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Parasite-induced colour alteration of intermediate hosts increases ingestion by suitable final host species

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Abstract

Parasites with complex life cycles often alter the phenotypic appearance of their intermediate hosts in order to facilitate ingestion by the final host. However, such manipulation can be costly as it might increase ingestion by less suitable or dead-end hosts as well. Species-specific parasitic manipulation is a way to enhance the transmission to suitable final hosts. Here, we experimentally show that the altered body colouration of the intermediate host *Gammarus pulex* caused by its acanthocephalan parasite *Pomphorhynchus laevis* differently affects predation by different fish species (barbel, perch, ruffe, brown trout and two populations of three-spined stickleback) depending on their suitability to act as final host. Species that were responsive to colour manipulation in a predation experiment were more susceptible to infection with *P. laevis* than unresponsive species. Furthermore, three-spined stickleback from different populations responded to parasite manipulation in opposite directions. Such increased ingestion of the intermediate host by preferred and suitable hosts suggests fine-tuned adaptive parasitic manipulation and sheds light on the ongoing evolutionary arms race between hosts and manipulative parasites.

Keywords

visual manipulation, trophic transmission, *Gasterosteus aculeatus*, evolutionary arms race, fishes, dead-end hosts.

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1. Introduction

Parasites with complex life cycles often depend on the ingestion of their intermediate host by a final host (*trophic transmission*), where they reach sexual maturity and reproduce (Lafferty, 1999; Lafferty & Kuris, 2002; Moore, 2002). Therefore, parasites' interests strongly conflict with those of the intermediate host, leading to high selective pressures on parasites to manipulate their intermediate host in a way that increases the probability of trophic transmission (*parasitic manipulation*). Numerous studies show that parasites alter — often dramatically — the conspicuousness or (anti-predator) behaviour of their intermediate hosts (e.g., Lefevre et al., 2009; Poulin, 2010; Moore, 2013). For example, sporocysts of the digenean parasite *Leucochloridium macrostomum* turn the antennae of the snail *Succinea putris* into colourful blinker lamps (Wesołowska & Wesołowski, 2014), while nematode-parasitized tropical arboreal ants, (*Cephalotes atratus*) resemble ripe fruits in the rain forest (Yanoviak et al., 2008). Both manipulations eventually lead to an increased ingestion of the parasite by birds.

However, not all potential predators of the intermediate host are equally suited as final host for the parasite. Thus, parasite-induced phenotypic alteration of the intermediate host might not only increase predation by the favoured final host, but also by less suitable or even non-host species (*dead-end hosts*, Mouritsen & Poulin, 2003; Poulin et al., 2005; Thomas et al., 2005; Seppälä et al., 2008). Indeed, some studies showed increased predation of infected individuals by non-hosts compared to uninfected individuals (Milinski, 1985; Poulin et al., 2005; Kaldonski et al., 2008), while others found evidence for infection-dependent predation by non-hosts (Seppälä et al., 2006). Consequently, the net benefit for the parasite resulting from the manipulation could be lower than expected, which might lead to a systematic overestimation of the adaptive significance of parasitic manipulation (Seppälä & Jokela, 2008; Cézilly et al., 2010).

However, parasitic transmission will be increased when suitable final hosts preferentially prey upon manipulated intermediate hosts (host-specific manipulation) (Levri, 1998; Seppälä et al., 2006). Testing this host-specific manipulation hypothesis requires to link suitability to act as final host of different predators with their responsiveness to parasite-manipulated intermediate hosts. Such studies are scarce thus far (but see Seppälä et al., 2008).

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Acanthocephalans represent a well-described example of manipulative parasites infecting arthropods as intermediate hosts and vertebrates as final hosts (Bethel & Holmes, 1973, 1977; Crompton & Nickol, 1985; Stone & Moore, 2014; Bakker et al., 2017). In these systems, inter- and intraspecific variation in host responses have been described repeatedly (e.g., Thomas et al., 2011; Poulin, 2013; Thünken et al., 2018). The acanthocephalan *Pomphorhynchus laevis* uses *Gammarus* species as intermediate hosts — in which they develop into the infectious cystacanths — and different fish species as final hosts. Gammarids are common in many freshwater ecosystems and represent an important food source for many fishes and birds (Wootton, 1990; MacNeil et al., 1999). Consequently, a broad array of fish species is exposed to *P. laevis* infected gammarids under natural conditions. Based on field studies from different rivers, Kennedy (2006) listed a minimum of 16 fish species that can serve as final hosts for *P. laevis*. Interestingly, these species seem to differ in suitability, e.g., due to variation in fish immune defence or gastrointestinal morphology (Lagrue et al., 2011). Barbel (*Barbus barbus*) and chub (*Leuciscus cephalus*) are highly suitable hosts of *P. laevis* (Kennedy, 2006). However, other species can serve as host for the parasite as well. Thus, the usage of a species as final host differs between populations depending on ecological factors like the composition of fish communities (Kennedy, 2006; Thomas et al., 2010).

