

## A comparative study of ectoparasites occurrence between grass carp and silver carp in guilan province culture ponds, Iran

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Parasitic infection is among the most common problems for carp cultivation. They are also important for the principal entrance of other hazardous infections as well. This study was carried out for determining of parasitic fauna of two major carp known as silver and grass carp with the comparison of prevalence value and intensity rate of parasites among them, alongside the relationship between the biometric characteristics and host sex with the infection level. For this purpose, a total of 94 fish samples were caught randomly using a fishing net, from Guilan ponds during spring and summer of the year 2018 and transported alive to the laboratory. Upon arriving, the biometric characteristics and genus of each carp were measured individually. Specimens were then acquired from the skin, gills, and eyes of the carp and examined according to standard parasitology methods. Recovered parasites were observed under a light microscope and then fixed for identification. As the result, the occurrence and intensity in the higher length group were comparatively greater than the lower one. Also, the prevalence and intensity of total parasites in male carp were higher than in females. In this research, *Dactylogyrus hypophthalmichthys* and *Dactylogyrus aristichthys* were observed in silver carp and *Dactylogyrus lamellatus* was detected in grass carp. In the paper below, we found that the host specificity varies in different species of *Dactylogyrus* isolated from grass carp and silver carp.

**Key words:** Disease, Parasitic fauna, Host, *D. lamellatus*, *Ctenopharyngodon idella*, Cultivation

### Introduction

The province of Guilan represents a hub of carp breeding in the country. Most low-income people prefer to eat farmed fish rather than marine or river fish. The herbivorous grass carp (*Ctenopharyngodon idella*) had been introduced into Iran aquaculture for biological control of aquatic weeds harboring the waterways. There are various diseases and infections involving carp that cause mortality and heavy losses in the region. Parasites can have a considerable impact on growth and behavior of fish (Scholz, 1999)

and cause mortality in cases of high parasitic burden. This phytophagous fish was noticed to be a target for many parasitic forms (Musselius and Strelkov, 1968; Molnar, 1971; Stepanova, 1971; Shirman and Smith, 1983; Abdel-Meguid, 1989, 1995). *Dactylogyrus* has been shown to cause mass mortality of fry, small and brood fish (Paperna, 1991). *Dactylogyrus* spp. are one of the most important parasitic pathogens causing gill-rot disease of grass carp (Bao *et al.*, 2016). Cudmore and Mandrak (2004) stressed that, a large number of disease-causing organisms such as *Dactylogyrus lamellatus* are known to infect Grass carp. It can induce severe damages to the gill filaments by causing hyperplastic proliferation, vacuolar degeneration, edema and necrosis (Molnar,

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1971; Ramadan *et al.*, 1995). The aim of this paper was to identify the parasitic fauna of two carp species, grass carp and silver carp, by investigating parasite infection on a number of warm water fish ponds in Iran aquaculture, as well as their epizootiological aspects through calculating their prevalence, intensity, abundance, and dominance. In the following manuscript, we discuss host-specific intensity conditions on certain prominent *Dactylogyrus* spp. in a couple of carp species.

## Material & methods

Fish samples were caught by random selection, using a fishing net, from Guilan warm water fish ponds during spring and summer of the year 2018 and transported alive to the laboratory. For this purpose, 94 specimens of grass carp and silver carp *Hypophthalmichthys molitrix* were studied. Upon arriving, the biometric characteristics and sex of each carp were measured individually. Specimens were then acquired from the skin, gills, and eyes of fish and examined carefully according to the standard parasitology method. Recovered parasites were observed through a light microscope and then fixed for identification. Methods used for collecting, fixing, staining, and mounting of parasite specimens were as follows: For the detection of protozoa, the mucus was (scraped separately from the skin, gills) onto a microslide and then spread the mucus with a cover slip. The protozoa to exposed a fixative for about 15 minutes and then washed for several minutes in alcohol containing a drop of added Iodine solution. Next, after dehydration both wet and dry smears were mounted in Canada balsam in accordance with Fernando *et al.*, (1972). Monogenea; fish gills were cut out and examined under a microscope at 40x-100x magnification. Vigorously moving worms were separated from the gills with a pipette and fixed under a cover-slip according to Fernando *et al.*, (1972) and Gussev (1983) in ammonium picrate and glycerol-gelatine

