



## OCCURRENCE AND INTENSITY OF PARASITES IN GOLDFISH (*Carassius auratus* L.) FROM GUILAN PROVINCE FISH PONDS, NORTH IRAN

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### ABSTRACT

In this survey 109 specimens of goldfish (*Carassius auratus*) were collected from Guilan fish ponds during 2012-13. After recording biometric characteristics, common parasitology methods were used. In the present study 11 parasite species were recovered from goldfish. Parasitofauna consisted of two protozoans: *Ichthyophthirius multifiliis* and *Trichodina* sp.; one digenean trematodes: *Diplostomum spathaceum*; six monogenean trematodes: *Dactylogyrus vastator*, *Dactylogyrus formosus*, *Dactylogyrus baueri*, *Dactylogyrus anchoratus* and *Gyrodactylus* sp.; one crustacean: copepodid stage of *Lernaea cyprinacea* and one nematodes larvae. All the monogeneans found during the current study are considered new locality records for goldfish in Guilan province, Iran. Mean intensity of infection and abundances of parasite species (with prevalences >10%) among seasons were tested by the Kruskal-Wallis test (KW, multiple comparisons) and Conover-Inman test. Results have shown that monogeneans had the highest prevalence values (49.54%) in goldfish in Guilan fish ponds.

#### How to Cite

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### INTRODUCTION

Goldfish (*Carassius auratus* L.), a freshwater fish in the Cyprinidae family, is one of the earliest cultured ornamental fish in the world (Tu et al., 2015). This species originally inhabits rivers, lakes, ponds and ditches with stagnant or slow flowing water. Goldfish occur in eutrophic waters, well vegetated ponds and canals. Their feeding is mainly on plankton, benthic invertebrates, plant material and detritus (Kottelat and Freyhof, 2007). This species has been brought to Iran from Eastern Asia for ornamental purposes about 80 years ago. Iranian people buy a few goldfish in a crystal bowl for celebrating the New Year known as Nowruz. For this reason in Iran goldfish is regularly cultured as a commodity fish in some of the warm-water fish ponds. Parasites of goldfish in different parts of the world have been reported by several authors (Ogawa and Egusa, 1979; Jalali and Molnar, 1990;

Levsen, 1995; Di Cave et al., 2000; Koyun and Altunel, 2007; Koyuncu, 2009 and Borisov, 2013). The monogenean fauna of goldfish in Japan was studied by Ogawa and Egusa (1979) who found six species of *Dactylogyrus* (*D. dulkeiti*, *D. formosus*, *D. baueri*, *D. intermedius*, *D. anchoratus* and *D. vastator*). Koyun and Altunel (2007) studied parasites of goldfish in Enne Dam Lake of Turkey and they discovered that goldfish were infected with two species of monogenean (*Dactylogyrus anchoratus* and *Gyrodactylus katherineri*) and one nematode (*Contracaecum* sp.). Borisov (2013) investigated the representatives of the Dactylogyridae family in imported goldfishes to Bulgaria from Singapore and he found five typical *Dactylogyrus* for goldfish (*D. vastator*, *D. formosus*, *D. intermedius*, *D. anchoratus* and *D. baueri*) as well. It can be concluded from these researches that goldfish can be considered one of the most common hosts of dactylogirids.

Parasites can have a considerable impact on the growth and behavior of fish (Scholz, 1999) and cause mortality in cases of high parasitic burden. The aim of the present study was to investigate the helminth fauna of one of the most popular ornamental fish *C. auratus* in Guilan fish ponds, as a main center of goldfish cultivation in Iran, as well as their epizootiological aspects through calculating their prevalence, intensity, abundance and dominance.