Pomphorhynchus spec. manipulate their amphipod intermediate hosts in several ways (Crompton & Nickol, 1985; McCahon et al., 1991; Kennedy, 2006). For example, uninfected *Gammarus pulex* avoid the odour of fish predators, whereas individuals infected with *P. laevis* prefer the scent of the parasite's final host (Baldauf et al., 2007; Perrot-Minnot et al., 2007). Besides behavioural manipulation, *P. laevis* also alters the visual appearance of its intermediate host (Bakker et al., 1997). The cystacanth of *P. laevis* is visible through the gammarid's cuticle and appears as a conspicuous orange dot in the dorsal coelom. Such colouration increases the conspicuous of intermediate hosts to fish predators (Bakker et al., 1997; Kennedy, 2006). Bakker et al. (1997) provided experimental evidence that the altered colouration of *G. pulex* caused by *P. laevis* increases predation by fishes and, thus, facilitates transmission to final hosts. Uninfected gammarids that were painted with an orange spot mimicking a *P. laevis* infection were more frequently eaten by predatory three-spined stickleback (*Gasterosteus aculeatus*) than control-treated gammarids (Bakker et al., 1997). This finding strongly

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supports the hypothesis that parasite-induced alteration of body colouration increases trophic transmission. Interestingly, a subsequent study applying a similar experimental approach found no evidence for increased predation of parasite-mimics by brown trout (*Salmo trutta*) (Kaldonski et al., 2009). As different fish species appear to vary in responsiveness to *P. laevis*-induced colour alteration and in suitability to act as final host, the described host-parasite system is ideal to test the host-specific manipulation hypothesis.

In the present study, we investigate variation in the predatory response of different potential fish hosts to *P. laevis*-induced colour alteration of the intermediate host *G. pulex*. We experimentally manipulated gammarids' colouration by painting an orange spot on the cuticle of uninfected individuals, mimicking a *P. laevis* infection (cf., Bakker et al., 1997; Kaldonski et al., 2009). Examined fishes include barbel, the preferred final host of *P. laevis* as well as perch (*Perca fluviatilis*), ruffe (*Gymnocephalus cernuus*), brown trout and three-spined stickleback. Additionally, in order to investigate intra-specific variation, a stationary and a migratory stickleback population was examined. Finally, we aimed to link fishes' behavioural responsiveness to their susceptibility to *P. laevis* infections. Therefore, we tested the susceptibility of each of the fish species to infection with *P. laevis* parasites under standardised laboratory conditions. Following the host-specific manipulation hypothesis, we predict that fishes' responsiveness is positively related to their susceptibility to parasitic infection and, thus, suitability to act as final hosts.

2. Material and methods

To examine the effect of colour alteration on the predation risk of the intermediate host independent from other infection related factors, such as parasite-induced changes of behaviour, we experimentally manipulated the colouration of uninfected *G. pulex*. To estimate fishes' suitability to act as final host, we fed them with *P. laevis*-infected *G. pulex* and subsequently examined the presence of adult *P. laevis* in the intestine.

2.1. Experimental animals

2.1.1. Intermediate host Gammarus pulex

Several thousand uninfected *G. pulex* were sampled with a hand net in February 2010 from the Katzenlochbach brook (50°41′59.03″N, 7°4′54.27″E) near

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Bonn, Germany, by kick sampling (Heynes 1954). Subsequently, the gammarids were transported to the laboratory where they were kept under standardised winter conditions (light/dark regime: 8.5/15.5 h, temperature 12 ± 2°C). Dead leaves served as shelter and nutrition. Only uninfected *G. pulex* with a body size larger than 5 mm were used in the colour-manipulation experiments.