respectively. Digenea; metacercaria were collected in a 0.6% saline solution. The sample was placed with a little saline on a glass slide and appropriate pressure applied. It was fixed with 90% alcohol and washed in 70% alcohol and then stained with alum haematoxylin according to Fernando *et al.*, (1972). The identification of whole parasites was achieved in accordance with the keys given by Gussev (1985) and Lom & Dykova (1992). Classical epidemiological variables (prevalence, intensity and abundance) were calculated according to Bush *et al.*, (1997). The length sizes of the all fish were divided into two group ranges;  $12.5 \leq L_1 \leq 36.5$  and  $37.5 \leq L_2 \leq 49$ . Results are expressed as mean  $\pm$  standard deviation (SD). Kruskal-Wallis H Test analysis of variance was applied to the data to determine significant differences in the prevalence, intensity and abundance of parasites concerning sex and length groups. Statistical analyses were performed by using the SPSS 22.0 version program and the confidence was kept at 95% level. Typically, a value of less than 0.05 is considered significant.

## Results and Discussion

In this study, a total of 94 individual fish (70 silver carp and 24 grass carp) were randomly collected from Guilan warmwater fish ponds. Of these about 40 out of 94 fish were infected with different parasite species. The total number of parasites was 98. The number of infected fish in silver carp was 22 and in grass carp was 18. *Dactylogyrus* species recovered from silver carp were *D. hypophthalmichthys* and *D. aristichthys* and those in grass carp was *D. lamellatus* (Table 1). The occurrence, intensity, abundance, and parasite range in the higher length group comparatively were greater than those in comparison with the lower length group (Table 2), but statistically ( $p=0.439$ ) not significant ( $p>0.05$ ). Also, the result declared that the prevalence, intensity, abundance, and parasite range of total parasites in male

Table 1. Infection amount of parasites in silver carp and grass carp based on different Parasite species from the Guilan province farms in spring and summer of the year 2018

Parasite infection	Prevalence value (%)	Mean intensity $\pm$ SD	Mean abundance $\pm$ SD	Parasite range	Fish species
<i>Diplostomum spathaceum</i>	2.12	1 $\pm$ 0	0.02 $\pm$ 0.14	1	<i>Hypophthalmichthys molitrix</i> N=70
<i>Dactylogyrus</i> spp. ( <i>hypophthalmichthys</i> & <i>aristichthys</i> )	23.4	5.63 $\pm$ 4.15	1.31 $\pm$ 3.09	1-14	
<i>Ichthyophthirius multifiliis</i>	4.25	3.5 $\pm$ 2.12	0.14 $\pm$ 0.77	2-5	
<i>Trichodina</i> sp.	-	-	-	-	
<i>Lernaea</i> sp.	-	-	-	-	
<i>Nematode</i>	2.12	2 $\pm$ 0	0.04 $\pm$ 0.29	2	<i>Ctenopharyngodon idella</i> N=24
<i>Diplostomum spathaceum</i>	0.68	2 $\pm$ 0	0.04 $\pm$ 0.29	2	
<i>Dactylogyrus lamellatus</i>	17.02	9.62 $\pm$ 5.50	1.63 $\pm$ 4.23	1-16	
<i>Ichthyophthirius multifiliis</i>	-	-	-	-	
<i>Trichodina</i> sp.	2.12	5 $\pm$ 0	0.10 $\pm$ 0.72	5	
<i>Lernaea</i> sp.	6.38	10.3 $\pm$ 4.50	0.65 $\pm$ 2.72	6-15	
Total	42.5	12.35 $\pm$ 11.81	5.25 $\pm$ 9.78	1-34	94

