

# Ecological study of *Carassotrema schistorchis* in wild silver carp, *Hypophthalmichthys molitrix*

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## ABSTRACT

*Carassotrema schistorchis* is a digenea parasite whose juvenile and adult stages parasitize the intestinal tract of definitive host fish. There is no available ecological information about this parasitic infection in wild host fish. The present work aimed to investigate the infection rate and distribution of *C. schistorchis* in wild silver carp, *Hypophthalmichthys molitrix*, and to assess the influence of environmental factors on infection by the parasite. From October 2016 to May 2019, a total of 554 wild silver carp were collected from two reservoirs. These collected fish were weighed, measured, and dissected to observe the presence of *C. schistorchis* following standard methodology. Ecological analysis results showed that the frequency distribution of *C. schistorchis* in the host population was aggregated distribution. The abundance of *C. schistorchis* exhibited a positive correlation with the host age and condition factor of silver carp, but it showed no relationship with water temperature, pH or DO. The condition factor value of infected fish was significantly higher than that of uninfected fish. These results indicated that food intake, not environmental factors, directly determined infection by the parasite in the host, indicating that an increased food intake would increase the risk of infection. Thus, the number of parasites could be used as an indirect biological marker for assessing the effectiveness of silver carp in filter-feeding on plankton. The following analysis indicated that silver carp with a body length of 50–55 cm showed the highest efficiency in controlling phytoplankton biomass. This study provides important information for understanding the ecology of *C. schistorchis* in wild silver carp.

## 1. Introduction

*Carassotrema schistorchis*, a parasitic helminth, was first reported by Wang and Pan (1984). The parasite is mainly distributed in the intestinal tracts of silver carp, *Hypophthalmichthys molitrix*, bighead carp, *Aristichthys nobilis*, and grass carp, *Ctenopharyngodon idellus*. A previous study indicated that the life cycle of members of genus *Carassotrema* consists of seven stages: egg, miracidium, rediae, cercariae, metacercariae, juvenile parasite, and adult parasite (Tang and Lin, 1979). The eggs are eaten by the first intermediate host, *Stenothyra toucheana*, and successively develop into rediae and cercariae in the body of the intermediate host. Cercariae are released from the intermediate host into water and adhere to phytoplankton or other tender plants on the bottom of the water body, where they become metacercariae. When fish feed on these phytoplankton, the metacercariae infect the definitive host and

successively develop into juvenile and adult stages. The mature adult parasites produce eggs in the intestinal tract of the host, and the eggs are excreted from the intestinal tract into water to start a new life cycle (Tang, 2005). Observations of the life cycle indicate that *Carassotrema* has only one intermediate host, an aquatic snail, and one definitive host, a planktivorous fish.

Silver carp is one of the most popular planktivorous fish consumed by humans (Xie, 2003). A number of studies have demonstrated that these fish can reduce phytoplankton biomass, control nuisance blooms, and promote nutrient regeneration (Hambright et al., 2002; Mátyás et al., 2003; Xie and Liu, 2001). Thus, silver carp has been introduced into reservoirs and lakes worldwide due to its excellent performance in controlling phytoplankton biomass and promoting ecosystem health (Xie, 2003; Zhang et al., 2019). The Shenzhen Reservoir (SR) and Yantian Reservoir (YR) are two important reservoirs that supply drinking

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water to Shenzhen and Hong Kong. The catchment areas of SR and YR are 60.5 and 18.9 km<sup>2</sup>, respectively. To prevent eutrophication and improve water quality, filter-feeding silver carp were introduced into the two reservoirs. Our preliminary investigation showed that *C. schistorchis* was the only digenean parasite infecting the intestinal tract of silver carp in SR and YR.

The present study aimed to investigate the relationship between the parasite and silver carp, the dynamic changes in the parasite, and the responses of the parasite to environmental factors. Therefore, this work provides important information on host-parasite interactions to understand the ecology of *C. schistorchis* in silver carp.

