Effects of host competence and schooling behavior on parasite transmission in a fish-gyrodactylid system: test of the dilution effect

Shun Zhou¹, Xiao Jin¹, Ming Duan¹, Hong Zou¹, Ming Li¹, David Marcogliese¹, Guitang Wang¹, and Wenxiang Li²

¹Affiliation not available
²Institute of Hydrobiology Chinese Academy of Sciences

November 17, 2023

Abstract

High species diversity in a community may reduce risk of infectious disease, termed the dilution effect. However, the generality of dilution effect in different disease systems remains controversial. Besides host competence, behavior of fish hosts also may play an important role in dilution or amplification of disease. Using the goldfish (Carassius auratus)-Gyrodactylus kobayashii system, effects of host competence and schooling behavior on parasite transmission were investigated while holding focal host density constant. Following competency tests of 12 fish species as potential hosts for the parasite, infection of G. kobayashii was determined on fins of goldfish mixed with each one of three different species based on their level of host competence, including the silver crucian carp, C. auratus gibelio (low competence), the grass carp, Ctenopharyngodon idellus (incompetence), the swordtail Xiphophorus helleri (incompetence), and all four species combined. Compared with mean abundance (85.8 ± 25.1) on goldfish in the control group, there was a significant decrease in the silver crucian carp group (30.0 ± 16.5), but no significant changes in the swordtail group (70.0 ± 22.2), the grass carp group (116.1 ± 33.2), or the multi-species group (75.9 ± 30.8) during the 10-day experiment. The parasite was also found on C. auratus gibelio in the silver crucian carp group and the multi-species group at a mean abundance of 7.1 and 10.9, respectively. Analysis of swimming behavior showed that the goldfish mixed well in schools with the silver crucian carp. However, the goldfish maintained separation from the grass carp and swordtail when mixed together. Distance between goldfish increased, and swimming speed and contact time decreased with the additional fish for all groups. The results suggested that the presence of a low-competence host in sufficient numbers was a necessary condition for a dilution effect due to encounter reduction, and the dilution effect may also be enhanced by changes in schooling behavior of goldfish in the presence of the low competence hosts. However, the presence of incompetent hosts did not result in any dilution effect owing to the specialist nature of the parasites and the lack of mixing with schools of goldfish.

Introduction

There is increasing attention on the dilution effect of biodiversity on infectious diseases. The dilution effect hypothesis (DEH) suggests that high species diversity in a community including low competent and incompetent hosts can reduce risk of infectious disease (Ostfeld & Keesing 2000). Encounter (contact) reduction, transmission reduction (following encounters), and susceptible host regulation were proposed as underlying mechanisms of the DEH (Keesing et al. 2006; Ostfeld & Keesing 2012), although altered susceptibility due to stressors may also play a role (Buss & Hua 2023). However, the generality of the DEH remains controversial given that increasing host species also introduces more pathogens in complex systems (Randolph & Dobson 2012; Wood & Lafferty 2013; Wood et al. 2014). Meta-analysis suggested that the magnitude of the dilution effect on plant diseases varied between ecosystem type, pathogen type, study design (observational versus manipulative), parasite life history and latitude (Liu et al.2020). In contrast, the magnitude of the dilution effect on zoonotic diseases was independent of host density, study design, and type and specialization of parasites, but related to the relative abundance of the focal host (Civitello et al. 2015). Therefore, in addition
to identifying patterns using correlative studies, mechanisms should be experimentally tested in different disease systems to determine when and where the DEH occurs (Halsey 2019).

A meta-analysis of published studies revealed that the dilution effect occurs commonly on plant, wildlife and human diseases (Civitello et al. 2015). The DEH also has been observed in aquatic host-parasite systems involving anuran, teleost and molluscan hosts infected with fungal, monogenean and trematode parasites (Johnson et al. 2008; Thieltges et al. 2008; Johnson & Hartson 2009; Johnson et al. 2009; Johnson & Hoverman 2012; Dargent et al. 2013; Venesky et al. 2014; Gendron & Marcogliese 2017). The occurrence of a dilution effect may rely on the specific composition of the host community (Johnson et al. 2013; Salkeld et al. 2013), as variation in host quality is a condition necessary for reduction in pathogen transmission (Keesing & Ostfeld 2021a).