Table 2. Variation of occurrence and infection intensity of parasites in silver carp and grass carp due to average length groups from the Guilan province farms in the spring and summer of the year 2018

Infection \ Length*	L <sub>2</sub> = 42.41 $\pm$ 3.78 N=42	L <sub>1</sub> = 29.37 $\pm$ 7.24 N=32	L <sub>t</sub> = 36.7 $\pm$ 8.53 *N=74
Occurrence %	14.86	6.75	21.62
Mean intensity SD $\pm$	9.45 $\pm$ 8.17	5 $\pm$ 3.67	8.06 $\pm$ 7.26
Mean abundance SD $\pm$	2.81 $\pm$ 6.14	0.67 $\pm$ 2.12	3.48 $\pm$ 6.19
Parasite range	1-29	1-10	1-29

\*74 samples length out of 94 were measured

\*( $p=0.439$ ) not significant ( $p>0.05$ )

carp were higher than those in females (Table 3).

It is usually known that external parasites constitute the largest group of pathogenic organisms in warm water fish (Snieszko and Axelrod, 1971). It seems that various factors are involved in the severity and infection type of these parasites. The most important of them are; water quality, fish density, host diet, body physiology and parasite life cycle. In this study, the prevalence value of parasites in silver carp, *Hypophthalmichthys molitrix*, was higher than grass carp, *Ctenopharyngodon idella*, but intensity rate, mean abundance, and parasite range were lower (Table 1). Probably the reason is *Ctenopharyngodon*

*idella* inhabits near the surface layer of the water column where it is less exposed to parasitic agents. The grass carp is an herbivore that initially feeds with zoo and phytoplankton and later exclusively on aquatic plants. Apparently, the prevalence value of *Dactylogyrus* sp. in the gills of silver carp was higher than grass carp in the present research, but other parameters were lower (Table 1). On the contrary, previous studies emphasized that the prevalence of *Dactylogyrus* sp. in grass carp was higher than in silver carp.

In the current study, the occurrence value of *Diplostomum spathaceum* in silver carp was higher than that in grass carp (2.12% and 0.68% respec-

Table 3. Variation of occurrence and infection intensity of parasites in silver carp and grass carp due to sex groups in the spring and summer of the year 2018

sex	Male N=26	Female N=14	Total *N=40
Infection			
Occurrence %	50	15	65
Mean intensity SD±	13.3±9.65	11±6.5	12.75±8.76
Mean abundance SD±	6±9.25	1.65±4.5	7.65±9.24
Parasite range	2-30	5-18	2-30

\*40 out of 94 samples sex were determined

tively), but the intensity rate in silver carp (1) was lower than in grass carp (2) (Table 1). In agreement with our research, on a survey in fish farms of Kerman province, the occurrence of this digenea in silver carp (21.1%) was higher than in grass carp (11.9%) too (Ezatkah, 2014). Unlike grass carp, silver carp inhabits near the soil bed of the pond, where the cercariae of *Diplostomum* sp. is located, that's because silver carp is more sensitive to monogeneans and digenea rather than grass carp. Eye fluke, *Diplostomum* sp. is known as a blindness factor in Iran's cultivated system and is now widespread throughout Iran (Pazooki *et al.*, 2007). Kiskaroly and Tafro (1983,1988) established the presence of *Diplostomum spathaceum* in *Ctenopharyngodon idella* from fish farms in Bosnia and Hercegovina.