## 2. Materials and methods

### 2.1. Sampling of silver carp and *C. schistorchis*

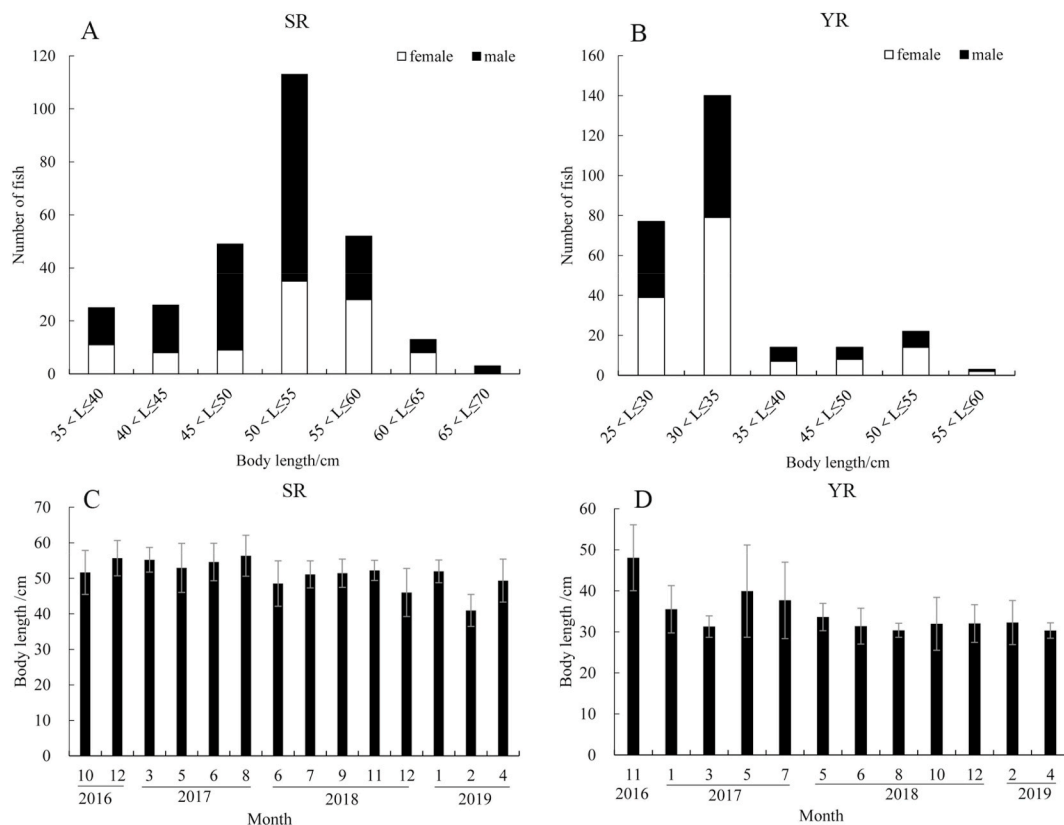
This study was conducted in SR (N22°34'44.05" E114°09'1.87") and YR (N22°39'46.60" E114°10'0.71") in Guangdong Province, China. At least 20 silver carp were collected with a fishing net in each sampling month from October 2016 to May 2019. Temperature, dissolved oxygen (DO), and pH in the two reservoirs were measured using YSI (6600). The collected fish were maintained in several 100 L tanks before parasite collection, and 50 L of water from the reservoir of origin was added to each tank, which was equipped with an automatic aerator to supply oxygen.