2.1.2. Predatory fishes

In the present study, we used five different fish species, all of which are described to prey on small invertebrates like *Gammarus* under natural conditions (Kottelat & Freyhof, 2007). *P. laevis* is present in every natural fish population examined. The suitability of three-spined stickleback as hosts for *P. laevis* is ambiguous thus far. In the Appendix, we therefore provide unpublished results of a study in which three-spined stickleback from a Swiss population were artificially exposed to *P. laevis* (Mazzi & Bakker, 2003). In short, 76% of the *P. laevis*-exposed fish were infected with a median number of 2 parasites per fish. 20% of the female parasites carried eggs indicating that sticklebacks are suitable final hosts for *P. laevis*. Perch, ruffe, and three-spined stickleback (Rhine population) were wild-caught fish captured in the river Rhine close to Grieth, Germany (51°47′15.17″N, 6°19′7.32″E). Different populations of the same species might face variation in environmental conditions and thus may differ in responsiveness to parasitic manipulation. To shed light on this variation we investigated a second stickleback population that was collected from a small brook close to Euskirchen, Germany (Kuchenheim population; 50°40′8.05″N, 6°49′35.82″E). Brown trout and barbel were purchased from fish farms (brown trout: Stolberg-Schevenhütte, Germany; barbel: Gersfeld, Germany) at the age of one year. As hatchery-reared and wild trout show similar feeding habits (Johnsen & Ugedal, 1990), they are suitable for the experiment. Further, to habituate experimental fish to gammarids as food they were fed daily with defrosted gammarids (*Hyalella azteca*) and with live uninfected *G. pulex* for several months. Fish were kept under similar conditions as *G. pulex* in tanks of variable size (adapted to fish size), except for trout that were kept in a large outdoor tank with continuous water flow.

2.2. Variation in fishes' response to parasite-induced colouration

2.2.1. Colour manipulation

We aimed at creating highly standardised prey mimics that imitate the natural reflectance of *P. laevis*. Therefore, the reflectance of the parasite cystacanth

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was measured through the cuticle of 10 naturally infected gammarids originating from a brook near Müllekoven, Germany (50°46′49.9″N, 7°6′36.1″E) with a spectrophotometer (Avantes USB 2000) connected to a deuterium-halogen light source (Avantes DH-2000). Reflection measurements were taken perpendicular to the body surface with a 1 mm probe under standardised conditions relative to a Spectralon white standard. For each individual a mean reflectance value deriving from 20 single measurements was taken. Subsequently, the mean reflectance value of the ten individuals was calculated, which served as a reference for the reflection values of the colour used for imitating infection on uninfected mimics. Similarly, the reflection reference for uninfected control mimics was calculated. Here, the mean reflectance of the cuticle of uninfected *G. pulex* ($N = 20$) served as a reference.

The imitation of the cystacanth's colour was achieved by mixing acrylic model paint colours (Revell enamel colour; yellow (order number 32 112), brown (32 180), orange (32 130) and black (32 107)), until spectrophotometrical readings confirmed that the colour matched with natural reflection values (see Figure A1 in the Appendix). This was achieved with a ratio of 18:4:0.25:0.2 of the respective colours. For the control, a clear, transparent colour was used (Revell Email enamel colour clear (32 101)).

Twenty-four hours before the start of the experiments, uninfected gammarids were haphazardly caught from their habitat tank. They were anesthetised with carbonated water, dried on a cellulose cloth, and their length was measured. A colour spot of approx. 1.5 mm diameter mimicking a *P. laevis* cystacanth was applied with a toothpick on a position where natural infections would be visible (colour manipulation treatment). In the control treatment, gammarids received a transparent spot in order to represent uninfected prey. The procedures took no longer than three minutes per individual. Gammarids of both treatment groups were transferred to small holding tanks containing tap water. Only gammarids that recovered and showed similar swimming behaviour as untreated gammarids were used in the experiment the next day. Gammarids of the two treatment groups did not differ in body size (Wilcoxon rank-sum test: $N_{\text{mimics}} = 2220$, $N_{\text{control}} = 2220$, $W = 2\,499\,698$, $p = 0.39$).

2.2.2. Experimental setup and procedure

Experiments were conducted in tanks (50 × 30 × 30 cm) that were laminated with a brown, self-adhesive foil, which matched in its reflection with spectrometric readings of the substrate from the natural gammarid habitat (own

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measurements). The foil was fixed to the bottom of the tanks and the lower 10 cm of the sidewalls to simulate sheltered and unsheltered areas as present in the natural habitat. The test tank was surrounded by opaque polystyrene plates in order avoid visual disturbance from outside. A fluorescent tube with daylight spectrum without UV (Sylvania Luxline plus Daylight de Luxe F36 W1860) was installed 75 cm above the test tank. Twenty-four hours before experimental trials started, one haphazardly chosen individual of each fish species was isolated in a tank that was of similar size and appearance as the test tank to acclimate to the experimental conditions. Each test tank was filled with aged tap water up to a height of 20 cm. Then, 20 colour-manipulated gammarids and 20 gammarids from the control treatment group were placed in each tank. Subsequently, the test fish was placed in a cylinder in the middle of each test tank (diameter 20 cm). In total, 15 barbel, 19 perch, 20 ruffe, 17 brown trout and 20 three-spined stickleback from each population were tested. After 30 minutes of acclimatization the cylinder was removed. The fish was allowed to swim freely and to prey upon the gammarids. With the exception of barbel, each fish species was allowed to prey upon the gammarids for one hour after release from the cylinder. Pilot studies revealed that barbel needed 2 hours after release from the cylinder to show predatory behaviour. Thus, this species was tested for three hours. After the trial, fish were removed from the test tanks and all surviving colour-manipulated gammarids and control gammarids were counted.

