typical of species abundance data.
Results

THE S. SOLIDUS POPULATIONS DIFFER IN GROWTH AND THEIR IMPACT ON THE HOST

In the laboratory experiment, where tapeworms were sampled while still growing, the worms from the Norwegian population (HG) were 226% larger on average than those from the German population (LG) (population main effect in an ANCOVA: \( F_{1,73} = 182, P < 0.001 \)). In the field experiment, where worms were sampled after completing the majority of their growth, they were 31% larger (\( F_{1,64} = 25.3, P < 0.001; \) Fig. S1, Supporting information).

We present the analyses of host condition and survival extensively in the supplementary material but mention the main results here. In the laboratory experiment, fish infected with HG S. solidus had relatively small livers, an indication of lower condition, whereas fish infected with either population of S. solidus had larger spleens and head kidneys, an indication of a more stimulated immune system (Fig. S2). Most of these treatment differences were smaller or absent in the field experiment (Fig. S2). Fish from the field experiment had relatively smaller livers and larger spleens than those from the laboratory experiment (Fig. S2), consistent with them having lower condition and a more stimulated immune system.

Fifty-four per cent of the fish in the field experiment died. We recovered fewer HG-infected fish from the cages than expected based on the infection rate in the laboratory experiment (the observed vs. expected infection rate was 55% vs. 73%, \( \chi^2 = 5.52, P = 0.019 \)) (Fig. S3). A similar pattern was seen for LG-infected fish (53% vs. 64% expected; Fig. S3), but this difference was not significant (\( \chi^2 = 1.25, P = 0.26 \)).

THE EFFECT OF S. SOLIDUS ON OTHER PARASITES IS POPULATION-DEPENDENT

In the laboratory experiment, neither initial fish weight (GLM, \( \chi^2 = 2.17, P = 0.14 \)) nor fish sex (\( \chi^2 = 2.00, P = 0.16 \)) affected D. pseudospathaceum abundance, but fish family (\( \chi^2 = 46.04, P < 0.001 \)) and treatment did (\( \chi^2 = 54.8, P < 0.001 \)). Initial weight and sex were not measured for every fish (mostly weights were missing), so when we excluded these terms from the model the sample size increased from 166 to 221. Both fish family (GLM, \( \chi^2 = 54.3, P < 0.001 \)) and treatment (GLM, \( \chi^2 = 88.9, P < 0.001 \)) were still significant with this larger sample; fish infected with the HG S. solidus had more D. pseudospathaceum metacercariae in their eyes than fish infected with the LG S. solidus or uninfected fish (Fig. 1).

Of the 324 fish we put into cages in the field experiment, 148 survived, and using microsatellites we were able...
to successfully assign 142 of these fish to a treatment (DNA extraction failed in a few samples). We found 21 parasite species (Table S1). Multivariate tests indicated that parasite communities differed significantly between treatments (Wald = 12.3, P < 0.001), between cages (Wald = 21.5, P < 0.001) and between fish of different sizes (Wald = 6.2, P = 0.007), but not between sexes (Wald = 3.9, P = 0.59). Table S2 summarizes the effect sizes and significance of each model term for each parasite species. Differences between cages were common; the abundances of 10 parasite species varied significantly among cages (Table S2).

After adjusting P-values for multiple tests and after controlling for the effects of cage, fish sex and fish size, we identified four species whose abundances differed significantly between treatments: the eye fluke *D. pseudospathaceum* sensu lato (Wald = 4.45, P = 0.008), another fluke occurring mainly in the eyes *Apatemon cobitidis* (Wald = 6.81, P < 0.001), a fluke in the gut *Sphaerostomum globiporum* (Wald = 4.07, P = 0.022) and an intestinal tapeworm *Proteocephalus filicollis* (Wald = 4.11, P = 0.021; Fig. 2, other common parasites from the community are plotted in Fig. S4). Several congeneric *Diplostomum* species can occur as indistinguishable metacercariae in the eye lenses of sticklebacks, so we cannot be sure that all the flukes recovered are *D. pseudospathaceum* sensu lato.

