

# Population growth of *Gyrodactylus kobayashii* in goldfish (*Carassius auratus*) associated with host density

## Research Article

**Cite this article:** Jin X, Zou H, Li M, Wu S, Wang G, Marcogliese DJ, Li W (2022). Population growth of *Gyrodactylus kobayashii* in goldfish (*Carassius auratus*) associated with host density. *Parasitology* **149**, 1057–1064. <https://doi.org/10.1017/S003118202200052X>

Received: 10 January 2022

Revised: 7 April 2022

Accepted: 11 April 2022




First published online: 21 April 2022

### Key words:

Density-dependent transmission; detachment; gyrodactylids; reinfection

### Author for correspondence:

Wenxiang Li, E-mail: [liwx@ihb.ac.cn](mailto:liwx@ihb.ac.cn)

Xiao Jin<sup>1,2</sup> , Hong Zou<sup>1</sup>, Ming Li<sup>1</sup> , Shangong Wu<sup>1</sup>, Guitang Wang<sup>1</sup>, David J. Marcogliese<sup>3,4</sup> and Wenxiang Li<sup>1,2</sup> 

<sup>1</sup>Key Laboratory of Aquaculture Disease Control, Ministry of Agriculture, and State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, People's Republic of China; <sup>2</sup>University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China; <sup>3</sup>Aquatic Contaminants Research Division, Water Science and Technology Directorate, Science and Technology Branch, Environment and Climate Change Canada, St. Lawrence Centre, 105 McGill, 7th floor, Montreal, Quebec H2Y 2E7, Canada and <sup>4</sup>St. Andrews Biological Station, Fisheries and Oceans Canada, 125 Marine Science Drive, St. Andrews, New Brunswick E5B 0E4, Canada

### Abstract

Host density is a key regulatory factor in parasite transmission. The goldfish (*Carassius auratus*)-*Gyrodactylus kobayashii* model was used to investigate effects of host density on population growth of gyrodactylids. A donor fish infected by five gravid gyrodactylids was mixed with 11 parasite-free goldfish at five host densities. There was a significant positive correlation between host density and mean abundance of *G. kobayashii* throughout the 58-day experiment. During early infection (days 15–24), mean abundance in medium high (0.5 fish L<sup>-1</sup>) and high host density groups (1 and 2 fish L<sup>-1</sup>) was significantly higher than that in the low host density groups (0.125 and 0.25 fish L<sup>-1</sup>). At high host density, prevalence increased more rapidly, and the peak prevalence was higher. Fitting of an exponential growth model showed that the population growth rate of the parasite increased with host density. A hypothesis was proposed that higher host density contributed to increased reinfection of detached gyrodactylids. A reinfection experiment was designed to test this hypothesis. Both mean abundance and prevalence at a host density of 1 fish L<sup>-1</sup> were significantly higher than those at 0.25 fish L<sup>-1</sup> on days 1 and 3, which suggested that more reinfections of *G. kobayashii* occurred at the higher host density. Density-dependent transmission during the early infection was an important contributor of population growth of *G. kobayashii*, as well as density-dependent reinfection of the detached gyrodactylids.

## Introduction

Numerous host features affect parasite abundance, including body size, host behaviour, the density or size of the host population, and the host immune response (Poulin, 2007). Epidemiological models predict that density-dependent transmission has a principal influence on abundance of directly transmitted parasites (Anderson and May, 1979, 1991). Furthermore, parasite population dynamics have been related to host density among a number of helminths (Krkošek, 2010). Host density has been associated with abundance of directly transmitted strongylid nematodes both within and across mammalian species (Arneberg *et al.*, 1998) and the indirectly transmitted tapeworm (*Anoplocephala gigantea*) across 18 populations of black rhinoceros (*Diceros bicornis*) (see Stringer and Linklater, 2015). Yet, studies examining the relationship between host density and abundance of helminths are relatively rare (but see Krkošek (2010) for other examples), and theory does not predict a simple relationship between them, due to temporal variation that results in complex host–parasite dynamics (Arneberg *et al.*, 1998). Furthermore, the complexities of many helminth life cycles make them difficult to examine experimentally.

Ever since the pioneering work of Scott (1982), the guppy (*Poecilia reticulata*)-*Gyrodactylus* spp. (Monogenea) host–parasite system has been the subject of numerous investigations examining the effects of host factors on the population dynamics of a helminth parasite (Scott and Anderson, 1984; Scott, 1985a, 1985b; Harris, 1988, 1989; Johnson *et al.*, 2011; Richards *et al.*, 2012). Various other studies made use of different fish–gyrodactylid systems to examine different aspects of parasite population biology. The salmon (*Salmo salar*)-*Gyrodactylus salaris* host–parasite system was used to determine parameters of parasite population growth (Cable *et al.*, 2000). The three-spined stickleback (*Gasterosteus aculeatus*)-*Gyrodactylus gasterostei* system was used to describe the effects of detachment and feeding on embryo development and survival (Cable *et al.*, 2002b). In addition, the goldfish (*Carassius auratus*)-*Gyrodactylus kobayashii* was used to evaluate the effect of host population size on parasite transmission (Zhou *et al.*, 2017). The ease of manipulation and quantification of this fish–helminth system makes it ideal to experimentally examine questions related to the population

**Table 1.** Total mean abundance and mean prevalence of *Gyrodactylus kobayashii* on caudal fin of goldfish (*Carassius auratus*) at five densities (0.125, 0.25, 0.5, 1, and 2 fish L<sup>-1</sup>) maintained the same population size of 12 over the 58-day experiment

Host density (no. fish L <sup>-1</sup> )	N	TP	Mean prevalence (%)	Total mean abundance (±s.d.)
0.125	6	2941	59.3	3.0 ± 1.0
0.25	5	7125	55.0	8.7 ± 11.9
0.5	4	13 182	81.4	24.8 ± 14.0
1	6	39 284	78.0	41.8 ± 38.8
2	5	24 102	75.9	29.2 ± 9.5

N, number of replicates; TP, the total number of *G. kobayashii* examined on caudal fin of goldfish; s.d., standard deviation.

dynamics and transmission of parasites, especially under confined conditions such as aquaculture.