Silver carp were anesthetized with tricaine methanesulfonate (MS-222) at a concentration of 150 mg/L. Then, the sex of the host was determined by the presence of the nuptial tubercle on the surface of the first fin ray of the pectoral fin. Male fish exhibit a nuptial tubercle, which causes the first fin ray to feel rough when touched with the fingers. In contrast, female fish do not have any nuptial tubercles, and the first fin ray therefore feels smooth. Next, the body length and body weight of the

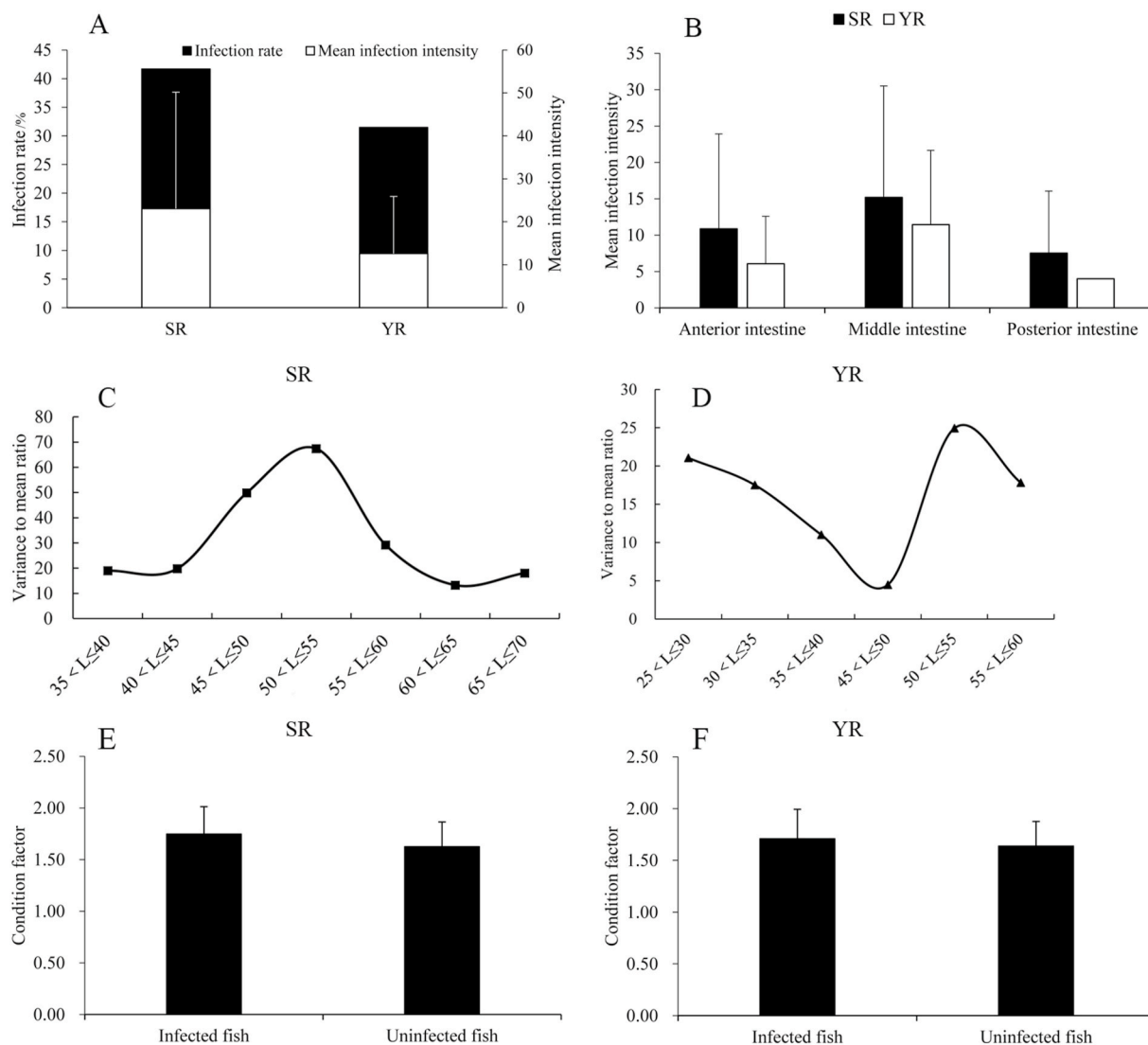
fish were measured. The age of the fish was determined with the growth ring on scales. The intestinal tract was divided into three parts for the examination of parasites. *Carassotrema schistorchis* was collected according to Pan and Zhang (1990). The collected parasites were washed with normal saline, preserved in 75% ethanol, and transported to the Department of Ecology, Jinan University, Guangzhou, China. The parasites were stained with hematoxylin, dehydrated through a graded ethanol series, cleared with xylene, and sealed with neutral gum. The morphological characteristics of the parasites were analyzed using a microscope (Nikon Ti inverted microscope). The morphological identification of the samples followed the previous literature (Tang, 2005; Wang and Pan, 1984).