The communities of unexposed controls and exposed but uninfected fish were not significantly different (Wald = 4.18, P = 0.05), suggesting that the treatment effect was driven by differences between uninfected fish and the two groups of *S. solidus*-infected fish (Fig. 2). The fish infected with LG *S. solidus* were similar to uninfected fish, with the exception of harbouring more *P. filicollis* and fewer *S. globiporum* (Fig. 2c,d). The HG-infected fish had the most distinct community, with fewer *A. cobitidis* (Fig. 2a), more *D. pseudospathaceum* s.l. (Fig. 2b), more *P. filicollis* (Fig. 2c) and fewer *S. globiporum* (Fig. 2d) than the other groups.

*Apatemon cobitidis* forms large, smooth metacercarial cysts, but during dissections we started noticing a number of irregular and seemingly degraded cysts. Although we only recorded the presence/absence of these irregular cysts in a little more than half of the fish we dissected (n = 88; the treatments were represented about proportionally in this subsample), there was nonetheless a conspicuous association between the presence of irregular cysts and infection with HG *S. solidus* (GLM, $\chi^2_{13} = 46.3, P < 0.001$; Fig. 3).

**RELATIONSHIP BETWEEN S. SOLIDUS GROWTH AND SUSCEPTIBILITY TO OTHER PARASITES**

In the laboratory experiment, a generalized linear model including fish family and tapeworm population was slightly improved by adding *S. solidus* weight (likelihood ratio test, $\chi^2 = 3.4, P = 0.041$); the positive coefficient ($=0.007$) suggested that *D. pseudospathaceum* intensity increased with worm weight within each *S. solidus* population. This may be a false positive, however. The trend
was not clear when plotting the data (Fig. 4a), and the effect of worm weight disappeared when fish family was excluded from the model ($\chi^2 = 0.01, P = 0.90$), suggesting it was entirely dependent on controlling for family effects. Also, the number of *D. pseudospathaceum* s.l. did not increase with worm weight within each *S. solidus* population in the field experiment (Wald = 1.33, $P = 0.91$; Fig. 4b).

The number of *A. cobitidis*, the other parasite species that differed between LG- and HG-infected fish, was unrelated to *S. solidus* size (likelihood ratio test, Wald = 2.56, $P = 0.21$) (Fig. 4c). Among the other parasite species observed in the field experiment, only *P. filicollis* exhibited a significant relationship with *S. solidus* size (Wald = 4.87, $P < 0.001$), with more of these gut-dwelling tapeworms occurring in fish harbouring larger *S. solidus* (Fig. 4d). Adding an interaction between *S. solidus* size and population did not significantly improve the model for any parasite species in the field experiment (all $P > 0.06$). The one species with a marginally significant interaction term was *D. pseudospathaceum* s.l., but this should not be ascribed much importance as the relationship between this parasite, *S. solidus* size and *S. solidus* population was not consistent between the two experiments (Fig. 4a,b). The worm weight by population interaction term was not significant in the laboratory experiment ($\chi^2 = 2.43, P = 0.12$).

**Discussion**

Infection with the tapeworm *S. solidus* alters the energy demands (Walkey & Meakins 1970; Lester 1971), foraging behaviour (Milinski 1984; Godin & Sproul 1988; Cunningham, Tierney & Huntingford 1994; Barber &
Huntingford 1995) and immune function of sticklebacks (Scharsack et al. 2004, 2013; Scharsack, Koch & Hammerschmidt 2007), and we hypothesized that such changes affect the probability that infected fish acquire other parasites. Both our experiments supported this, but they also revealed that the effect of *S. solidus* varied in direction and was population-dependent. In the field experiment, *S. solidus*-infected fish had more *P. filicollis* but fewer *S. globiporum*, irrespective of the *S. solidus* population. Other changes only occurred in fish infected with the fast-growing *S. solidus* (HG population). These fish had more *D. pseudospathaceum* in both experiments and fewer *A. cobitidis* in the field experiment compared to uninfected fish and to the fish harbouring the slower-growing *S. solidus* (LG population). The differences between *S. solidus* populations are not obviously linked to growth, though, because the changes were not correlated with *S. solidus* size within each population.