The viviparous reproduction, where gyrodactylids proliferate directly on the host without any free-living developmental stages or intermediate hosts, and short generation time can lead to rapid population growth of these parasites (Scott, 1982; Scott and Anderson, 1984). Indeed, the life history characteristics of *Gyrodactylus* spp., their overall pathogenicity, asexual and sexual reproduction (Harris, 1989; Cable and Harris, 2002), and the induction of acquired resistance by the host are more similar to microparasites (e.g., bacteria, protozoans) than they are to helminth macroparasites (Tadiri *et al.*, 2019), and Scott (1982) makes the point that gyrodactylids do not conveniently fit in either category. However, it is precisely these characteristics which render them a suitable model to examine disease dynamics in a helminth parasite.

Transmission of gyrodactylids mainly depends on contacts among fish hosts (Bakke *et al.*, 1992; Tadiri *et al.*, 2019). Attachment on the body surface of fish makes it feasible to monitor the presence of gyrodactylids in a non-lethal manner (Harris, 1988). Numerous studies have made use of the guppy-*Gyrodactylus* laboratory model to examine effects of parasite density, host immune response, challenge infections, population connectivity, parasite aggregation, host social behaviour, and pollution on parasite population dynamics (Scott, 1982, 1985a, 1985b; Scott and Anderson, 1984; Scott and Robinson, 1984; Gheorgiu *et al.*, 2006; Richards *et al.*, 2010; Johnson *et al.*, 2011; Tadiri *et al.*, 2018). Given the importance of host density in epidemiological models of parasite transmission (Anderson and May, 1979; McCallum *et al.*, 2001), another study demonstrated that population size of *Gyrodactylus turnbulli* increased with guppy density under laboratory conditions, although the occurrence of epidemics was independent of host density (Johnson *et al.*, 2011). However, other than this latter work, no study has directly addressed the effects of host density on population dynamics of a gyrodactylid on fish.

In addition to the most important transmission pathway by host–host contact, contact between hosts and detached gyrodactylids is also an important transmission route (Bakke *et al.*, 1992; Cable *et al.*, 2002a). Gyrodactylids may become detached from their hosts as a result of host immune response (Lester, 1972; Scott and Anderson, 1984), host death (Cable *et al.*, 2002a), or during the host–host contact and locomotion on the host (Bakke *et al.*, 2007).

*Gyrodactylus kobayashii* is the dominant species on fins, scales and gills of the goldfish (Li *et al.*, 2014). The parasite requires three to four weeks to reach maximum mean abundance in a host population under experimental conditions (Zhou *et al.*, 2017). Here, the goldfish-*G. kobayashii* model was used to investigate effects of host density on population growth of gyrodactylids in an experimental model system and the role of detached parasites in transmission. We predicted (1) that transmission of *G. kobayashii* to goldfish would be density-dependent, and (2)

that detached parasites could successfully infect fish in this model system.

## Materials and methods

### Preparation of fish and establishment of infection mode

Goldfish were collected from a fish farm in Wuhan, China, and stocked at density of 3 fish L<sup>-1</sup> in several 100 L aquaria for two weeks. To obtain gyrodactylid-free goldfish, all fish were treated in three consecutive baths of 1:10 000 formalin solution for 12 h at 48-h intervals. After 30 days of feeding, 10 treated goldfish were randomly selected, anaesthetized with 0.02% MS-222 (tricaine methane sulphonate) and examined with a dissecting microscope to confirm their gyrodactylid-free status. Specimens of *G. kobayashii*, which was identified as Li *et al.* (2014), then were inoculated onto the caudal fin of anaesthetized goldfish to initiate a stock source of parasites. To obtain more specimens of *G. kobayashii* for the experiment, some gyrodactylid-free goldfish were regularly introduced to the aquarium with infected goldfish (Zhou *et al.*, 2017).

### Effect of host density on infection

Gyrodactylid-free immature goldfish with a mean standard length 4.9 ± 0.4 cm were randomly assigned to five groups, each containing 12 fish to maintain the same population size. To obtain densities of 2, 1, 0.5, 0.25 and 0.125 fish L<sup>-1</sup> (Table 1), five different sizes of aquaria were used: 20 × 28 × 11 cm, 29 × 28 × 15 cm, 58 × 28 × 15 cm, 46 × 36 × 29 cm and 67 × 44 × 33 cm. If goldfish died during the experiment, the dead fish were left in the aquarium for 12 h, and then replaced with gyrodactylid-free goldfish (not included in the data analysis). A single goldfish in each aquarium was chosen as the primary fish (donor fish) for parasite inoculation. The donor fish was anaesthetized with 0.02% MS-222 and placed in a Petri dish. Using a dissecting microscope, each donor fish was inoculated with five gravid *G. kobayashii* on the caudal fin, and then returned to its original aquarium. Among the head, gills, body surface, pectoral fin, ventral fin, anal fin, dorsal fin and caudal fin of goldfish, the highest proportion with 37.0% was found on the caudal fin, and the proportion changed little during different infection periods (Zhou, 2018). To ensure consistent and repeatable parasite observation on the same fish and minimize handling, *G. kobayashii* was screened non-invasively only on caudal fins of goldfish. On each sampling day, each fish was examined for *G. kobayashii* using a dissecting microscope to minimize handling after anaesthetization with 0.02% MS-222. These fish subsequently were returned to their own aquarium for further parasite screening. Fish were maintained in dechlorinated tap water at 20 ± 1°C and 12 h light–dark cycle, and fed with commercial pellet feed at 2% of the estimated total fish biomass every day. The water was stirred by an

aeration device during the entire experiment to prevent sedimentation of food particles and provide oxygenation. Faeces and uneaten feed were removed regularly, and one-third of the water was changed every 3 days. The fish were examined every two days during the first month, and every six days during the second month. The experiment was run for 58 days.