Encounter reduction, considered one of the most common mechanisms behind the dilution effect (Johnson & Thieltges 2010), may occur via a number of means, including mortality of free-living parasite stages, mortality of parasitic stages on hosts, or alterations in host behavior and activity (Johnson & Thieltges 2010). Changes in host behavior may affect the likelihood of parasite encounter and subsequent risk of infection (Johnson & Thieltges 2010). For example, the presence of predators affects anticercarial behavior in tadpoles, contributing to the increase in exposure rates to trematode parasites (Rohr et al. 2015). Sex differences in the degree of shoaling behavior and inter-individual contact rates determines relative infection levels of Gyrodactylus turnbulli in the guppy (Poecilia reticulata) males and females (Richards et al., 2010). Given that shoaling behavior in guppies affects transmission of gyrodactylids (Richards et al., 2010; Johnson et al., 2011), any changes in schooling behavior will likely affect the risk of infection. How one fish species with schooling behavior responds to the presence of other fish species may then result in the occurrence of a dilution effect. However the effects of host schooling on the occurrence of the dilution effect have never been tested in previous empirical studies and predictive models.

The monogenean Gyrodactylus kobayashii is the most common gyrodactylid on ornamental goldfish (Carassius auratus) (Liet et al. 2014), and the goldfish-G. kobayashii model has been established to study the transmission of the parasite under laboratory conditions (Zhou et al. 2017). While monogeneans tend to show high specificity to a particular host, gyrodactylids are known to infect other host species under experimental conditions, albeit with low infection success (King & Cable 2007; King et al. 2009). The viviparous gyrodactylids mainly transmit via contact between hosts, and have the capability of continuous transmission during their entire lifetime (Boeger et al. 2005).

Using this system, we first determined if host competence was a necessary condition for the dilution effect to occur in the goldfish-G. kobayashii model system. To test the influence of host competence on parasite transmission, we added other fish species of varying competence to the experimental system. This experiment also examined effects of host diversity and abundance on transmission.

Goldfish display schooling behavior, and mean distance among individuals and schooling pattern varies with the population size of goldfish (Leem et al. 2012; Jeon et al. 2013). Importantly, the frequency of social contact affects gyrodactylid transmission in guppies (Johnson et al. 2011). We thus examined if schooling behavior in goldfish plays a role in parasite transmission and, potentially, the occurrence of the dilution effect in the presence of other host species of varying competence.

Materials and methods

Preparation of fish and gyrodactylids

Gyrodactylids were obtained by periodically introducing gyrodactylid-free goldfish to aquariums with G. kobayashii-infected goldfish (Zhou et al. 2017). In addition to the goldfish, fingerlings or immature individuals of 12 fish species (Table 1) were transferred to the laboratory and separately stocked in several 100-L aquariums each equipped with an aerator. To remove ectoparasites, fish were treated with an appropriate concentration of formalin or salt solution. After 30 days in the aquariums, 10 individuals of each species were
anaesthetized with 0.02% MS-222 (tricaine methanesulfonate) and examined to confirm their gyrodactylid-free status using a stereomicroscope.

**Tests of host competence**

Standard length of each fish was measured, and then a single fish was stocked in a tank with 0.5 L of dechlorinated tap water. Gyrodactylids were collected from heavily infected goldfish and two worms were transferred to the caudal fin of each fish anaesthetized with MS-222 as described by King and Cable (2007). Following 5 min inoculation, the caudal fins of 30 individuals of each species were examined using a stereomicroscope. On day 1 and day 7, gyrodactylids were examined on caudal fins of these fish anaesthetized with MS-222. If a fish was infected with at least one gyrodactylid on day 1 and three gyrodactylids on day 7, it was recorded as attachment success and establishment success, respectively, which was modified from King and Cable (2007). Attachment and establishment success rates were calculated as the number of infected fish divided by the number of exposed fish on day 1 and day 7, respectively. Mean abundance, the mean number of parasites per fish, infected or not, also was calculated after establishment. If establishment success rate and mean abundance were significantly lower than those on the goldfish in the control group, the species was considered as a low competent host, whereas any species without any establishment success was defined as an incompetent host.