Interspecific associations between parasite species can arise in different ways, such as covariance in transmission rates (Janovy, Clpton & Percival 1992; Lotz, Bush & Font 1995), direct competition between parasites (Knowles et al. 2013), or indirect ‘apparent’ interactions mediated by immune suppression or cross-reactivity (Poulin 2001; Pedersen & Fenton 2007; Hoverman, Hoye & Johnson 2013). We experimentally infected fish, so a fish’s exposure to *S. solidus* was independent of its exposure to other parasites (i.e. associations were not a consequence of correlated transmission). The effect of *S. solidus* on the parasite community is thus a consequence of either (i) *S. solidus* infection affecting the probability of encountering other parasites (e.g. by changing fish behaviour; Barber, Walker & Svensson 2004) or (ii) *S. solidus* infection affecting the physiological susceptibility of fish to other parasites (e.g. by immunomodulation; Scharsack et al. 2004; Scharsack, Koch & Hammerschmidt 2007; Scharsack et al. 2013).

Both mechanisms may be important. As a possible example of the first, fish that harboured larger *S. solidus* had more *P. filicollis* (Fig. 4d). Hopkins (1959) concluded that *P. filicollis* abundance is mainly determined by stickleback diet. To meet the energy demands of having a large *S. solidus*, fish may increase foraging and feeding (Godin & Sproul 1988), which could in turn increase the likelihood of consuming copepods infected with the transmission stage of *P. filicollis*. If fish infected with large *S. solidus* simply have elevated feeding rates, we would expect them to have higher rates of infection with all tropically transmitted parasites. That was not the case, though, as the abundance of the trematode *S. globiporum* was lower in *S. solidus*-infected fish and not correlated with *S. solidus* size. Fish with *S. solidus* preferentially forage on smaller prey items (Milinski 1984; Cunningham, Tierney & Huntingford 1994; but see Ranta 1995), so perhaps infected fish consumed relatively more of the small copepods that are the intermediate hosts of *P. filicollis* than the benthic macroinvertebrates that transmit *S. globiporum*. We also cannot rule out the possibility that *S. solidus* affects the physiological susceptibility of fish to infection with *P. filicollis* or *S. globiporum*.

The positive association between HG *S. solidus* and *D. pseudospathaceum* clearly has a physiological basis, because it was detected with a fixed infection dose in the laboratory experiment. The striking association between HG *S. solidus* and irregular, degraded *A. cobitidis* cysts also suggests a physiological, possibly immunological basis for this pattern. The different abundances of *D. pseudospathaceum* s.l. and *A. cobitidis* in fish infected with LG and HG *S. solidus* is not obviously attributable to the growth differences between the *S. solidus* populations, given that their abundances were not correlated with *S. solidus* growth within each population (Fig. 4a-c). An alternative is that antigenic differences between HG and LG *S. solidus* affect susceptibility; for example, HG worm tissue induces a stronger innate immune reaction than LG tissue in stickleback leucocytes cultured in vitro (Franke et al. 2014).

There were two cases in the field experiment where a treatment group had an elevated abundance of one parasite but a reduced abundance of another: (i) *S. solidus*-infected fish had more *P. filicollis* but fewer *S. globiporum* and (ii) the HG-infected fish had more *D. pseudospathaceum* s.l. but fewer *A. cobitidis*. Indirect effects could produce such a pattern. For example, interspecific competition between flukes inhabiting the eyes of fish has been documented (Kennedy 2001; Désilets et al. 2013), so an *S. solidus*-mediated increase in one eye fluke (*D. pseudospathaceum* s.l.) might result in a decrease in another (*A. cobitidis*). Our data do not support this idea, though. Fish with more *D. pseudospathaceum* s.l. did not have fewer *A. cobitidis*, if anything they had more (spearman correlation for all fish: \( \rho = 0.20, P = 0.02 \), just for HG-infected fish: \( \rho = 0.54, P = 0.002 \)). The intensities of *P. filicollis* and *S. globiporum* were also uncorrelated at the level of individual fish (spearman correlation for all fish: \( \rho = -0.10, P = 0.22 \), just for fish with *S. solidus*: \( \rho = -0.08, P = 0.52 \)). Thus, the associations between *S. solidus* and these four species are not obviously the indirect by-product of those four species’ interactions with each other.