### Fitting of the exponential model for population growth

Intrinsic population growth of gyrodactylids was determined on individual, isolated goldfish. More than 100 gyrodactylid-free goldfish were stocked at density of 3 fish  $L^{-1}$  under the same laboratory conditions as above to ensure availability of enough infected fish for the experiment. On day 0, each fish was anaesthetized and inoculated with five gravid *G. kobayashii* on the caudal fin, then stocked separately in a  $20 \times 12 \times 10$  cm tank containing 0.5 L dechlorinated water. To record all the gyrodactylids, *G. kobayashii* was examined on the gills, fins and body surface of each goldfish. 7–10 fish were killed after anaesthetization and gills were removed for parasitological examination each day. To reduce effects of the host immune response (Zhou *et al.* 2018), the experiment was completed on day 9. A total of 85 goldfish was used in the experiment.

An exponential-growth model ( $P_t = P_0 \times e^{rt}$ ) was used to fit the population growth of gyrodactylids, where  $P_t$  is the parasite population size on all goldfish in a tank on a sampling day,  $P_0$  is the initial population size and  $r$  is the population growth rate of gyrodactylids (Scott, 1982; Scott and Anderson, 1984). The data used for model fitting included both the parasite number on the caudal fin of the isolated goldfish over 9 days and the goldfish used in the host-density experiment. A previous study showed that mean abundance on goldfish declined between 21 and 28 days, indicating the immune response is causing the death rate to exceed the birth rate (Zhou *et al.*, 2018). Furthermore, given that mean abundance first peaked at 24 days before declining at a density of 0.5 fish  $L^{-1}$ , only the data from day 0 to day 24 in the host-density experiment were used in the analysis of population growth of *G. kobayashii* to reduce the effects of host resistance. Intrinsic population growth rate of *G. kobayashii* was also estimated by the exponential-growth model using the parasite numbers on the gills, body surface and fins of the isolated goldfish over 9 days. Data analysis was performed by MATLAB®.

### Reinfection of detached gyrodactylids

To test whether detached *G. kobayashii* could reattach on goldfish and if the reinfection was associated with host density, a cage ( $10 \times 10 \times 10$  cm) with a single goldfish infected with 20 gyrodactylids on the caudal fin was placed in the upper water of an aquarium ( $50 \times 36 \times 29$  cm) containing 11 gyrodactylid-free goldfish. The cage was covered with double plastic mesh (1 cm) to avoid contact between the goldfish inside and outside of the cage. Two densities (0.25 and 1 fish  $L^{-1}$ ) of goldfish were used by controlling water volume ( $46 \times 36 \times 29$  and  $11 \times 36 \times 29$  cm). The goldfish were maintained as described above. Parasites were counted on the caudal fin of each goldfish after anaesthetization, and each fish was returned to its own aquarium for subsequent parasitological examination later. The sampling was performed on days 1, 3, 5 and 7.

To determine effects of detachment time on survival and infection ability of *G. kobayashii*, gyrodactylids were detached from the caudal fin using a vortex instrument with 1000 r/min within 30 s. A preliminary experiment established that about 90% gyrodactylids maintain infectivity, which are higher than those collected by fine tweezers. A single gyrodactylid was put in a 6-well plate with dechlorinated water, and 150–300 worms were included in each

group. The parasites were examined when detached for 0 (control group), 3, 12, and 24 hours. Worms with visible body movement were considered to be alive and recorded. Then three living worms were inoculated onto the caudal fin of each goldfish with 12–14 replicates. If a gyrodactylid was detected on the gills and body surface of goldfish which was killed after anaesthetization on day 3, the living worm was considered to be infective. Then the percentages of survival and successful infection were calculated.

### Statistical analysis

Prevalence (the percentage of the goldfish population infected with *G. Kobayashii* excluding the donor fish) and mean abundance (the average number of *G. kobayashii* per fish, including uninfected fish) were calculated on each sampling day (Bush *et al.*, 1997). Mean prevalence and total mean abundance were also calculated throughout the 58-day experiment (average value of all the sampling days).

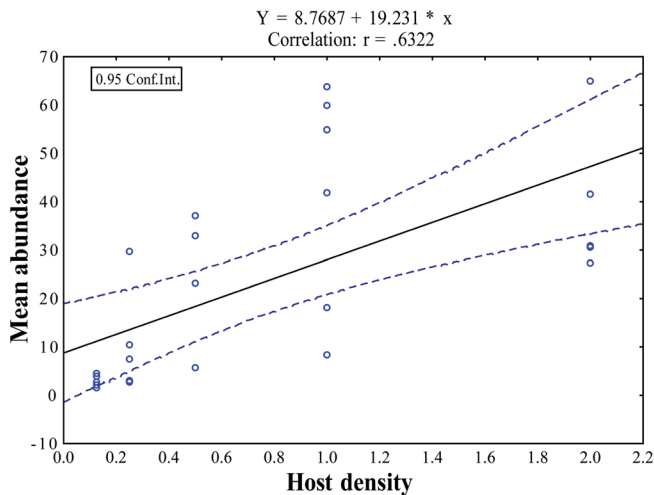
The relationship between host density and mean abundance of gyrodactylids was analysed using the Pearson correlation coefficient. Linear mixed-effects models (LMM) were used to analyse effects of host density and infection time on mean abundance of parasites (following log-transformation  $\log[x + 1]$ ) using the R package nlme (R Core Team, 2017). In all models, the aquarium was used as a random factor, and host density and infection time were the explanatory variables. The models were compared by the Akaike's information criterion (AIC) using the 'anova' function in nlme package and the model with the lowest AIC was deemed the best-fit model for the data. The  $P$  values for the variables in all models were also computed by the 'anova' function. Multiple comparisons were performed using least significant differences (LSD). Differences in prevalence were tested using the chi-square test among different host densities. In the detachment experiment, differences in mean abundance of *G. kobayashii* were assessed using the independent samples  $t$ -test between the two host densities. Statistical analysis was performed by the software Statistics 10.0 (StatSoft, Inc.). In all cases, the level of significance was set at  $P < 0.05$ .