**Effect of host competence on infection of G. kobayashii**

Given that decreases in density of susceptible hosts may lead to a dilution effect (Keesing *et al.* 2006; Johnson & Thieltges 2010; Johnson *et al.* 2013), goldfish densities were maintained constant in the experiments. Based on different competences of the 12 fish species (Table 1), three were selected for the mixed experiment with goldfish, including the silver crucian carp (*Carassius auratus gibelio*) with low establishment success, the swordtail (*Xiphophorus helleri*) and the grass carp (*Ctenopharyngodon idellus*), both without establishment success. Then these fish were randomly assigned to five groups, including a control group (10 goldfish), a silver crucian carp group (10 goldfish and 10 *C. auratus gibelio*), a grass carp group (10 goldfish and 10 *C. idellus*), a swordtail group (10 goldfish and 10 *X. helleri*) and a multi-species group (10 goldfish, 3 *C. auratus gibelio*, 4 *C. idellus* and 3 *X. helleri*), which is an additive design. Each group was maintained at a constant density of goldfish and replicated five times. Fish were maintained in a round tank [60 (diameter) × 60 (depth) cm] containing 50-L dechlorinated tap water at 20 ± 1 °C, and fed twice daily with commercial pellet feed. To keep the water in good condition, feces and uneaten food were removed regularly, and one-third of the water was changed every two days.

After five days of acclimation, a donor goldfish (marked with particular body color) heavily infected with *G. kobayashii* (n = 170-180) on its fins was introduced into each tank. After 10-days, gyrodactylids were counted on fins of each fish anaesthetized with MS-222 except for the donor goldfish using a stereomicroscope. Since the majority of *G. kobayashii* were found on fins of goldfish in the preliminary experiments, only fins were included in the parasitological examination to reduce potential effects of manipulation on the fish. Mean abundance was calculated on each infected species, while total mean abundance was determined at the host community level.

**Effects of additional fish on schooling behavior of goldfish**

To determine changes of goldfish swimming behavior in the presence of other fish species, the ectoparasite-free fish were randomly assigned to four groups, including the control group (12 goldfish), the silver crucian carp group (6 goldfish and 6 silver crucian carp), the grass carp group (6 goldfish and 6 grass carp), and the swordtail group (6 goldfish and 6 swordtail). Each group was replicated ten times and maintained in a square tank (67 × 44 × 33 cm) containing 50-L dechlorinated tap water with a minimum of 5 mg/L dissolved oxygen at 20 ± 1 °C. A web camera (BASLER, acA1920-155uc, 25 frames/s) was placed over each fish tank at a 1.5 m distance. Behavior of the fish in each tank was recorded continuously for 10 min. Average speed, average adjacency distance and cumulative contact time (less than 1 cm distance between two goldfish) among the six goldfish in each tank were calculated from the videos using the program Ethovision XT10.0 (Noldus, Netherlands). Trajectory heatmap of a randomly selected goldfish was also generated from the videos using
the software Ethovision.

**Statistical analysis**

Significant differences were analyzed using the G-test of heterogeneity in establishment success rate (Sokal & Rohlf 2012), and the non-parametric Mann-Whitney U test in mean abundance between the test group and the control group. One-way ANOVA with Tukey’s multiple comparison test was performed to compare differences in mean abundance at the population and community levels, average speed, average adjacency distance, and cumulative contact time of goldfish between the control group and the multispecies groups.

**Results**

The control goldfish group had 100% establishment success and a mean abundance of 17.0 ± 13.2. Of the 12 fish species, 8 were infected by *G. kobayashii* with 6.7-83.3% attachment success (Table 1). But only *C. auratus gibelio* became infected with 56.7% establishment success and 3.0 ± 4.4 mean abundance (Table 1), which were both significantly lower than those on the control group (*P* < 0.05). The silver crucian carp is defined as a low-competence host, while the other species with no establishment success are considered as incompetent hosts.