2.3. Variation in susceptibility to the P. laevis parasite in fishes

Ten individuals of each fish species (except three-spined stickleback) were captured randomly to examine their susceptibility to *P. laevis*. For the Rhine stickleback population seven fish were used, for the Kuchenheim stickleback population 11. All fish were food-deprived for one day. After determining the standard length (SL) and body mass (M), each fish was placed individually in a tank where it was allowed to ingest ten *G. pulex* infected with mature *P. laevis* originating from the brook in Müllekoven. A gammarid was determined as infectious if it showed a clearly visible orange spot through the cuticle. The fish stayed in the tank until it had ingested all infected gammarids. After the infection procedure, fish were transferred to a community pool with other infected conspecifics. Here, fish were daily fed with a mixture of defrosted uninfected gammarids and chironomid larvae. Fish were kept under standardised winter conditions (light/dark 8.5/15.5 h, temperature 12 ± 2°C). Four months (±5 days) after exposure, fish were killed by

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a blow to the head, followed by immediate decapitation. Subsequently, the intestine as well as abdominal cavity and viscera were searched for living *P. laevis* or their remains. The total number of parasites found in the intestine and abdominal cavity of each fish was recorded.

2.4. Statistical analysis

In the analysis, we refer to the different fishes as fish populations (one trout, perch, ruffe and barbel population each and two three-spined stickleback populations). In order to analyse whether fish populations differ in responsiveness to colour-manipulated gammarids Manly's alpha was calculated (Manly, 1974; see also Klecka & Boukal, 2012). Manly's alpha takes into account that during a trial some prey items are eaten, and thus accounts for changes in the proportions of available prey classes. We excluded all fish that consumed more than 50% of the gammarids in a trial, as these would have been forced to feed against their potential preferences (see Bakker et al., 1997). Therefore, 5 perch, 5 ruffe and 10 trout were not included in this analysis. Including these fish would not qualitatively change the results (data not shown). Three-spined stickleback and barbel never consumed more than 20 prey items in a given trial. Differences between populations were examined by an ANOVA. Responsiveness of each fish population was investigated by one-sample t-tests. Significant deviation from 0.5 thus reports responsiveness (preference or avoidance of colour-manipulated gammarids, respectively) to colour-manipulated gammarids.

The total number of ingested gammarids by fish of the different populations was analysed by fitting a GLM with quasi-Poisson distribution to account for overdispersion of the data. Population and fish size were used as explanatory variables. Additionally, we investigated whether fish responsiveness was related to the total number of ingested gammarids. To this end, the mean number of ingested gammarids per species was calculated. A linear model was fitted with mean number of ingested mimics as dependent variable, which was explained by responsiveness (population responded to colour manipulation yes/no). A population was considered as responsive to manipulation when fish discriminated between coloured and uncoloured gammarids (see one-sample t-tests in the paragraph above).

To analyse differences in susceptibility to *P. laevis* infection between fishes of the different populations, a GLM was fitted with quasi-Poisson distribution to account for overdispersion of the data. Total number of parasites

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found in the intestine and abdominal cavity served as dependent variable and fish population as explanatory variable. Body size was added as covariate. The total number of parasites (intestine plus body cavity) significantly correlated with the number of parasites found in the intestine, where the parasite reaches sexual maturity (Spearman rank correlation: $N = 58$, $S = 8718$, $p < 0.001$).

In order to link fishes' susceptibility to *P. laevis* infection to their responsiveness to colour manipulation, a GLMM with binomial distribution was fitted with parasites presence (yes/no) in the individual fish as dependent variable. As we had no behavioural data of these individual fish, we entered whether an individual belongs to a species that responded to colour manipulation or to an unresponsive species as explanatory variable. Fish population was entered as random factor to control for the multiple uses of fish originating from the same population.

In all models, likelihood ratio tests ("LRT") assessed whether the removal of a variable caused a significant decrease in model fit. Non-significant variables were removed, while significant ones remained in the model. In case of binomial test, χ^2-values are reported. For models assuming quasi-Poisson distribution we report F-values. All given test probabilities are two-tailed throughout. All analyses were performed using R 3.02 statistical package (R Core Team, 2013).

2.5. Ethics

Permissions were obtained to artificially infect and dissect fishes with *P. laevis* (LANUV NRW 8.87-51.04.20.09.352). Fish were killed according to §4 of the German animal welfare act.