## Results

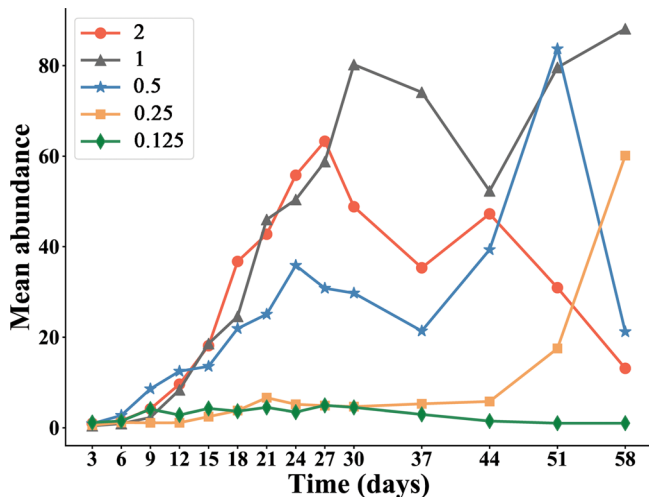
### Effect of host density on transmission

Throughout the experiment, two goldfish died in the 2 fish  $L^{-1}$  group (on days 21, 37) and one goldfish died in the 0.125 fish  $L^{-1}$  group (on day 24). Total mean abundance was 3.0, 8.7, 24.8, 41.8 and 29.2 at host densities of 0.125, 0.25, 0.5, 1 and 2 fish  $L^{-1}$ , respectively, over the 58-day experiment (Table 1). Total mean abundance in medium (0.5 fish  $L^{-1}$ ) and high-density groups (1 and 2 fish  $L^{-1}$ ) was significantly higher than that in low host density groups (0.125 and 0.25 fish  $L^{-1}$ ) ( $P < 0.05$ ). There was a significant positive correlation ( $n = 26$ ,  $R^2 = 0.3997$ ,  $P < 0.05$ ) between host density and mean abundance of *G. kobayashii* throughout the 58-day experiment (Fig. 1). The mean prevalence (75–81%) at high and medium-high host densities was higher than that (55–59%) at low densities throughout the 58-day experiment (Table 1).

At a host density of 0.125 fish  $L^{-1}$ , mean abundance was always low (<4.9). At 0.25 fish  $L^{-1}$ , mean abundance was low (<6.6), but increased rapidly after day 44. Mean abundance never reached a peak at the two lowest densities over the 58 days. In other density groups, mean abundance increased to a high level on days 24–30, after which it fluctuated within a high range (Fig. 2). During the first 24 days, there were significant differences in mean abundance among the five host densities (Table 2). On days 18–24, mean abundance at high and medium-



**Fig. 1.** Relationship between host density and mean abundance of *Gyrodactylus kobayashii* on all goldfish (*Carassius auratus*) in each treatment throughout the 58-day experiment. The correlation is significant.



**Fig. 2.** Changes in mean abundance of *Gyrodactylus kobayashii* over time on goldfish (*Carassius auratus*) at five host densities (0.125, 0.25, 0.5, 1, and 2 fish L<sup>-1</sup>).

high host densities (0.5–2 fish L<sup>-1</sup>) was significantly higher than that at low host densities (0.25 and 0.125 fish L<sup>-1</sup>) ( $P < 0.05$ ). At high and medium–high host densities, prevalence increased rapidly and peaked on days 12–24. The maximum prevalence was higher than 90%, and then stabilized at a high level. At low host densities, the maximum prevalence, which was observed on days 15–27, was lower than 90%, and then decreased to a medium level (Fig. 3).

**Exponential model fitting for population growth**

Population growth of gyrodactylids fitted well with the exponential model on the isolated goldfish and grouped goldfish at densities of 0.25, 0.5, 1 and 2 fish L<sup>-1</sup> (Fig. 4), but not at 0.125 fish L<sup>-1</sup> owing to the low goodness of fit (Table 3). Population growth rate of gyrodactylids increased from 0.042 to 0.235 (/parasite/day) with host density over 24 days (Table 3). Intrinsic population growth rate was 0.362 on the whole body and 0.260 on the caudal fin of individual, isolated fish over 9 days.

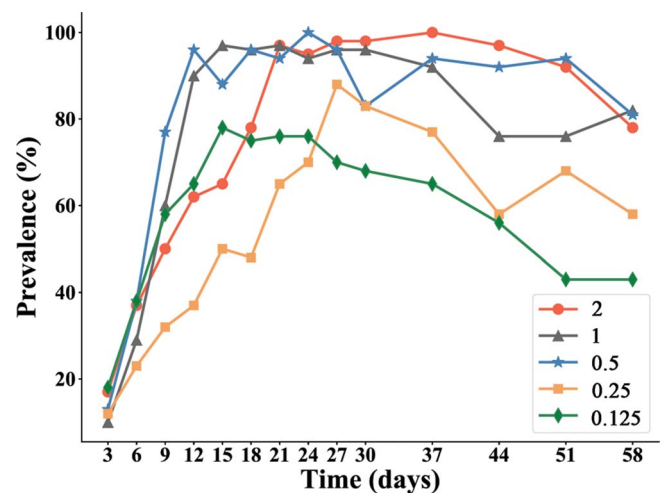
**Reinfection of detached gyrodactylids**

Following isolation of the donor fish in the cage, *G. kobayashii* was detected on the body surface of the other goldfish in the

**Table 2.** Summary of linear mixed-effects model outputs for the measures of mean abundance of *Gyrodactylus kobayashii* on caudal fin of goldfish (*Carassius auratus*) during the first 24 days

Model	df	AIC	logLik	P
Outcome : mean abundance				
Random effect (aquaria)	5	652.7	-312.3	-
Infection time	9	645.35	-237.6	<0.0001
Host density	12	499.3	-313.7	0.0056
Infection time × host density	44	419.4	-165.6	<0.0001

df, degree of freedom of the model; AIC, Akaike's information criterion; logLik, log-likelihood ratios.



**Fig. 3.** Changes in prevalence of *Gyrodactylus kobayashii* over time on goldfish (*Carassius auratus*) at five host densities (0.125, 0.25, 0.5, 1, and 2 fish L<sup>-1</sup>).