In the mixed-species culture, no *G. kobayashii* was detected on grass carp and swordtail (Fig 1). Compared with the goldfish in the control group (85.8 ± 25.1), the mean abundance of *G. kobayashii* fins of goldfish was lower in the swordtail group (70.0 ± 22.2), the multi-species group (75.9 ± 30.8) and the silver crucian carp group (30.0 ± 16.5), but higher in the grass carp group (116.1 ± 33.2) (Table 2). There were significant differences in mean abundance on goldfish between the silver crucian carp group and all other groups (*P* < 0.05) (Fig 1). Mean abundance of *G. kobayashii* on *C. auratus gibelio* in the silver crucian carp group (7.1 ± 5.6, 66.0% prevalence) and the multi-species group (10.9 ± 3.8, 86.7% prevalence) were both significantly lower than that on *C. auratus in* the corresponding group (*P* < 0.05). Total population size of *G. kobayashii* was 857.8, 370.4, 1160.8, 698.8 and 791.4 in the five groups, respectively. At the host community level, the mean abundance was 18.5 (± 10.7) and 39.6 (± 15.1) in the silver crucian carp and the multi-species groups, respectively (Table 2). A significant difference in total mean abundance on all hosts was detected between the control group and the silver crucian carp group (*P* < 0.05) and the multi-species group (*P* < 0.05).

Observation of schooling patterns showed that population of goldfish mixed well with the silver crucian carp, but not with the grass carp and the swordtail (S Video). The average adjacency distance among goldfish significantly increased, while the average speed and cumulative contact time significantly decreased in all mixed groups when compared with those in control group (*P* < 0.05) (Fig 2). The trajectory heatmap showed that the goldfish tended to school along the tank wall both in the control and silver crucian carp groups. In contrast, goldfish were uniformly distributed in the whole tank in the grass carp group and in part of the tank in the swordtail group (Fig 3). A schematic model of the spatial distribution of the fishes under the different experimental conditions is presented in Fig 4, with the school of goldfish alone (Fig 4A), the goldfish school mixed with low-competence silver crucian carp (Fig 4B), and goldfish schooling separately from incompetent grass carp (Fig 4C).

**Discussion**

Results suggest that in this fish-*Gyrodactylus* system, the presence of additional species leads to a dilution effect, but only in the presence of a low competent host. A significant decrease in mean abundance of *G. kobayashii* on goldfish was detected in silver crucian carp group, a low competent host, but not significantly in the presence of non-competent hosts. However, no dilution effect was observed in the four-species treatment that included silver crucian carp.

Species with low competence play key roles in the dilution effect (Thieltges et al. 2008). While dilution effect occurred commonly in communities with higher diversity (Ostfeld & Keesing 2000; Johnson & Thieltges 2010; Keesing & Ostfeld 2021a), the important determinant of parasite transmission was the specific composition of the host community which affected the strength of the dilution effect (Ostfeld & Keesing 2000; Logiudice
et al. 2008; Johnson et al. 2013; Salkeld et al. 2013). Variation in host quality is a condition necessary for a reduction in pathogen transmission (Keessing & Ostfeld 2021a). In the Lyme disease system, low-quality hosts in high-diversity communities deflected ticks away from the most competent hosts (Keessing et al. 2009; Keessing et al. 2010). The presence of low competence treefrogs (Hyla versicolor) reduced Ribeiroia ondatrae abundance in toads (Bufo americanus) through encounter reduction (Johnson et al. 2008). In a field investigation, the presence of the invasive round goby (Neogobius melanostomus), itself infected by the eyeflake Diplostomum spp. at low infection levels, sharply reduced the prevalence and abundance of the eyeflukes in native fishes from the St Lawrence River, Canada (Gendron & Marcogliese 2017). In our study, a dilution effect was not observed in the community with the highest species richness, but only in that with sufficient numbers and/or density of silver crucian carp, a low-competence host. Therefore, results further confirmed that the specific composition in a community, including species with low competence, was a necessary condition leading to the dilution effect, rather than simply diversity. However, abundance and/or density of the low competent host also appears important, as the mix-species assemblage also contained silver crucian carp, but at lower abundance and density (Thieltges et al. 2008). The relative abundance of low-competence hosts compared to competent hosts is an important component for the dilution effect (Ostfeld & Keessing 2012). Curiously, a higher density of all potential hosts (competent plus low competence) did not enhance parasite transmission, despite the fact that density of primary host play a roles in transmission of gyroactylids (Johnson et al. 2011; Jin et al. 2022).