*Ichthyophthirius multifiliis* is the most dangerous and concordant, the most prevalent protozoan of the country's freshwater fish farms which produces heavy damages to the aquaculture industry especially fingerlings (Fig. 1). In the current research, this parasite was only recovered from silver carp, while the other protozoa, *Trichodina* sp. was only observed in grass carp (Table 1). The disease due to *I. multifiliis*, commonly known as *Ichthyophthiriasis*, is widespread and has been reported from different freshwater fish species in Iran (Pazooki *et al.*, 2006; Jalali, 1997; Raissy *et al.*, 2010). Grass carp fingerlings which are kept in the pond or tank with heavy density are susceptible to be infected by *Ichthyophthirius*. *Ichthyophthirius multifiliis* and *Trichodina pediculus* were iden-

tified from the gills of silver carp in Bangladesh (Alam *et al.*, 2012). *Ichthyophthirius* sp. with an occurrence rate of 7.1%, density of 5, and mean abundance of 0.36 was also reported from the skin of grass carp in Bangladesh (Akhter *et al.*, 1997). In the following work, the prevalence, and intensity of *Ichthyophthirius multifiliis* in the gills of silver carp were 4.2% and 3.5 respectively, which are lower than the previous study with various hosts and different microhabitat.

The absence of *Trichodina* sp. in the fishpond is an indication of water pollution lack and proper pool sanitary management. *Trichodina* sp. with an occurrence rate of 10.4%, density of 4.1, and mean abundance of 0.43 was recorded from the skin of silver carp in Bangladesh (Akhter *et al.*, 1997). Also, *Trichodina* sp. with an occurrence of 8.9%, density of 5, and mean abundance of 0.45 was detected from



Fig. 1. *Ichthyophthirius multifiliis* from the gill of silver carp mag. 100x.

the gill of grass carp in Bangladesh (Akhter *et al.*, 1997). In the available experiment, the prevalence of *Trichodina* sp. (2.1%) in the gills of grass carp was quite lower, but density (5) was consistent with the former inquiry in Bangladesh.

*Lernaea* species often causes more heavy mortality of bighead (*Aristichthys nobilis*) rather than grass carp and silver carp, but in this study *Lernaea cyprinacea* was only separated from grass carp. Yin *et al.*, (1963) found that different species of anchor worms were parasitize on the silver carp and grass carp, as *L. ctenopharyngodontis* infects grass carp and *L. cyprinacea* infects silver carp. In the present survey, the prevalence level and intensity of *Lernaea* sp. in grass carp were 6.3% and  $10.3 \pm 4.50$  respectively. The parasite was isolated from the skin of silver carp in ponds of Bangladesh (Alam *et al.*, 2012). Also, *Lernaea* sp. was reported from grass carp in Mahabad dam (Abdi *et al.*, 1997). Freshwater fish in Iran are generally encountered by this parasite throughout the year, but severe parasite infestation with mortality occurs only during the warm months of the year and this is due to the fact that ecologically, the evolution of the life cycle of the parasite occurs at high degrees of temperature.

Dove and Ernst (1998) considered that *Dactylogyrus* is one of the largest genera of parasitic helminths of which 95% are parasites of gills in the fish of the family Cyprinidae. In present study, 3 species of *Dactylogyrus* spp. were collected from the gills of silver carp and grass carp, consisting *D. hypophthalmichthys* (Fig. 2), which is host-specific for silver carp, *D. aristichthys* (Fig. 4) which is host-specific for bighead but separated from silver carp and one host-specific species of *D. lamellatus* (Fig. 3) recovered from grass carp. The occurrence rate and mean intensity of mixed *Dactylogyrus* spp. in silver carp were 23.4% and 5.63 respectively, while the occurrence and intensity with *Dactylogyrus lamellatus* in grass carp were 17.02% and 9.62 respectively. In a study on Kerman province's farms (Ezatkah,

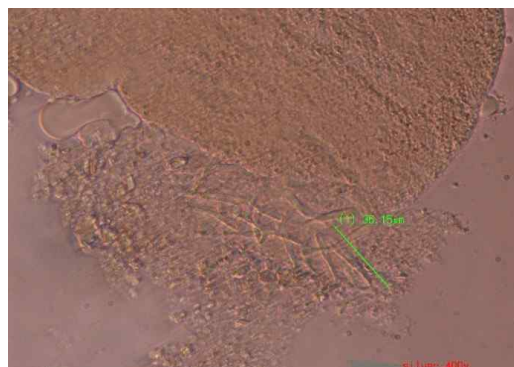


Fig. 2. *D. hypophthalmichthys* from the gill of silver carp mag. 400x.