3. Results

3.1. Variation in response to parasite-induced colouration

The proportion of ingested colour-manipulated and control gammarids was different between fish populations (ANOVA: df = 5, $F = 7.075$, $p < 0.001$, Figure 1). Barbel ($N = 15$) and three-spined stickleback ($N_{\text{Kuchenheim}} = 20$, $N_{\text{Rhine}} = 20$) were responsive to the colour manipulation and their prey choice deviated from random (Figure 1). Colour-manipulated gammarids were more often eaten than control gammarids by barbel (one-sample t-test, df = 13, $t = 5.076$, $p < 0.001$, Figure 1) and by the Kuchenheim stick-

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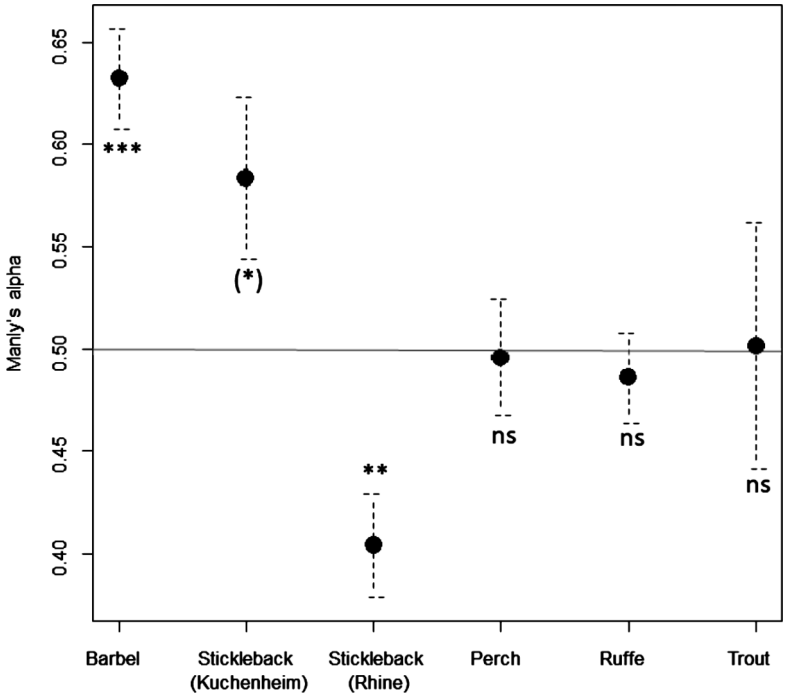

Figure 1. Manly's alpha (means ± SE) representing selective predation of the different fishes on colour-manipulated gammarids. In each trial, 20 colour-manipulated gammarids and 20 control gammarids were offered. Values greater than 0.5 indicate higher consumption of colour-manipulated gammarids. Asterisks indicate whether consumption significantly deviated from random predation (0.5). ns indicates $p > 0.5$; $^{(*)}0.05 < p < 0.1$; $^{**}p < 0.01$; $^{***}p < 0.001$.

lebacks (one-sample t-test, df = 18, $t = 1.995$, $p = 0.062$, Figure 1). By contrast, Rhine sticklebacks avoided the consumption of colour-manipulated gammarids (one-sample t-test, df = 18, $t = -3.605$, $p = 0.002$, Figure 1). Perch ($N = 14$), ruffe ($N = 15$) and brown trout ($N = 7$) did not show any significant discrimination between experimental groups (all p values > 0.53, Figure 1).

The total number of ingested gammarids varied between populations (LRT: df = 5, $F = 16.924$, $p < 0.001$, Figure A2 in the Appendix) and was positively correlated to fish body size (LRT: df = 1, $F = 27.370$, $p < 0.001$, Figure A2 in the Appendix). The mean number of ingested gammarids per population was significantly related to the respective responsiveness for vi-

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sual manipulation, with responsive fishes eating a lower total amount of gammarids (LRT: $F = -14.084$, $p = 0.019$, Figure A2 in the Appendix).

3.2. Variation in susceptibility to *P. laevis* parasites in fishes

The number of parasites found in the intestine and body cavity (ruffe: no parasites; perch: 1 parasite in 1 fish; trout: 3 parasites in 2 fish; Kuchenheim stickleback: 4 parasites in 3 fish; Rhine stickleback: 8 parasites in 3 fish; barbel: 20 parasites in 7 fish) differed between fish populations (LRT: df = 5, $F = 5.862$, $p < 0.001$), and was independent from fish body size (LRT: df = 1, $F = 0.174$, $p = 0.678$). Fishes that were responsive to colour manipulation had a higher probability to be infected by *P. laevis* than unresponsive ones (LRT: $\chi^2 = 5.945$, $p = 0.015$).