Incompetent hosts (decoys) can interfere with parasite transmission and distract free-living parasite stages away from suitable hosts, leading to wasted transmission events (Johnson & Thieltges 2010). Abundance of trematode (Himasthla elongata) in cockle hosts (Cerastoderma edule) was significantly reduced by the presence of incompetent hosts (Crepidula fornicata, Mya arenaria and Crassostrea gigas) that acted as decoy hosts for cercariae (Thieltges et al. 2008). Interestingly, the presence of incompetent hosts did not result in a dilution effect in our experiments, unlike other systems (Johnson & Thieltges 2010). Decoy hosts may not divert infective parasites away from goldfish in this system, likely because the parasites are host specialists. This lends support to the idea that the dilution effect is weaker with host specialists, with little or no wasted transmission (Ostfeld & Keessing 2012).

Several mechanisms have been proposed for how biodiversity reduces disease risk, such as by reducing encounters between hosts and parasites, reducing establishment after encounters occurred, or decreasing numbers of susceptible hosts (Keessing et al. 2006). Addition of low competence treefrogs significantly reduced infection of R. ondatrae in toads by limiting transmission of the cercariae to toads likely via a strong immune defense against them in treefrogs, a form of encounter reduction (Johnson et al. 2008; Johnson & Hartson 2009). In our experimental system, transmission to goldfish likely was reduced by the presence of sufficient numbers of low competent hosts via encounter reduction, as the infective gyroactylids may have been deflected away from the goldfish to the crucian carp, where they then only established in low numbers. A dilution effect was observed not only at the population level on the goldfish hosts (population level) but also at the host community level on the goldfish and crucian carp combined (Buss & Hua 2023).

The presence of low competence or incompetent hosts may influence the behavior and activity of a competent host (Johnson & Thieltges 2010; Keessing et al. 2010). For pathogens transmitted via host contact, theoretical models supported the DEH under the condition that additional host species mixed well with the focal hosts (Keessing et al. 2006; Keessing & Ostfeld 2021b), promoting encounter reduction between infected and susceptible hosts. In the present study, observations of schooling behavior showed that the silver crucian carp mixed well with the goldfish. Thus, the low competent host was in close proximity to goldfish, potentially attracting infective gyroactylids away from susceptible goldfish, leading to encounter reduction. Furthermore, the average adjacency distance among goldfish significantly increased, and the cumulative contact time significantly decreased following the addition of the silver crucian carp. The higher adjacency distance and lower contact time may contribute to a reduction in transmission of gyroactylids between goldfish. However, changes in schooling behavior alone are insufficient to cause a dilution effect, as they were also observed in the presence of non-competent hosts. This may in part be due to the fact that the incompetent hosts did not mix with goldfish. The lack of contact between the host species may have also served to
prevent wasted transmission events. Host behavior has been shown to be important in the transmission of other gyrodactylids. The frequency of social contacts through shoaling behavior in the guppy determined the outbreak of epidemics in *G. turnbulli* (Johnson *et al.* 2011). Indeed, more than one mechanism contributing to a dilution effect may be at play at the same time in a particular system (Johnson & Thieltges 2010).

Although no dilution effect was detected in the presence of the two incompetent hosts, the higher mean abundance on goldfish in the grass carp group may result from reinfection of detached gyrodactylids in the water column due to spatial distribution of goldfish in the whole tank (Jin *et al.* 2022).