## MATERIALS AND METHODS

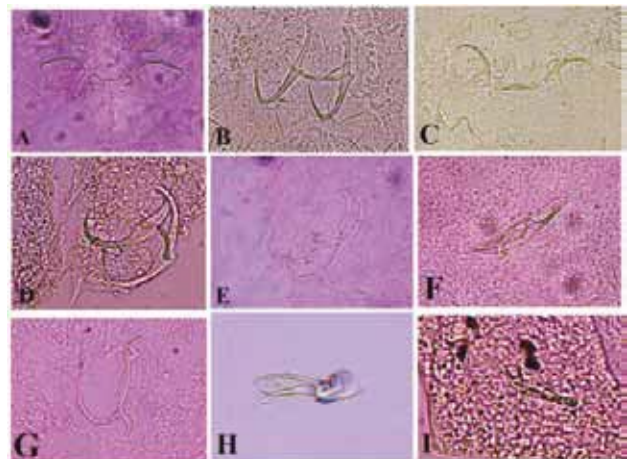
A total of 109 goldfish *Carassius auratus* were collected from Guilan province fish farms, north Iran. The fishes were caught from September 2012 to August, transported to the Parasitology Laboratory of National Inland Water Aquaculture Research Institute in Anzali city alive in water obtained from the collection site. A dissolved oxygen saturation of approximately 85-90% was maintained during transport. Upon arrival, fish were killed by a sharp blow to the head, weighed and measured and then examined externally for gross signs of parasitism. If no gross signs were observed, skin biopsies were prepared from the entire length of the lateral body wall. Goldfish (109 in number) averaged 18.44 g ( $\pm 34.51$  g, range=1-308 g) in weight and averaged 9.25 cm ( $\pm 3.62$  cm, range = 3.5-28 cm) in total length. A gill biopsy was collected from the second arch of specimens. A fin biopsy was collected from the caudal fin of specimens. Wet mounts of all biopsied tissues were prepared for further analysis.

After recording biometric characteristics, standard necropsy and parasitology methods were used (Stoskopf, 1993). All organs of the fish were examined except for blood. Live trematodes were relaxed in distilled water at 4°C for 1 h and fixed in 10% hot buffered formalin. The worms were identified using parasite identification keys (Yamaguti, 1961; Bykhovskaya-Pavlovskaya et al., 1962; Moravec, 1994) and then deposited at the Laboratory of Fish Diseases of National Inland Water Aquaculture Research Institute (Iran). Classical epidemiological variables (prevalence, intensity and abundance) were calculated according to Bush et al. (1997). The dominance of a parasite species was calculated as  $N/N_{sum}$  (where  $N$  = abundance of a parasite species and  $N_{sum}$  = sum of the abundance of all parasite species found) and expressed as a percentage (modified after Leong & Holmes, 1981). The dominance values were used for classification of parasites as eudominant (>10%), dominant (5.1% -10%), subdominant (2.1%-5%), recedent (1.1%-2%) and subrecedent (<1.0%) of given species (Niedbala and Kasparzak, 1993). Mean intensity of infection and abundances of parasite species (with prevalences >10%) among seasons were tested by the Kruskal-Wallis test (KW, multiple comparisons) and Conover-Inman test. Results were considered significant at the 95% level ( $p < 0.05$ ). Computations were performed using the SPSS. 15 programme.

## RESULTS

In the present study two protozoan and nine metazoan parasites, including members of the parasite taxa: Nematoda, Digenea, Monogenea and Copepoda, were determined (Table 1). The prevalence (P), mean intensity of infection (MI), range, mean abundance (MA) and dominance percentage of the parasites are presented in Table 1. The eudominant parasite of goldfish (Table 1) were monogeneans and *Trichodina* sp. (Dominance =  $D = 47.33\%$  and  $42.78\%$ , respectively). The subdominant parasites were *D. spathaceum* ( $D=4.03$ ), *I. multifiliis* ( $D=2.86\%$ ) and *L. cyprinacea* ( $D= 2.86\%$ ). The only subrecedent parasite was nematoda larvae ( $D= 0.13$ ).