4. Discussion

The present study demonstrates variation in the responsiveness of different predatory fish species to artificially coloured *G. pulex* mimicking an infection by *P. laevis*. This variation was positively related to the susceptibility of the respective fish species to *P. laevis* infection. Although infection with a parasite does not guarantee that the parasite will reproduce (Pattersson et al., 2011, 2013), our results are in accordance with other studies on the suitability of different fishes to act as final host (i.e., allowing parasitic reproduction). The preferred host of *P. laevis* under natural conditions, the barbel (Britton & Pegg, 2011), preferentially consumed colour-manipulated gammarids and showed highest susceptibility to artificial infections. This result indicates that the colour alteration of the intermediate host caused by *P. laevis* increases ingestion by the optimal host and, thus, that colour alteration increases transmission of the parasite to a suitable final host. Therefore, our study supports the adaptive manipulation hypothesis (Bakker et al., 1997). In that scope, we were also able to confirm the results of Bakker et al. (1997), who reported that three-spined stickleback respond to the colour manipulation of *P. laevis*. The present study shows that three-spined stickleback are suitable hosts for *P. laevis* (see below). Furthermore, we found support for the results of Kaldonski et al. (2009), who reported that parasite-induced colour alteration plays no role for parasite transmission to brown trout. Brown trout, perch and ruffe showed very low infection rates in our experiment. Also, under natural conditions they do not seem to be the main hosts of *P. laevis* (e.g., Kennedy

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et al., 1978; Kennedy, 2006). These species did not response to colour manipulation. Therefore, our study provides a solution to the conflicting results of the different studies. It implies that colour alteration of the intermediate hosts represents a strategy of parasites to increase transmission to suitable final hosts without increasing transmission to less suitable hosts.

Specific manipulation by the parasite could be achieved, for example, by exploiting physiological differences in the visual system of fishes (Lythgoe, 1979). This could lead to different responses to specific colours such as to the orange spot of *P. laevis* cystacanths. Future experiments could address whether (natural) variation in carotenoid-based colouration of the parasite depend on the final host species present in the system, the production costs of the colouration and potential fine-tuned species-specific feeding preferences of final hosts. An alternative explanation for the link between fishes' responsiveness and susceptibility is a possible secondary adaptation of the parasites to the intestinal tract of predatory fishes that are attracted by the colour of infected gammarids and thus increasingly prey on them. Future studies, based on phylogenetic comparisons, could address these questions.

Intriguingly, fish from the two three-spined stickleback populations responded to parasite manipulation, but in opposite directions. This result suggests intra-specific variation between populations in the evolutionary arms race between hosts and parasites. The difference in the behavioural response might depend on variation of the costs for the stickleback host that are associated with different parasite prevalence in host populations. Although an acanthocephalan infection does not seem to seriously harm most fish hosts (Kennedy, 2006), *P. laevis*, which deeply penetrates the tissue of the digestive tract, can cause considerable damage, particularly in small fishes like three-spined stickleback (Schmidt et al., 1974; Mazzi & Bakker, 2003). Increased activity of the immune system of the host following an infection and associated changes in the metabolism (Dezfuli et al., 2002) can indirectly affect fitness-related traits like the intensity of ornamentation, which plays a central role in sexual selection (Bakker & Milinski, 1993). This should select for counter-adaptation by the host, like strategies to actively avoid infected prey as it has been shown in three-spined stickleback from a stationary Swiss population (Mazzi & Bakker, 2003). Host responses to parasitic manipulation are assumed to differ between populations due to differences in selective pressure exerted by parasites. For instance, the relative abundance of intermediate hosts as well as the composition of the fish community may

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vary between different habitats influencing the prevalence of infection of final hosts (Kennedy, 2006). Three-spined stickleback populations from the Rhine and from Kuchenheim differ in both aspects. The Rhine harbours a much higher fish diversity than the small Kuchenheim brook. This should also affect *P. laevis* prevalence across as well as within species. The results of the present study encourage future work on the interplay between variation in parasite prevalence and fish diversity (Thomas et al., 2011).