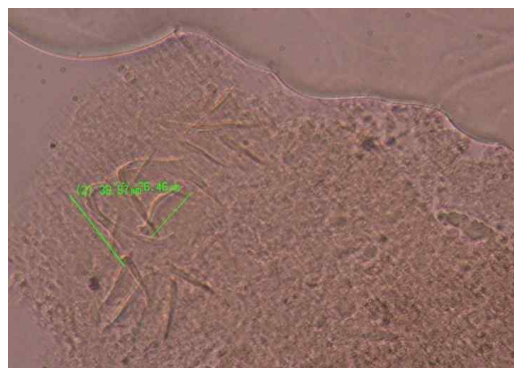


Fig. 3. *D. lamellatus* from the gill of grass carp mag. 400x.



Fig. 4. *D. aristichthys* from the gill of silver carp mag. 400x.

2014), the occurrence of *Dactylogyrus* sp. in silver carp (25.3%) was very close to that of the present result, but in the case of grass carp (26.7%) was rela-

tively higher than in our study. In other research on farms of Bangladesh, the occurrence of *Dactylogyrus* sp. in silver carp was 10%, which is lower than the current result, and intensity was 5 which is similar to this work, while the occurrence and intensity of *Dactylogyrus* sp. in grass carp were 13.2% and 4.32 which are lower than our research (Alam *et al.*, 2012). The difference in infection rate in studying fish species may be due to differences in biology, nutrition, the behavior of fish, and also environmental conditions.

*D. aristichthys* have been reported from fish reproduction centers of the Caspian sea basin (Jalali & Molnar, 1990). *D. hypophthalmichthys* was identified from silver carp in reproduction centers of Caspian sea basin (Jalali & Molnar, 1990), and *D. lamellatus* was recovered from grass carp in Behshti hatchery and breeding center (Jalali & Molnar, 1990). Infection of *Dactylogyrus lamellatus* is responsible for high mortalities observed in fry and fingerling production (Shamsi *et al.*, 2009). *Dactylogyrus lamellatus* was found in *Ctenopharyngodon idella* by Sengupta and Dalwani (2008), Shamsi *et al.*, (2009) and Bozorgnia *et al.*, (2012) in fish farms of Iran and by Yao and Nie (2004) in fish farms in China with prevalence higher than 60% and the highest culmination of the mean intensity in April. The highest infection level of *D. lamellatus* was also observed in a field study in Poland (Pojman'ska, 1995). The host factors like fish size and crowding have a strong influence on infection levels of monogeneans on their fish hosts (Özer and Erdem, 1999). There are different views on the effect of length and weight of the fish on parasitic infection level, some researchers such as our finding (Table 2), believe that infection level increases with increasing weight and length (Bichi and Ibrahim, 2009; Imam and Dewu, 2010), but there is no significant difference between the average length groups and parasite abundance of the examined fish ( $p>0.05$ ). In the following work, we discovered that host specificity correlation due to sil-

ver carp is weaker than against bighead so that, it was able to adopt one species from bighead (*D. aristichthys*), in addition to keeping its own host-specific species (*D. hypophthalmichthys*). Conversely, in the case of grass carp, the host-specific severity is so strong that it cannot receive and accept other host-specific species of carp-type *Dactylogyrus* unless its own specific species, *D. lamellatus*.

**Conclusion:** In the present research we found that the host specificity varies in different species of *Dactylogyrus* isolated from grass carp and silver carp. To avoid entry of exotic monogeneans especially *Dactylogyrus* species into the carp fish ponds, introduction of imported live fish to the country should be strictly controlled. Also, to prevent *Dactylogyrus* and carp infestation with these monogeneans pathogen, the entry of *Carassius gibelio* and goldfish into the carp ponds, due to the fact that *Carassius* species can be the reservoir of many *Dactylogyrus* species for carp species, should be prohibited.

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