As it is shown in Table 1, monogeneans (including *D. vastator*, *D. intermedius*, *D. formosus*, *D. baueri*, *D. Anchoratus* and *Gyrodactylus* sp.) indicate the highest prevalence values (49.54%) in goldfish (Fig. 1).



**Fig 1.** Monogeneans recovered from goldfish (*Carassius auratus*) in Guilan fish ponds (A to E consist of the attachment apparatus: A) *D. vastator* B) *D. baueri* C) *D. intermedius* D) *D. formosus* E) *D. anchoratus*; F to I - the copulatory organ: F) *D. vastator* G) *D. baueri* H) *D. intermedius* I) *D. formosus*)

According to Table 2, the abundance of *Lernaea cyprinacea* in summer was significantly higher than in other seasons (Conover-Inman test,  $p < 0.05$ ). It was also true for abundance of *Trichodina* sp. in autumn which was significantly higher than in summer (Conover-Inman test,  $p < 0.05$ ).

In Table 3 the prevalence of parasites in different length groups had varying values and the differences between them were significant for *D. spathaceum*, *Trichodina* sp. and monogenean parasites ( $p < 0.05$ ). There was a positive correlation between fish length, fish weight and infection to *Trichodina* sp. and *D. spathaceum*. Conover-Inman test also demonstrated most differences are between small fish and the large ones.

**Table 1.** The prevalence, mean intensity, range, abundance and dominance of some parasites in goldfish (*C. auratus*)

Parasite	Prevalence(%)	Mean±SD	Range	Abundance±SD	Dominance(%)
<i>D.spathaceum</i> N=31	11.00	2.58±1.31	1-5	0.28±0.91	4.03
<i>Trichodina sp.</i> N=329	32.11	9.4±12.22	1-74	3.01±8.15	42.78
<i>I. multifiliis</i> N=22	4.58	4.4±6.06	1-15	0.2±1.48	2.86
Monogenean* N=364	49.54	6.53±4.85	1-25	3.21±4.73	47.33
<i>L. cyprinacea</i> N=22	11.92	1.69±0.94	1-3	0.20±0.63	2.86
Nematoda N=1	0.91	1±0	1	0.009±0.095	0.13

\* Consisting of *D. vastator*, *D. intermedius*, *D. formosus*, *D. baueri*, *D. anchoratus*, and *Gyrodactylus* sp.

**Table 2.** The prevalence, mean intensity, range and abundance of some parasites of *C. auratus* in different seasons

Parasite	<i>D. spathaceum</i>	<i>Trichodinasp.</i>	<i>I. multifiliis</i>	Monogenean*	<i>Lernaea</i>	Nematoda
	Prevalence(%) Mean±SD	Prevalence(%) Mean±SD	Prevalence(%) Mean±SD	Prevalence(%) Mean±SD	Prevalence(%) Mean±SD	Prevalence(%) Mean±SD
Seasons	Abundance±SD Range	Abundance±SD Range	Abundance±SD Range	Abundance±SD Range	Abundance±SD Range	Abundance±SD Range
<b>Summer</b> (N=48)	6.25 2.0±1.73 0.12±0.60 <sup>a</sup> 1-4	9.17 4.40±3.30 0.91±2.31 <sup>a</sup> 1-10	4.16 2.50±2.12 0.10±0.59 <sup>a</sup> 1-4	45.83 5.45±3.60 2.50±3.65 <sup>a</sup> 1-13	27.08 1.69±0.94 0.45±0.89 <sup>b</sup> 1-3	0 <sup>a</sup>
<b>Autumn</b> (N=53)	16.98 2.77±1.20 0.47±1.15 <sup>a</sup> 1-5	45.28 11.62±14.15 5.26±11.07 <sup>b</sup> 2-74	3.77 1.0±0.0 0.03±0.19 <sup>a</sup> 1	49.05 7.56±6.06 3.56±5.61 <sup>a</sup> 1-25	0 <sup>a</sup>	0 <sup>a</sup>
<b>Winter</b> (N=8)	0 <sup>a</sup>	12.5 6.00± 0.75±2.12 <sup>ab</sup> 6	12.5 15.0± 1.87±5.30 <sup>a</sup> 15	75.00 6.83±2.31 5.12±3.72 <sup>a</sup> 3-10	0 <sup>a</sup>	12.5 1.00± 0.12±0.35 <sup>a</sup> 1

\* Consisting of *D. vastator*, *D. intermedius*, *D. formosus*, *D. baueri*, *D. anchoratus* and *Gyrodactylus* sp.