### 2.2. Statistical analyses

The infection rate, mean infection intensity, and mean abundance of the parasite were determined following Bush et al. (1997). (1) The condition factor was calculated using the formula  $k = (W/L^3) \times 100$ , where  $k$  is the condition factor,  $W$  is body weight, and  $L$  is body length (Jones et al., 1999). (2) The dispersion of the parasite in the fish was determined on the basis of the variance-to-mean ratio; Parameter  $k$  of negative binomial distribution was calculated using a maximum likelihood estimate method, and the fitting of negative binomial distribution was tested using chi-square. (3) The correlations between abundance and host age, the host condition factor, and environmental factors were analyzed via Spearman's rank correlation analysis. (4) The mean body length among different months was analyzed using One-way ANOVA; The infection rate among different months was tested using the chi-squared test; The mean infection intensity among intestinal sections was analyzed using k-independent samples nonparametric tests. (5) The mean abundance and mean infection intensity among different months were analyzed through covariance analysis with the body length of the



**Fig. 1.** Distribution of silver carp body length (A, B) and the mean body length of the fish in each sample month (C, D) in the two reservoirs. SR: Shenzhen Reservoir; YR: Yantian Reservoir.



**Fig. 2.** Mean infection intensity (A, B) and variance-to-mean ratio (C, D) of *Carassotrema schistorchis* in the host as well as the influence of the parasite on the host condition factor (E, F). A: Mean infection intensity and infection rate of the parasite in the two reservoirs; B: Distribution of the parasite in different parts of the intestine; C, D: Variance-to-mean ratio of *Carassotrema schistorchis* in silver carp; E, F: Condition factor of infected and uninfected fish. SR: Shenzhen Reservoir; YR: Yantian Reservoir.

fish as the covariable. Assumptions of normality and homoscedasticity were checked with Shapiro-Wilk's and Levene's tests, respectively. The results were considered statistically significant at  $p < 0.05$ . All data were analyzed in Excel (2016) (Microsoft Corporation) and SPSS Statistics 24 (International Business Machines Corporation).

### 3. Results

#### 3.1. Hosts

A total of 554 silver carp were collected from the two reservoirs, including 281 from SR and 273 from YR. The body lengths of the silver carp in the two reservoirs ranged from 35 cm to 70 cm for SR and 25–60 cm for YR, with average body lengths of  $51.31 \pm 6.28$  and  $34.84 \pm 3.89$  cm, respectively (Fig. 1 A, B). The average body lengths of the fish in the two reservoirs differed significantly among sampling months (SR:  $F = 13.54$ ,  $p < 0.05$ ; YR:  $F = 13.40$ ,  $p < 0.05$ ) (Fig. 1 C, D).

#### 3.2. Intensity and frequency distribution of *C. schistorchis* in the hosts

The total mean infection intensity of *C. schistorchis* in SR and YR was

23.1 and 12.6, respectively (Fig. 2 A), with the infection rate of 41.7% and 31.5%, respectively. The mean infection intensity of *C. schistorchis* in the anterior intestine and middle intestine was significantly higher than that in the posterior intestine (Fig. 2 B;  $p < 0.05$ ). The mean infection intensity of *C. schistorchis* in the anterior, middle, and posterior intestine were 10.9, 15.2, and 7.5, respectively, in SR, whereas they were 6.1, 11.4, and 4 in YR, respectively (Fig. 2 B).