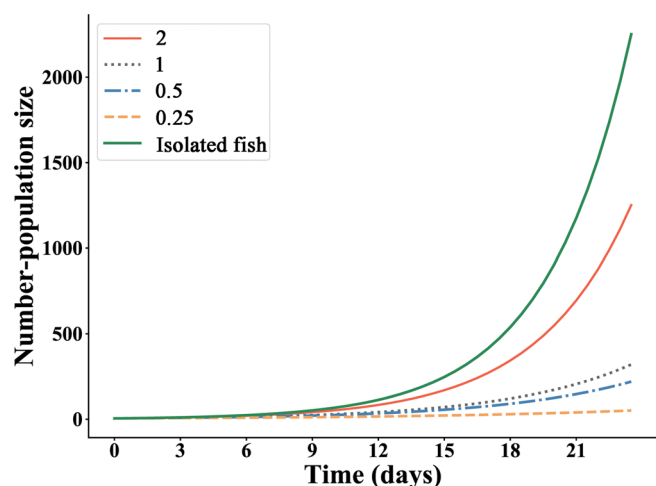
aquarium. On each sampling day, both mean abundance and prevalence at 1 fish L<sup>-1</sup> were significantly higher than those at 0.25 fish L<sup>-1</sup> ( $P < 0.05$ ) (Fig. 5).

When detached for 0 and 3 hours, more than 90% gyrodactylids were alive and 85–100% had infection capacity. After 12 and 24 hours of detachment from their hosts however, about 38% gyrodactylids were alive and only 15–20% were successfully infective (Fig. 6).

**Discussion**

Experimental results indicate that transmission of *G. kobayashii* on goldfish was density dependent at densities of 0.125–2 fish L<sup>-1</sup>. Population growth was exponential up to 24 days, and was also dependent on host density. Furthermore, experiments showed that transmission occurred *via* detached parasites, and this was greater at the higher density. Detached gyrodactylids maintained high survival and infection capacity within 3 h of detachment, indicating a limited window for reinfection of detached parasites.

A significant positive correlation was found between host density and mean abundance of *G. kobayashii* throughout the experiment, and mean abundance in high host density groups was significantly higher than that in low host density groups. These results suggested that population growth of *G. kobayashii* is dependent on host density under laboratory conditions. Under laboratory conditions, total population size of *G. turnbulli*



**Fig. 4.** Fitting of the exponential model for population growth of *Gyrodactylus kobayashii* on the caudal fin of the individual, isolated goldfish (*Carassius auratus*) over 9 days and all fish held in aquariums at four densities (0.25, 0.5, 1, and 2 fish  $L^{-1}$ ) over the first 24 days of the experiment.

**Table 3.** Population growth rate, estimated from fitting of the exponential model, of *Gyrodactylus kobayashii* on the isolated goldfish (*Carassius auratus*) before day 9 and those held at five densities before day 24.  $r$ , population growth rate;  $R^2$ , goodness of fit

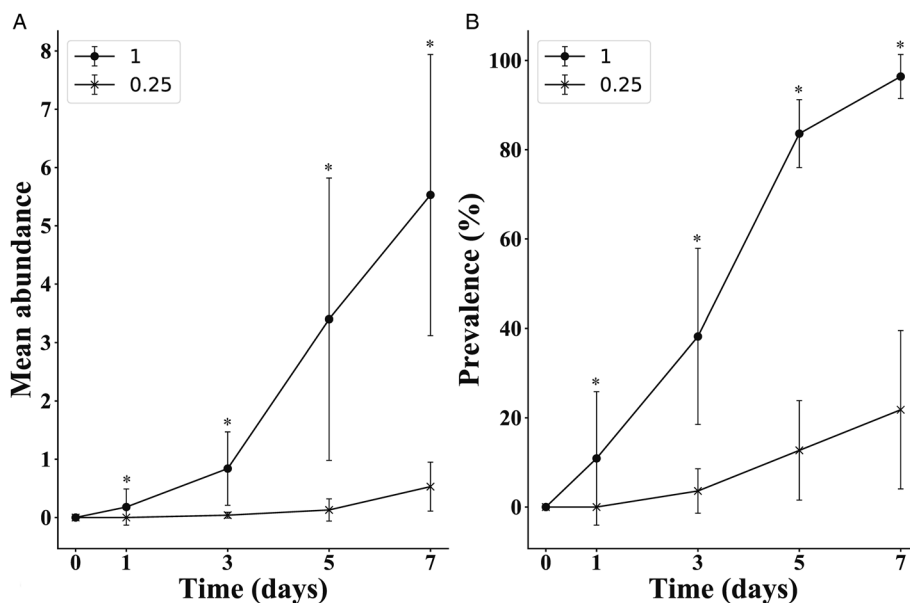
Host density (fish $L^{-1}$ )	$r$ (95% confidence)	$R^2$
0.125	0.042 (0.002–0.083)	0.548
0.25	0.099 (0.049–0.149)	0.848
0.5	0.161 (0.087–0.234)	0.853
1	0.177 (0.101–0.253)	0.912
2	0.235 (0.222–0.248)	0.999
Isolated fish		
On caudal fin	0.260 (0.207–0.313)	0.963
On gills and body surface	0.362 (0.310–0.414)	0.985

on guppies increased with host density at those ranging from 0.07 to 0.6 fish  $L^{-1}$  (Johnson *et al.*, 2011). Our study differed from the latter in that Johnson *et al.* (2011) found that the occurrence of epidemics in the guppy was independent of host density, and this was likely due to the mating behaviour of the guppy, compared to the goldfish. Population growth of parasites is closely related to transmission strategy. For trophically transmitted parasites and directly transmitted parasites with an infective larva stage, physical contacts between parasites and hosts determine transmission and population growth (Barlow, 1996). However, for the viviparous gyrodactylids, transmission occurs primarily *via* contacts between infected and susceptible hosts, followed by parasite reproduction directly on the host (Bakke *et al.*, 2007). Therefore contacts among hosts and the reproductive capacity, along with the survival of these parasites on their hosts, determine the population growth of gyrodactylids (Scott, 1982). Using the guppy-*Gyrodactylus* model, Tadiri *et al.* (2019) found that parasite population growth was one of the most important factors affecting the strength and severity of infection. Population growth rates observed in our study (0.235 at the highest density) were generally comparable to those for *G. turnbulli* on guppies at 27.5°C and *G. salaris* on salmon at 19°C, which ranged from 0.22 to 0.23 (Scott and Nokes, 1984; Jansen and Bakke, 1991). In field surveys, positive correlations were observed between