\*\* Data shown with different letters in each column are statistically significant at the p<0.05 level.

**Table 3.** The prevalence, mean intensity, standard deviation and maximum intensity of parasites of *C. auratus* in different sizes

Parasite	<i>D. spathaceum</i>	<i>Trichodina sp.</i>	<i>I. multifiliis</i>	Monogenean*	<i>Lernaea</i>	Nematoda
Size class	Prevalence (%) Mean±SD	Prevalence (%) Mean±SD	Prevalence (%) Mean±SD	Prevalence (%) Mean±SD	Prevalence (%) Mean±SD	Prevalence (%) Mean±SD
TL (cm)	Max intensity	Max intensity	Max intensity	Max intensity	Max intensity	Max intensity
<b>6&lt;</b> <b>Small</b> (N=17)	0 <sup>a</sup>	5.88 0.58±2.42 <sup>a</sup> 10	0 <sup>a</sup>	35.29 2.29±3.53 <sup>ab</sup> 11	23.52 0.52±1.06 <sup>a</sup> 3	0 <sup>a</sup>
<b>6.1-12</b> <b>Medium</b> (N=78)	10.25 0.28±0.95 <sup>ab</sup> 5	33.33 3.29±9.23 <sup>ab</sup> 74	3.84 0.07±0.47 <sup>a</sup> 4	55.12 3.67±5.11 <sup>b</sup> 25	11.53 0.16±0.54 <sup>a</sup> 3	1.28 0.01±0.11 <sup>a</sup> 1
<b>12.1&gt;</b> <b>Large</b> (N=14)	28.57 0.64±1.15 <sup>b</sup> 3	57.14 4.42±5.44 <sup>b</sup> 18	14.28 1.14±3.99 <sup>a</sup> 15	28.57 0.50±0.94 <sup>a</sup> 3	0 <sup>a</sup>	0 <sup>a</sup>

\* Consisting of *D. vastator*, *D. intermedius*, *D. formosus*, *D. baueri*, *D. anchoratus*, and *Gyrodactylus* sp.

\*\* Data shown with different letters in each column are statistically significant at the p<0.05 level