The variance to mean ratios were fitted to aggregated distribution, but the frequency distribution of the parasite in host population failed to be described with the negative binomial distribution (SR:  $k = 0.18$ ,  $\chi^2 = 46.78 < \chi^2_{0.05[3]} = 48.305$ ,  $p < 0.05$ ; YR:  $k = 0.126$ ,  $\chi^2 = 11.226 < \chi^2_{0.05[3]} = 11.591$ ,  $p < 0.05$ ). The highest variance-to-mean ratio values were observed in hosts with body lengths of 50–55 cm in both reservoirs, and the typical convex curve was observed in SR, but not in YR (Fig. 2 C, D).

#### 3.3. Influence of the parasite on the host condition factor

The condition factors of infected and uninfected silver carp were 1.99 and 1.86, respectively, in SR, whereas they were 1.93 and 1.83 in YR (Fig. 2 E, F). The condition factor of infected fish was significantly

**Table 1**

Correlation between the abundance of *Carassotrema schistorchis* and environmental factors, host age, and the host condition factor in the two reservoirs.

Shenzhen Reservoir					
	HA	CF	temp	pH	DO
n	281	281	14	14	14
r	0.234	0.143	−0.061	−0.162	−0.113
p	0.025	0.016	0.83	0.564	0.689
Yantian Reservoir					
	HA	CF	temp	pH	DO
n	273	273	12	12	12
r	0.289	0.173	−0.095	0.457	0.417
p	0.000	0.004	0.747	0.1	0.138

HA: host age; CF: condition factor; temp: temperature; DO: dissolved oxygen; n: number of samples, 281 and 273 represent number of samples of host age and condition factor; 14 and 12 represent number of samples of the temperature, pH, and dissolved oxygen from each sample month; The total number of *C. schistorchis* of 281 fish in SR were 2722, and the total number of *C. schistorchis* of 273 fish in YR were 1071; r: correlation coefficient; Differences were considered significant at  $p < 0.05$  and  $p < 0.01$ .

higher than that of uninfected fish in both reservoirs ( $p < 0.05$  for SR;  $p < 0.01$  for YR).

### 3.4. Parasite abundance correlations

There was a significant positive correlation between host age and the abundance of *C. schistorchis* in both SR ( $p < 0.05$ ) and YR ( $p < 0.01$ ) (Table 1). The condition factor showed a significant positive correlation with the abundance of the parasite in the two reservoirs (SR:  $p < 0.01$ ; YR:  $p < 0.01$ ) (Table 1). However, there were no statistically significant differences between the abundance of *C. schistorchis* and the environmental factors in the two reservoirs (all  $p > 0.05$ ).