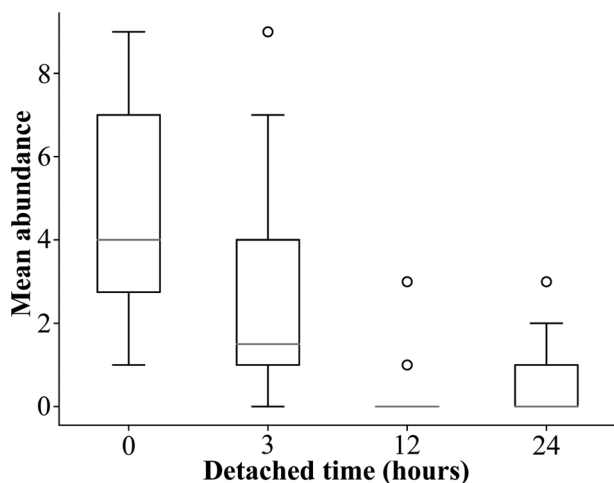
host population density and mean abundance of strongylid nematodes in 19 mammal species (Arneberg *et al.*, 1998), *A. gigantea* in black rhinoceros (Stringer and Linklater, 2015) and parasitic copepods (*Lepeophtheirus salmonis*) on Atlantic salmon (*S. salar*) (see Jansen *et al.*, 2012). The latter is of relevance to aquaculture, as are the results herein.

In the early days of infection, host density-dependent transmission was the main cause of population increase of *G. kobayashii*. Density-dependent transmission for directly transmitted pathogens has been supported by theoretical models (Anderson and May, 1979; Earn *et al.*, 2000; McCallum *et al.*, 2001; Smith *et al.*, 2009) and empirical evidence from field surveys (Begon *et al.*, 1998; Ramsey *et al.*, 2002; Stringer and Linklater, 2015). Generally, more contacts between parasites and hosts occur at higher density of hosts (Barlow, 1996). For pathogens transmitted by host contact, contact among host individuals is critical for transmission dynamics (McCallum *et al.*, 2001; Johnson *et al.*, 2011). For example, the contact rate between individual brushtail possum *Trichosurus vulpecula* during the breeding season decreased with reductions in host density (Ramsey *et al.*, 2002). At the same host density, effective contact rate among goldfish was inferred to be constant at different host population sizes (Zhou *et al.*, 2017). In the present study, the maximum prevalence was higher at high and medium-high host densities than that at low host densities, which suggested that host density-dependent transmission occurred during the first three to four weeks of the experiment. The increased contacts with density generally increase the number of new infected hosts (density-dependent transmission), but once established, gyrodactylids reproduce quickly and mean abundance increases further due to population growth (Scott, 1982; Scott and Nokes, 1984). The fact that prevalence never peaked at the lowest host densities and mean abundance remained low at the two lowest host densities for at least the first 45 days of the experiment also suggests that host-to-host transmission promotes population growth at higher densities. Overall, mean abundance will be density dependent as populations increase on each individual infected host, until immunity kicks in and parasite mortality increases (Scott, 1982, 1985a; Zhou *et al.*, 2017, 2018).

When the hosts become infected, population growth of gyrodactylids is generally determined by birth rate and death rate (Scott, 1982). Fecundity of gyrodactylids is dependent on water temperature (Scott and Nokes, 1984; Jansen and Bakke, 1991) and age structure of the parasite (Scott, 1982). Under the same laboratory conditions, birth and death rates are considered to be constant (Scott, 1982). However, accidental death may be variable with host density. Gyrodactylids may become detached from their hosts, which is common during host–host contact, locomotion on the host, or as a result of the host immune response or host death (Scott and Anderson, 1984; Bakke *et al.*, 1992). Furthermore, many parasites are lost during host-to-host transfer (Scott and Robinson, 1984). In the present study, the death rates of *G. kobayashii* were inferred to be variable with density based on the fitting of the population growth model. Compared with the intrinsic population growth rate ( $R = 0.260$ ) measured on the caudal fin of individual, isolated fish, the population growth rate ( $r$ ) declined from 0.235 to 0.042 with a decrease in host density (Table 3). Under the constant birth and death rate, accidental death rates ( $d = R - r$ ) were inferred to be 0.218, 0.161, 0.099, 0.083 and 0.025 at host densities of 0.125, 0.25, 0.5, 1 and 2 fish  $L^{-1}$ , respectively, which implies a high occurrence of accidental death at low host density during the early weeks of infection. Presumably, more parasites are lost during transfer, or more detached parasites cannot reinfect another host at low densities because contact rates are lower.



**Fig. 5.** Mean abundance (A) and prevalence (B) during reinfection of the detached *Gyrodactylus kobayashii* on goldfish (*Carassius auratus*) at host density of 0.25 and 1 fish L<sup>-1</sup> on days 1–7. The asterisk (\*) represents significant differences between the two host densities.



**Fig. 6.** Mean abundance of *Gyrodactylus kobayashii* on goldfish (*Carassius auratus*) inoculated with three gyrodactylids at four different detachment times on day 3 post-inoculation.

During the course of transmission, detachment has been observed in other experimental systems (e.g., Scott, 1985a). Large numbers of detached gyrodactylids have been observed in the water column and on the substrate (Scott and Anderson, 1984; Bakke et al., 1992). Detachment was also inferred from the reinfection experiment where uninfected goldfish became infected following addition of the donor fish, which was isolated from the other fish in a double-mesh cage, to the experimental aquarium. Furthermore, a large proportion of detached parasites was inferred based on the higher accidental death rate at low host density (see above).

Even though the proportion of detached *G. kobayashii* was not estimated in the reinfection experiment, reinfection was dependent on host density, even after one day. In addition to the most important direct transfer during host contact, there are three other potential transmission routes, such as contact between hosts and detached gyrodactylids on the substrate, contact between host and detached parasites in the water column, and contact between living hosts and infected dead hosts (Bakke et al., 1992). Up to 10% of transmission is estimated to occur via contact between host and detached parasites in the water

column in *G. gasterostei* (Harris, 1982). But for *G. salaris*, transmission routes such as indirect transmission from the substrate or direct contact transmission from infected live and/or dead fish (prevalence 44.4%, mean intensity 1.9), were relatively more important than transmission by drifting detached parasites (prevalence 6.4%, mean intensity 1.0) (Soleng et al., 1999). While we cannot compare the different routes of transmission of detached parasites, in our reinfection experiment mean abundance and prevalence at the higher host density were both significantly greater than those at low density, even after one day, prior to parasite reproduction.