Kaldonski et al. (2009) and Cezilly et al. (2010) concluded that parasite colour alteration of intermediate hosts generally plays a limited role for increased transmission to final fish hosts, based on the finding that brown trout did not respond to the manipulation. The authors argued that the contradicting results of Bakker et al. (1997) resulted (1) from methodological flaws while mimicking the parasite-infection and (2) from the usage of an unsuitable final host, i.e., three-spined stickleback. In the light of the present study, this criticism does not hold. Barbel, which is the most important host of *P. laevis*, were responsive to the colour alteration of *G. pulex* which clearly supports the hypothesis by Bakker et al. (1997), stating that colour alteration caused by the parasite increases transmission to final hosts. Based on data obtained from only three individuals, Hine & Kennedy (1974) prematurely labelled three-spined stickleback as non-suitable host of *P. laevis*. In their study, two out of three individuals carried a single parasite each, which thus excluded the presence of gravid female parasites, a criterion for the suitability of the host. In contrast, the data reported in the Appendix indicate that sticklebacks are physiologically compatible to serve as final host and, thus, are suitable hosts for *P. laevis* for reproduction in nature. Indeed, three-spined stickleback may be an important host for *P. laevis* under changing natural environments. Even under harsh conditions, when fish diversity decreases temporally (e.g., due to anthropogenic impacts) the robust three-spined stickleback is often able to maintain stable populations (Wootton, 1976), ensuring at least basic opportunities for parasites' reproduction and survival. Field data on natural infections, particularly from smaller rivers where three-spined stickleback are often the dominant species, are required to clarify their importance as final host for *P. laevis* under natural conditions.

Overall, larger fishes were less infected by *P. laevis* but consumed a higher absolute number of gammarids compared to the smaller, more infected fishes. The consequences for parasitic transmission in the wild are unclear and may depend on several factors like the proportion of host and

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non-host species and the general predation risk (see Seppälä & Jokela, 2008). Gammarids are an important component in the food web of aquatic systems (MacNeil et al., 1999), where they represent an important food source for many fishes. Parasites (particularly those with complex life cycles) have the potential to alter and maintain biodiversity (Lafferty et al., 2008) by adding complexity (Dunne et al., 2013). Our results suggest that manipulative parasites may contribute to population dynamics (see Heggelin et al., 2007) as increased conspicuousness of intermediate hosts induced by parasitic manipulation might increase consumption by predator species.

Besides changing colouration, *P. laevis* and other acanthocephalans are known to alter a range of behaviours of their intermediate gammarid host like geo- and phototatic behaviour or activity (e.g., Bethel & Holmes, 1973, 1977; Thünken et al., 2010, 2018; Bakker et al., 2017), or predator evasion (Baldauf et al., 2007; Perrot-Minot et al., 2007). In three-spined stickleback changes in colouration and behaviour of infected *G. pulex* alone lead to an increased risk of predation for the intermediate host (Mazzi & Bakker, 2003). Furthermore, intermediate and final hosts may respond in diverse manners to deal with the risk of parasitic infection (Kennedy, 2006). Thus, in order to evaluate the overall fitness consequences of parasitic infections integrative approaches are required combining laboratory experiments with field data. As we were interested in parasitic manipulation, we experimentally examined a specific trait of the intermediate host that is altered by the parasite (colouration) and analysed its impact on parasitic transmission independent from other host traits potentially affected by the infection.

Summarising, the present study shows that the altered colouration of the intermediate host *G. pulex* caused by its acanthocephalan parasite *P. laevis* differently affects predation by fishes, depending on their suitability to act as final host. This could be a result of parasitic adaptation to use predators preferring coloured gammarids as final hosts. However, our results could also hint towards specific parasitic manipulation of the intermediate host to reach a suitable final host. Thus, this study highlights the importance of parasite-induced colour alteration of the intermediate host for the evolution of successful transmission of the parasite to its final host.

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Appendix

Suitability of three-spined stickleback as a final host for Pomphorhynchus laevis

According to the classification of Hine & Kennedy (1974), three-spined stickleback are unsuitable as final hosts for *Pomphorhynchus laevis* (also referred by Kaldonski et al., 2009). However, this classification was based on only three individuals and thus must be considered with caution because parasite prevalence is often below 100%. Therefore, for the present study, we re-examined 60 *P. laevis* that were dissected out of artificially infected three-spined stickleback originating from the same population as the fish used in the study of Bakker et al. (1997). Details of the infection procedure are described in Mazzi & Bakker (2003). 76% of the exposed fish were infected with a median number of 2 parasites per fish. Worms were preserved in 70% ethanol. Digital photographs including a size standard were taken from the preserved individuals to determine body length. The sex of each parasite and presence of eggs was noted to analyse whether the parasite reaches sexual maturity in sticklebacks. The sex of one individual could not be determined, reducing the total sample size to 59 individuals. The body size of ethanol-preserved (shrunken) *P. laevis* (mean $\pm$ SD = 10.09 mm $\pm$ 1.54) largely

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conforms to the classification of Hine & Kennedy (1974) for body size of *P. laevis* found in suitable hosts (12–15 mm). The body size of males ($N = 25$, mean ± SD = 9.91 mm ± 1.59 mm) and females ($N = 34$, mean ± SD = 10.21 mm ± 1.55 mm; for one female the body size could not be determined) was comparable (t-test: $t = 0.74$, df = 57, $p = 0.46$). Seven out of 35 females (i.e., 20%) carried eggs. Fecund females were larger in body size than females without eggs (with eggs: mean ± SD = 11.59 mm ± 1.81 mm, without eggs: mean ± SD = 9.92 mm ± 1.35 mm, Student's t-test: $t = 2.60$, df = 32, $p = 0.014$). Hine & Kennedy (1974) state that hosts should be classified as suitable when ripe parasite females are frequently present. Accordingly, we consider three-spined stickleback as a suitable host for *P. laevis* reproduction.