## DISCUSSION

Some of the parasites isolated from goldfish in Guilan fish ponds are capable of making significant mortalities among captive and wild stocks, especially parasites which do not need an intermediate host, such as protozoan and monogeneans. Isolated parasites in this study may be transferred from cultivated fish to aquarium fish. In the present study, monogeneans were responsible for the highest prevalence (47.33%) in the gills and on the skin of sampled fish. Gills necropsies yielded the greatest diversity of parasites with eight taxa represented in this organ. *C. auratus* is a potential bearer of dactylogyrids and might be playing a role in their spreading not only in ornamental fish but also in fish from the natural ichthyofauna (Borisov, 2013). *Dactylogyrus vastator*, which is isolated from the sampled fish, is a highly pathogenic species and is usually observed on the gills of *Carassius carassius*, *Carassius auratus gibelio* and *Cyprinus carpio* (Gussev, 1985). *D. vastator* is rather widespread through freshwater lakes and fish farms where common carp inhabits or is cultivated (Shamsi et al., 2009). It is the most prevalent species of the monogeneans among fingerlings in the northern part of Iran (Guilan and Mazandaran provinces) and causes high injuries and mortalities (Jalali and Molnar, 1990). So the infection of Guilan goldfish with *D. vastator* is justifiable. On the other hand, *D. anchoratus* needs special attention due to its low host specificity and high tolerance to a wide range of temperature and salinity (Shamsi et al., 2009). This parasite attaches to the base of primary filaments where osmoregulating chloride cells are located. Therefore, infection with the parasite could be dangerous and harmful especially for the fry-size fish in hatcheries (Shamsi et al., 2009). *D. intermedius* is an important veterinary ectoparasite in Asia, Central Europe, Middle East and North America (Wang et al., 2011). *D. intermedius* may cause gill inflammation, excessive mucus secretions, accelerated respiration and mixed infections with another parasites and secondary bacterial infections. This parasite always leads to the loss of appetite, productivity and high mortalities (Wang et al., 2011). In recent years, the infestation of *D. intermedius* in freshwater fish has increased and caused serious economic damage (Wang et al., 2011). In Europe, *D. intermedius* has been found in goldfishes imported from Singapore (Di Cave et al., 2000) and in goldfishes from aquariums in Norway (Levsen, 1995). Typical for the species are anchors and a copulatory organ (Fig. 1, C and H). Comparing the results of the present study with the other researchers who referred that in past time just *Dactylogyrus dulikeity* was reported for *Carassius auratus* in Guilan fish ponds (Shamsi et al., 2009), but in the present study five other species (*D. vastator*, *D. intermedius*, *D. formosus*, *D. baueri*, *D. anchoratus*) are recovered. Goldfish parasite investigations in other parts of the country have shown that *D. baueri* was recovered previously in Semnan, Isfahan, Kashan, shahre-Kord and also from

*Carassius gibelio* in Guilan, *D. vastator* in goldfishes of Tehran, Semnan and *Cyprinus carpio* of Guilan, *D. anchoratus* in Isfahan, Kashan and shahre-Kord (Jalali and Molnar, 1990; Meshgi et al., 2006; Shamsi et al., 2009; Fadaei-Fard et al., 2010; Mehdizadeh-mud and Varian, 2012, Daghigh Roohi et al., 2014). So all the monogeneans found during the current study are considered new locality records for goldfish in Iran. As mentioned above, some of monogeneans were imported by goldfish from Singapore to Bulgaria. So identification of fish parasites and quarantine before import to each country can make a limitation to parasite distribution between different localities.

## ACKNOWLEDGMENT

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## Sažetak

### POJAVA I INTENZITET PARAZITA KOD ZLATNE RIBICE (*Carassius Auratus* L.) U RIBNJACIMA POKRAJINE GUILAN, SJEVERNI IRAN

U ovom je istraživanju prikupljeno 109 primjeraka zlatne ribice (*Carassius auratus*) iz ribnjaka pokrajine Guilan tijekom 2012.-13. godine. Nakon određivanja biometrijskih karakteristika, koristile su se uobičajene metode parazitologije. Utvrđeno je 11 vrsta parazita kod zlatnih ribica. Parazitofauna se sastojala od dvije protozoe: *Ichthyophthirius multifiliis* i *Trichodina* sp.; od jedne digenean trematode: *Diplostomum spathaceum*; šest jednorodnih metilja (Monogenea) trematoda: *Dactylogyrus vastator*, *Dactylogyrus formosus*, *Dactylogyrus baueri*, *Dactylogyrus anchoratus* i *Gyrodactylus* sp.; jednog raka: copepodne faze *Lernaea cyprinacea* i jedne ličinke nematode. Svi jednorodni metilji pronađeni tijekom ovog istraživanja prvi put su zabilježeni kod zlatnih ribica u pokrajini Guilan, Iran. Srednji intenzitet infekcije i brojnost vrsta parazita (u prevalenciji > 10%) među godišnjim dobima testiran je Kruskal-Wallis testom (KW, višestruke usporedbe) i Conover-Inman testom. Rezultati su pokazali da su jednorodni metilji imali najvišu prevalenciju (49.54%) kod zlatnih ribica u ribnjacima pokrajine Guilan.

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