Other species of gyrodactylids give birth to the first-generation offspring about one day after infection at 19–25°C (Scott, 1982; Jansen and Bakke, 1991). The next offspring are born on day 4 (Scott, 1982; Jansen and Bakke, 1991). Thus, an increase in infrapopulation numbers after day 1 could be attributed to reproduction, in addition to reattachment. Results herein also suggest that the reattached parasites were able to establish successful reproducing populations with a notable increase after three days at high density. In contrast, no reattachment was observed at low host density after one day and very little after three days, suggesting that reattachment is very limited at low host densities and probably relatively unimportant. This result implies that the contact rate of detached parasites and the recipient fish was greater at the higher density and extremely low at the lower density. The population growth trajectory of *G. kobayashii* that infected goldfish via detached worms at high density over seven days was comparable to that observed in the density experiment over the same time span at 20°C. Thus, colonization of gyrodactylid through reattachment is capable of leading to vigorous infrapopulations, and could be a significant contributor to parasite spread, at least at high densities.

The movement of detached *G. salaris* and subsequent establishment of infection in a new host population was restricted by survival time (Olstad et al., 2006). Similarly, infection ability of the detached *G. kobayashii* was maintained at a relatively high level only within the first 3 hours in our experiment. Thus, detached *G. kobayashii* have a narrow window to reattach to a new host. Scott (1985a) noted that detached *G. turnbulli* survived only a mean of 11.6 h. Only one of 14 detached *G. salaris* could infect Atlantic salmon after 24 h (Olstad et al., 2006). With such a limited window for reattachment, these results together with those from the reattachment experiment reinforce

the notion that reattachment may be significant only at high densities.

In summary, density-dependent population growth of *G. kobayashii* was confirmed under laboratory conditions. Both transmission to new hosts and population growth on individual hosts were density-dependent in the early weeks of infection. Reinfection of the detached gyrodactylids also was observed, but infections were negligible at low density after 3 days compared to high host density. Infectivity of detached gyrodactylids decreased greatly after 3 h. Gyrodactylid colonization through reattachment is capable of leading to vigorous infrapopulations, and could be an important contributor to parasite spread at high host densities.

**Acknowledgements.** The authors would like to thank the editor and the anonymous reviewer for their valuable suggestions and the time they have invested into reviewing the manuscript.

**Authors' contributions.** XJ performed the laboratory work, statistical analyses and wrote the article. WL designed the study and wrote the article. DJM revised the manuscript and interpreted the findings. HZ, ML, SW and GW contributed to the interpretation of the findings.

**Financial support.** This work was funded by the National Key Research and Development Program of China (Grant No. 2020YFD0900300), the National Natural Science Foundation of China (No. 31872604), and China Agriculture Research System of MOF and MARA.

**Conflicts of interest.** The authors declare there are no conflicts of interest.

**Ethical standards.** Fish feeding and anaesthetization were in accordance with the recommended guidelines for animal experimentation by the Chinese Association for Laboratory Animal Sciences.