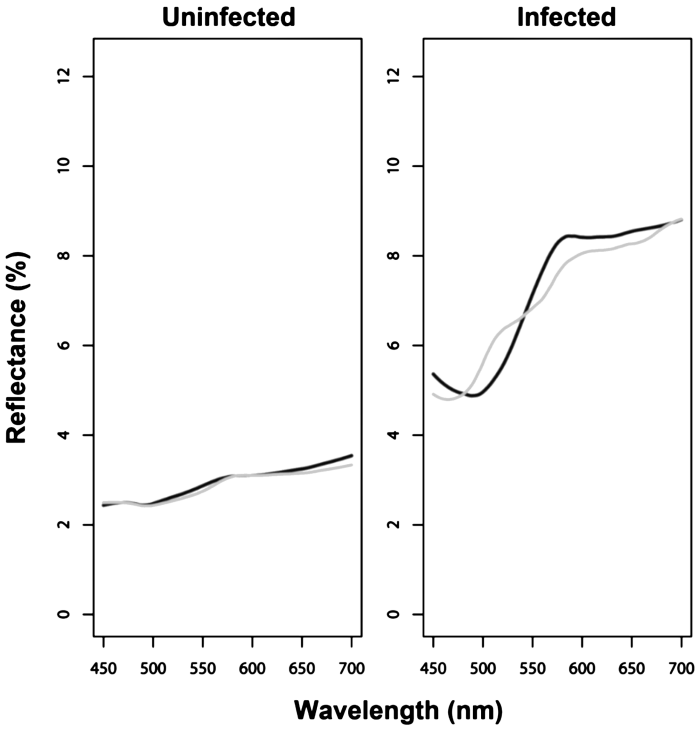

Figure A1. Mean reflectance spectra of natural *Gammarus pulex* (black lines) and experimentally treated gammarids (grey lines). The left panel shows the reflectance of the cuticle of naturally uninfected gammarids and control treated gammarids that were painted with a transparent spot. The right panel shows the reflectance of *P. laevis* cystacanths measured through the gammarid's cuticle and the colour-manipulated gammarid painted with an orange spot.

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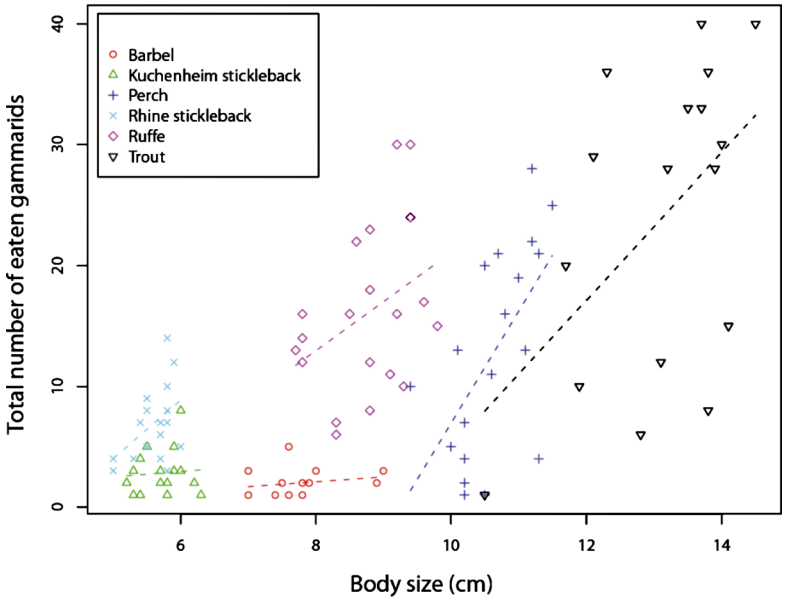

Figure A2. Relationship between body size of experimental fish and the total number of eaten gammarids by fish in the predation experiment (both gammarid treatment groups are pooled). Data for barbel, perch, ruffe, trout and the 2 three-spined stickleback populations (Rhine and Kuchenheim population) are shown. Fishes that responded to colour manipulation (barbel and the two stickleback populations) consumed on average less gammarids than unresponsive fishes (perch, ruffe and trout). Lines are least-square regression lines.

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