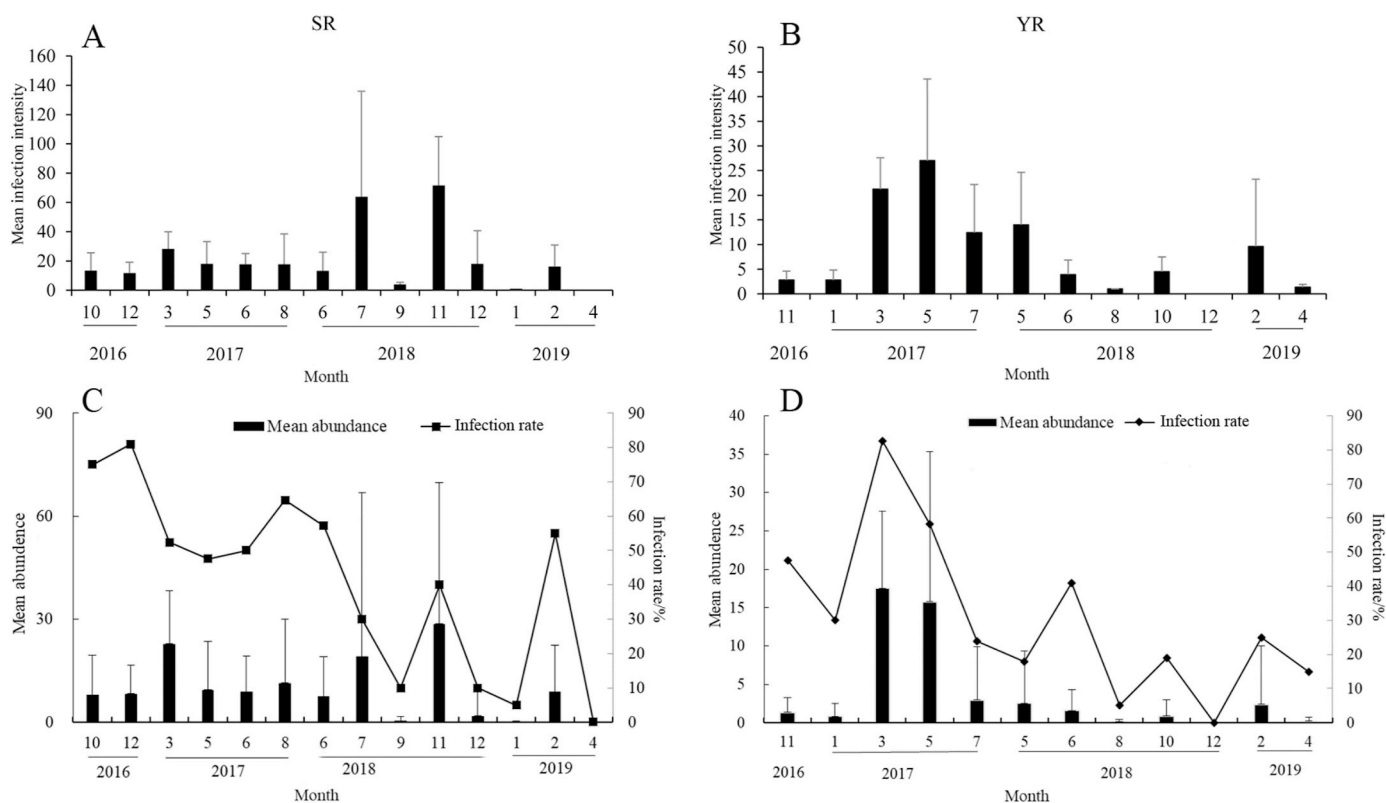
### 3.5. Seasonal dynamics of *C. schistorchis*

The infection intensity (Fig. 3 A, B), infection rate (Fig. 3 C, D), and the mean abundance (Fig. 3 C, D) of *C. schistorchis* in SR and YR showed significant differences among the sampling months (all  $p < 0.05$ ). The infection rate in 2016 and 2017 was higher than that in 2018 and 2019 in SR, but the highest mean abundance was observed in July and November 2018 (Fig. 3 C). In addition, the mean abundance and the infection rate of *C. schistorchis* in March and May 2017 were significantly higher than those in the other months in YR (all  $p < 0.05$ ) (Fig. 3 D).

## 4. Discussion

Abiotic factors such as water temperature, rainfall, pH, and salinity and biotic factors such as the age, sex, behavior, and mortality of the host may exert a strong influence on the prevalence and abundance of digenetic trematodes (Allen, 2011; Smith and Elizabeth, 2013). Among these factors, water temperature is the key abiotic factor controlling parasite dynamics in aquatic systems (Karvonen et al., 2013). Changes in water temperature may directly influence the rates of parasite establishment and development, the release of infective stages, and transmission between hosts (Karvonen et al., 2013). pH and DO are also related to the prevalence of aquatic parasites (Alsarakibi et al., 2014). In the present study, the abundance of *C. schistorchis* showed no correlation with water temperature, pH, or DO. This may be because SR and YR are located in the tropics, and the water temperatures in the two reservoirs are higher than 17 °C year round, which provides suitable conditions in which parasites can live and reproduce. The pH and DO levels in the two reservoirs showed no significant changes among the sampling months. Thus, the relatively stable environmental characteristics of the two reservoirs were suitable for *C. schistorchis* to complete life cycle, meaning that water temperature, pH, or DO had no significant influence on *C. schistorchis* infection in the two reservoirs.