## References

- Anderson RM and May RM (1979) Population biology of infectious diseases: Part I. *Nature* **280**, 361–367.
- Anderson MR and May RM (1991) *Infectious Diseases of Humans: Dynamics and Control*. New York: Oxford University Press.
- Arneberg P, Skorping A, Grenfell B and Read AF (1998) Host densities as determinants of abundance in parasite communities. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **265**, 1283–1289.
- Bakke TA, Harris PD, Jansen P and Hansen LP (1992) Host specificity and dispersal strategy in gyrodactylid monogeneans, with particular reference to *Gyrodactylus salaris* (Platyhelminthes, Monogenea). *Diseases of Aquatic Organisms* **13**, 63–74.
- Bakke TA, Cable J and Harris PD (2007) The biology of gyrodactylid monogeneans: the “Russian-doll killers”. *Advances in Parasitology* **64**, 161–460.
- Barlow ND (1996) The ecology of wildlife disease control: simple models revisited. *Journal of Applied Ecology* **33**, 303–314.
- Begon M, Feore SM, Brown K, Chantrey J, Jones T and Bennett M (1998) Population and transmission dynamics of cowpox in bank voles: testing fundamental assumptions. *Ecology Letters* **1**, 82–86.
- Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis, *et al.* revisited. *Journal of Parasitology* **83**, 575–583.
- Cable J and Harris PD (2002) Gyrodactylid developmental biology: historical review, current status and future trends. *International Journal for Parasitology* **32**, 255–280.
- Cable J, Harris PD and Bakke TA (2000) Population growth of *Gyrodactylus salaris* (Monogenea) on Norwegian and Baltic Atlantic salmon (*Salmo salar*) stocks. *Parasitology* **121**, 621–629.
- Cable J, Scott ECG, Tinsley RC and Harris PD (2002a) Behavior favoring transmission in the viviparous monogenean *Gyrodactylus turnbulli*. *Journal of Parasitology* **88**, 183–184.
- Cable J, Tinsley RC and Harris PD (2002b) Survival, feeding and embryo development of *Gyrodactylus gasterostei* (Monogenea: Gyrodactylidae). *Parasitology* **124**, 53–68.
- Earn DJD, Rohani P, Bolker BM and Grenfell BT (2000) A simple model for complex dynamical transitions in epidemics. *Science* **287**, 667–670.
- Gheorgiu C, Marcogliese DJ and Scott M (2006) Concentration-dependent effects of waterborne zinc on population dynamics of *Gyrodactylus turnbulli* (Monogenea) on isolated guppies (*Poecilia reticulata*). *Parasitology* **132**, 225–232.
- Harris PD (1982) *Studies of the Gyrodactyloldea (Monogenea)* (PhD). University of London.
- Harris PD (1988) Changes in the site specificity of *Gyrodactylus turnbulli* Harris, 1986 (Monogenea) during infections of individual guppies (*Poecilia reticulata* Peters, 1859). *Canadian Journal of Zoology* **66**, 2854–2857.
- Harris PD (1989) Interactions between population growth and sexual reproduction in the viviparous monogenean *Gyrodactylus turnbulli* Harris, 1986 from the guppy, *Poecilia reticulata* Peters. *Parasitology* **98**, 245–251.
- Jansen PA and Bakke TA (1991) Temperature-dependent reproduction and survival of *Gyrodactylus salaris* Malmberg, 1957 (Platyhelminthes: Monogenea) on Atlantic salmon (*Salmo salar*). *Parasitology* **102**, 105–112.
- Jansen PA, Kristoffersen AB, Viljugrein H, Jimenez D, Aldrin M and Stien A (2012) Sea lice as a density-dependent constraint to salmonid farming. *Proceedings of the Royal Society B-Biological Sciences* **279**, 2330–2338.
- Johnson MB, Lafferty KD, Van Oosterhout C and Cable J (2011) Parasite transmission in social interacting hosts: monogenean epidemics in guppies. *PLoS ONE* **6**, e22634.
- Krkošek M (2010) Host density thresholds and disease control for fisheries and aquaculture. *Aquaculture Environment Interactions* **1**, 21–32.
- Lester RJG (1972) Attachment of *Gyrodactylus* to *Gasterosteus* and host response. *Journal of Parasitology* **58**, 717–722.
- Li RR, Li WX, Wu XD and Wang GT (2014) Identification of *Gyrodactylus* species in goldfish (*Carassius auratus*) through morphological study and the analysis of the rDNA ITS sequence. *Acta Hydrobiologica Sinica* **38**, 903–909.
- McCallum H, Barlow N and Hone J (2001) How should pathogen transmission be modelled? *Trends in Ecology & Evolution* **16**, 295–300.
- Olstad K, Cable J, Robertsen G and Bakke TA (2006) Unpredicted transmission strategy of *Gyrodactylus salaris* (Monogenea: Gyrodactylidae): survival and infectivity of parasites on dead hosts. *Parasitology* **133**, 33–41.
- Poulin R (2007) *Evolutionary Ecology of Parasites*, 2nd Edn. Princeton: Princeton University Press.
- R Core Team (2017) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Ramsey D, Spencer N, Caley P, Efford M, Hansen K, Lam M and Cooper D (2002) The effects of reducing population density on contact rates between brushtail possums: implications for transmission of bovine tuberculosis. *Journal of Applied Ecology* **39**, 806–818.
- Richards EL, van Oosterhout C and Cable J (2010) Sex-specific differences in shoaling affect the rate of parasite transmission in guppies. *PLoS ONE* **5**, e13285.
- Richards EL, van Oosterhout C and Cable J (2012) Interactions between male guppies facilitates the transmission of the monogenean ectoparasite *Gyrodactylus turnbulli*. *Experimental Parasitology* **132**, 483–486.
- Scott ME (1982) Reproductive potential of *Gyrodactylus bullatarudis* (Monogenea) on guppies (*Poecilia reticulata*). *Parasitology* **85**, 217–236.
- Scott ME (1985a) Dynamics of challenge infections of *Gyrodactylus bullatarudis* Turnbull (Monogenea) on guppies, *Poecilia reticulata* (Peters). *Journal of Fish Diseases* **8**, 495–503.
- Scott ME (1985b) Experimental epidemiology of *Gyrodactylus bullatarudis* (Monogenea) on guppies (*Poecilia reticulata*): short- and long-term studies. In Rollinson D and Anderson RM (eds), *Ecology and Genetics of Host-Parasite Interactions*. New York: Academic Press, pp. 21–38.
- Scott ME and Anderson RM (1984) The population dynamics of *Gyrodactylus bullatarudis* (Monogenea) within laboratory populations of the fish host *Poecilia reticulata*. *Parasitology* **89**, 159–194.
- Scott ME and Nokes DJ (1984) Temperature-dependent reproduction and survival of *Gyrodactylus bullatarudis* (Monogenea) on guppies (*Poecilia reticulata*). *Parasitology* **89**, 221–228.
- Scott ME and Robinson MA (1984) Challenge infections of *Gyrodactylus bullatarudis* (Monogenea) on guppies, *Poecilia reticulata* (Peters), following treatment. *Journal of Fish Biology* **24**, 581–586.
- Smith KF, Acevedo-Whitehouse K and Pedersen A (2009) The role of infectious diseases in biological conservation. *Animal Conservation* **12**, 1–12.

- Soleng A, Jansen PA and Bakke TA** (1999) Transmission of the monogenean *Gyrodactylus salaris*. *Folia Parasitologica* **46**, 179–184.
- Stringer AP and Linklater WL** (2015) Host density drives macroparasite abundance across populations of a critically endangered megaherbivore. *Oecologia* **179**, 201–207.
- Tadiri CP, Scott ME and Fussmann GF** (2018) Microparasite dispersal in metapopulations: a boon or bane to the host population? *Proceedings of the Royal Society B-Biological Sciences* **285**, 20181519.
- Tadiri CP, Kong JD, Fussmann GF, Scott ME and Wang H** (2019) A data-validated host-parasite model for infectious disease outbreaks. *Frontiers in Ecology and Evolution* **7**, 307.
- Zhou S** (2018) *Ecology of Gyrodactylus kobayashii on goldfish (Carassius auratus) and the screening of anthelmintic drugs* (PhD). University of Chinese Academy of Sciences.
- Zhou S, Zou H, Wu SG, Wang GT, Marcogliese DJ and Li WX** (2017) Effects of goldfish (*Carassius auratus*) population size and body condition on the transmission of *Gyrodactylus kobayashii* (Monogenea). *Parasitology* **144**, 1221–1228.
- Zhou S, Li WX, Zou H, Zhang J, Wu SG, Li M and Wang GT** (2018) Expression analysis of immune genes in goldfish (*Carassius auratus*) infected with the monogenean parasite *Gyrodactylus kobayashii*. *Fish and Shellfish Immunology* **77**, 40–45.