Our experiment demonstrated that the dilution effect depends more on specific community composition than biodiversity in this host-parasite system. In this system, low competent hosts in sufficient number or density are required for a dilution effect. The primary mechanism in this case appears to be encounter reduction. Changes in host behavior in the presence of a low-competence host also may contribute to a dilution effect. In this host-parasite system, the presence of incompetent hosts did not lead to a dilution effect, supporting the idea that the dilution effect may not be as strong with host specialists. The occurrence of a dilution effect in the presence of the silver crucian carp schooling with the goldfish, but the absence of a dilution effect in the presence of additional non-competent hosts that segregated from the goldfish suggested that host schooling behavior plays a key role in restricting the dilution effect.

**Acknowledgments**

We would like to thank the anonymous reviewers for investing time and expertise into reviewing our manuscript.

**Author Contributions**

WL and GW conceptualized the study. SZ, XJ and MD performed the experiments and analysis. WL, DJM and ZS wrote the first draft of the manuscript and all authors contributed substantially to revision.

**Funding information**

This work was supported by the National Natural Science Foundation of China (32230109, 32373179), and the Earmarked Fund for CARS (CARS-45).

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are openly available Zenodo:

**References**


King, T.A. & Cable, J. (2007). Experimental infections of the monogenean *Gyrodactylus turnbulli* indicate that it is not a strict specialist. *International Journal for Parasitology*, 37, 663-672.


### Table 1. Attachment success and establishment success of *Gyrodactylus kobayashii* on various fish species.

The fish species in bold were chosen for the experiment of fish mix-culture.

<table>
<thead>
<tr>
<th>Fish species</th>
<th>N</th>
<th>Family</th>
<th>Standard length ± SE (cm)</th>
<th>Attachment success (%)</th>
<th>Establishment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Carassius auratus</em> (as control group)</td>
<td>30</td>
<td>Cyprinidae</td>
<td>4.7 ± 0.6</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>Carassius auratus gibelio</em></td>
<td>30</td>
<td>Cyprinidae</td>
<td>6.1 ± 0.7</td>
<td>83.3</td>
<td>56.7</td>
</tr>
<tr>
<td><em>Cyprinus carpio</em></td>
<td>30</td>
<td>Cyprinidae</td>
<td>9.8 ± 1.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Danio rerio</em></td>
<td>30</td>
<td>Cyprinidae</td>
<td>3.3 ± 0.6</td>
<td>6.7</td>
<td>0</td>
</tr>
<tr>
<td><em>Hemiculter leucichus</em></td>
<td>30</td>
<td>Cyprinidae</td>
<td>5.0 ± 0.8</td>
<td>20.0</td>
<td>0</td>
</tr>
<tr>
<td><em>Pseudorasbora parva</em></td>
<td>30</td>
<td>Cyprinidae</td>
<td>4.9 ± 0.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ctenopharyngodon idellus</em></td>
<td>30</td>
<td>Cyprinidae</td>
<td>6.8 ± 1.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Culter alburnus</em></td>
<td>30</td>
<td>Cyprinidae</td>
<td>8.4 ± 0.8</td>
<td>36.7</td>
<td>0</td>
</tr>
<tr>
<td><em>Xiphophorus helleri</em></td>
<td>30</td>
<td>Poeciliidae</td>
<td>3.8 ± 0.7</td>
<td>40.0</td>
<td>0</td>
</tr>
<tr>
<td><em>Gambusia affinis</em></td>
<td>30</td>
<td>Poeciliidae</td>
<td>2.8 ± 0.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Myxocyprinus asiaticus</em></td>
<td>30</td>
<td>Catostomidae</td>
<td>8.4 ± 1.0</td>
<td>30.0</td>
<td>0</td>
</tr>
<tr>
<td><em>Tachysurus fulvidraco</em></td>
<td>30</td>
<td>Bagridae</td>
<td>7.9 ± 1.2</td>
<td>30.0</td>
<td>0</td>
</tr>
<tr>
<td><em>Clarias gariepinus</em></td>
<td>30</td>
<td>Clariidae</td>
<td>6.1 ± 0.9</td>
<td>70.0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 2. Mean abundance (± SE) of *Gyrodactylus kobayashii* on goldfish (*Carassius auratus*) and silver crucian carp (*Carassius auratus gibelio*) (at the population level), and total parasites and mean abundance on all fish (at the community level) in the five groups. CG, control group, 10 *C. auratus*; TG1, 10 *C. auratus* + 10 *C. auratus gibelio*; TG2, 10 *C. auratus* + 10 grass carp (*Ctenopharyngodon idellus*); TG3, 10 *C. auratus* + 10 swordtail (*Xiphophorus helleri*); TG4, 10 *C. auratus* + 3 *C. auratus gibelio* + 4 *C. idellus* + 3 *X. helleri*.