*Schistoscephalus solidus* benefits from attaining a large size in fish because it increases fecundity (Schräer et al. 2001). Fish infected with HG worms seemed to have an increased mortality rate in the field experiment compared to fish infected with LG worms, hinting at growth costs. However, the difference in mortality rates was relatively small (18% vs. 11% lower than expected for HG and LG, respectively; Fig. S3), particularly given the conspicuous difference in final worm sizes (HG worms were 33% larger than LG worms in the field experiment). Fish infected with HG worms had more *D. pseudospathaceum* s.l., but fewer *A. cobitidis*, suggesting they did not suffer from a generally higher parasite burden. It is unlikely that *S. solidus* directly competes with these species for the same host resources, because *S. solidus* is in the body.
cavity and the two trematodes are primarily in the eyes. Additionally, all three of these parasites reproduce in fish-eating birds, so there is no conflict concerning transmission to the next host, at least when they are all infective. Interestingly, Diplostomum sp. is rare and Apatemon sp. is common in sticklebacks in the HG S. solidus population (M. Kalbe unpublished data), so perhaps the HG population is adapted to compete with Apatemon sp. but not Diplostomum sp. Additional experiments, like repeating the field experiment in the lake where the HG S. solidus were collected, would be needed to determine if the changes in the parasite community represent an adaptation to local levels of interspecific competition.

There is ample experimental evidence that parasite species can interact in their hosts (Holmes 1961; Holland 1987; Janovy 2002), but surveys of natural parasite communities often yield apparently random assemblages (Poulin 2007). The helminth communities of fish seem particularly unstructured (Muñoz, Mouillot & Poulin 2006; Kennedy 2009), with interspecific associations often being temporally or spatially variable (Janovy & Hardin 1988; Poulin & Valtonen 2002; Vidal-Martinez & Poulin 2003; Norton, Lewis & Rollinson 2004; Behnke et al. 2005). Although the cages in our field experiment were spaced only about ~5 m apart from one another, the fish from different cages harboured distinct parasite communities. For the four parasites significantly affected by S. solidus, the effect of cage was stronger than that of treatment (proportional reduction in deviance due to cage: 10%, 29%, 48% and 30% for P. filicollis, S. globiporum, D. pseudospathaceum s.l. and A. cobitidis, respectively, vs. that due to treatment: 6%, 5%, 11% and 14%; more details in Table S2). Fish families were grouped by cage, so the relatively large effect of cage suggests that spatial variation in exposure and/or genetic susceptibility are more important for shaping parasite communities than the interactions between parasite species within a host. Indeed, several studies have found host age or size, factors that influence exposure rates, to affect parasite community structure (Vidal-Martinez, Kennedy & Aguirre-Macedo 1998; Poulin & Valtonen 2001; Zelmer & Arai 2004; Pérez-del Olmo et al. 2008; Timi, Luque & Poulin 2010). Our results also suggest that interspecific interactions can be obscured by intraspecific variation. For instance, the effects of S. solidus on A. cobitidis and on D. pseudospathaceum s.l. would appear much smaller if we lumped the two S. solidus populations together. Such contingency may often reduce the net effect of one parasite species on another and thus lessen the probability of finding strong pairwise associations in naturally sampled parasite communities (Seppälä et al. 2012).

Our field experiment was a compromise between experimental and observational approaches that let us quantify how a large tapeworm affects parasite community assembly. Infection with S. solidus affected the abundance of four parasite species, but in two cases these effects were contingent on the S. solidus population, occurring only in fish infected with the HG S. solidus. We could not directly attribute the changes in the parasite community to the pronounced growth differences between HG and LG S. solidus, though. The impact of S. solidus on the parasite community was population-dependent and weak relative to cage effects, suggesting that, while interactions between parasite species occur, they may not be of primary importance in structuring the parasite community. To evaluate the relative role of interspecific interactions in parasite community assembly, we encourage further field experiments that manipulate parasite abundance while controlling host genetics and spatial location.

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Data accessibility

Data available from the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.bq8j8 (Benesh & Kalbe 2016).

References


supporting information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Plerocercoid weight as a function of initial fish weight.

Fig. S2. (a) Liver, (b) spleen, and (c) head kidney weight in the lab and field experiments.

Fig. S3. The expected and observed prevalence of *Schistocephalus*-infected fish in the field experiment.

Fig. S4. The abundances of 11 parasite species in sticklebacks from the field experiment.

Table S1. The parasites species found in the 142 fish dissected in the field experiment as well as their prevalence, mean abundance, and variance in abundance.

Table S2. Output from the analysis of the field experiment.