Condition factor, a length–weight model, is sensitive to small



**Fig. 3.** Seasonal dynamics of *Carassotrema schistorchis* in silver carp in two reservoirs. SR (A, C): Shenzhen Reservoir; YR (B, D): Yantian Reservoir.

changes in condition and used to predict growth and to assess nutritional status of fish (Jones et al., 1999). In previous studies, parasites infection decreased the condition factor of host (Xia et al., 1999; Zhang, 1993). For example, the infection of *Dactylogyrus vaginulatus* had negative effect on the condition factor of silver carp (Xia et al., 1999); the infection of *Camallanus cotti* significantly decreased the condition factor of host *Mystus macropterus* (Zhang, 1993). However, in this study, the condition factor of infected silver carp was significantly higher than that of uninfected fish, the phenomenon was different with previous studies. The further correlation analysis demonstrated that the abundance of *C. schistorchis* exhibited a strong correlation with the silver carp condition factor, suggesting that the fish condition factor was proportional to abundance of *C. schistorchis*. When two hosts present the same body length, the host with a greater body weight often exhibits better physiological and nutritional status as well as a higher condition factor value (Jones et al., 1999), meaning that the host with a high condition factor requires more food to satisfy its metabolic demands. It has been suggested that the host feeding habit determines endoparasite abundance, as hosts acquire these parasites mainly by ingesting food (Karling et al., 2012). Thus, the prevalence and abundance of *C. schistorchis* in silver carp are related to the fish diet. After the cercariae of *C. schistorchis* are released from the intermediate host snail into water, they adhere to phytoplankton or other tender plants at the bottom of the water body, where they become metacercariae (Tang, 2005). Metacercariae can infect silver carp when the fish filter these phytoplankton. We examined the intestinal tract contents of silver carp and found that algae, sediment, and plant fragments were food items in the diet of the fish. Therefore, the life cycle feature that the metacercariae of *C. schistorchis* adhere to the food of silver carp caused a high infection intensity of the parasite in the host with large food intake.

To prevent eutrophication and improve the water quality of reservoirs and lakes, juvenile silver carp are stocked with appropriate densities, and old individuals are caught by netting (Xie and Liu, 2001). Nutrients accumulate in the fish through the food chain and are removed from the water with the removal of fish (Xie, 2003). As mentioned above, *C. schistorchis* infects silver carp when the fish digest the plankton. The greater the food intake of the fish, the higher parasite abundance is. Thus, the number of parasites could be used as an indirect biological marker for assessing the effectiveness of silver carp filter-feeding on plankton. The variance-to-mean ratio of *C. schistorchis* among different host body lengths showed that silver carp with body lengths of 50–55 cm presented the highest parasite levels in both reservoirs, meaning that the degree of the aggregation of *C. schistorchis* in these fish was high. This result suggested that silver carp with a body length of 50–55 cm showed the highest efficiency of feeding on plankton.

In conclusion, the present work investigated the ecology of *C. schistorchis* in silver carp and clarified the distribution of the parasite in the host. The abundance of *C. schistorchis* was mainly determined by the food intake of the host, not by environmental factors. The host with a body length of 50–55 cm demonstrated the highest efficiency in decreasing phytoplankton biomass. This study provides important information for understanding the ecology of *C. schistorchis* in wild definitive hosts.

## Ethics approval

Usage of silver carp was approved by the Jinan University Laboratory Animal Ethics Committee (20160901–01). The Guide for the Care and Use of Laboratory Animals and the National Standards of Laboratory Animals of the People's Republic of China were followed by the authors.

## Declaration of competing interest

The authors declare that they have no competing interests.

## Acknowledgments

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