<table>
<thead>
<tr>
<th></th>
<th>CG</th>
<th>TG1</th>
<th>TG2</th>
<th>TG3</th>
<th>TG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean abundance on goldfish</td>
<td>85.8 ± 25.1</td>
<td>30.0 ± 16.5</td>
<td>116.1 ± 33.2</td>
<td>70.0 ± 22.2</td>
<td>75.9 ± 30.8</td>
</tr>
<tr>
<td>Mean abundance on silver crucian carp</td>
<td>\</td>
<td>7.1 ± 5.6</td>
<td>\</td>
<td>\</td>
<td>10.9 ± 3.8</td>
</tr>
<tr>
<td>Total parasites on all fish</td>
<td>857.8</td>
<td>370.4</td>
<td>1160.8</td>
<td>698.8</td>
<td>791.4</td>
</tr>
<tr>
<td>Mean abundance on all fish</td>
<td>18.5 ± 10.7</td>
<td>\</td>
<td>\</td>
<td>\</td>
<td>39.6 ± 15.1</td>
</tr>
</tbody>
</table>
Fig 1. Mean abundance of *Gyrodactylus kobayashii* on fins of goldfish (*Carassius auratus* ) and silver crucian carp (*C. auratus gibelio* ) in different combinations of fishes. Control group, 10 *C. auratus* ; TG1, 10 *C. auratus* + 10 *C. auratus gibelio* ; TG2, 10 *C. auratus* + 10 grass carp (*Ctenopharyngodon idellus* ); TG3, 10 *C. auratus* + 10 swordtail (*Xiphophorus helleri* ); TG4, 10 *C. auratus* + 3 *C. auratus gibelio* + 4 *C. idellus* + 3 *X. helleri* . Different letters on the grey bar indicate a significant difference in mean abundance among the five groups at 0.05 level.

Fig 2. Effects of additional fish species on average speed (A), average adjacency distance (B) and cumulative contact time (C) among six goldfish (*Carassius auratus* ). Different letters on the grey bar indicate a significant difference among the four groups at 0.05 level.

Fig 3. Trajectory heatmap of a goldfish (*Carassius auratus*) within ten minutes in goldfish (as control), silver crucian carp (*Carassius auratus gibelio*), grass carp (*Ctenopharyngodon idellus*) and swordtail (*Xiphophorus helleri*) groups. The warmer color on the right side of heatmap indicates much more time the goldfish stayed there.

Fig 4. Conceptual model of spatial distribution of fish with schooling behavior. (A) School of goldfish (*Carassius auratus*) (orange); (B) Goldfish school mixed with silver crucian carp (*Carassius auratus gibelio*) (grey) with low competence, reducing encounters between infected and uninfected goldfish; (C) Goldfish school separated from incompetent grass carp (*Ctenopharyngodon idellus*) or swordtail (*Xiphophorus helleri*) (green), hardly reducing encounters between infected and uninfected goldfish.

S Video. Relationship of fish schools between focal host and added fish species. (A) Schooling patterns of
goldfish (*Carassius auratus*) alone; (B) Schooling patterns of goldfish and silver crucian carp (*Carassius auratus gibelio*); (C) Schooling patterns of goldfish and swordtail (*Xiphophorus helleri*); (D) Schooling patterns of goldfish and grass carp (*Ctenopharyngodon idellus*).