

Occurrence and intensity of Parasites in Common Carp, *Cyprinus carpio*, from Anzali Wetland, southwest of the Caspian Sea, Iran

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Abstract

In this survey, 54 specimens of common carp (*Cyprinus carpio*) were collected from Anzali Wetland between August 2011 and July 2012. After recording biometric characteristics, routine necropsy and parasitology methods were used. Totaly, 3165 individuals of parasites were isolated and identified from common carp. Parasitofauna consisted of 10 species as follows: two protozoans, *Ichthyophthirius multifiliis* and *Trichodina* sp.; two digenean trematodes, *Diplostomum spathaceum* and *Posthodiplostomum* sp.; two monogenean trematodes, *Dactylogyrus extensus* and *Diplozoon nipponicum*; two crustaceans, *Lernaea cyprinacea* and *Argulus foliaceus*; one cestode, *Caryophyllaeus fimbriceps* and one nematode, *Raphidascaris acus* larvae. *D. extensus* had the highest prevalence, mean intensity, dominance and abundance. The abundance of *Trichodina* sp. and *C. fimbriceps* in the age class of > 2 years old were significantly greater than that in the younger age classes. *L. cyprinacea* (copepodid stage) and *C. fimbriceps* showed the greatest abundances in summer and autumn, respectively. *Trichodina* sp. and *L. cyprinacea* (copepodid stage) showed significantly greater abundances in the eastern parts of the wetland compared with abundances of other stations.

Keywords: Anzali Wetland, *Cyprinus carpio*, Parasite, Occurrence, Abundance

1. Introduction

The common carp (*Cyprinus carpio* L.1758) is one of the most widely distributed freshwater fishes in the world (Welcomme, 1988) primarily because of its large native area of Eurasian distribution (Balon, 1995) combined with historical translocations and introductions for farming, aquaculture, and sport fishing (Balon, 2006; Copp *et al.*, 2005; Hoffmann,

1999). Conversely, the wild carp is now listed as vulnerable due to a considerable loss in genetic variability resulting from both natural and human selection pressures in most of its native areas in Eastern Europe and Asia (Piria *et al.*, 2016).

Anzali Wetland (37° 25' N, 49° 28' E), with a surface area of about 15,000 ha is located at the southwest of the Caspian Sea in Guilan Province, Iran. Anzali Wetland is a vast reservoir which inhabits 49 fish species, including the native species,

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wild common carp. In recent years, however, the fish stocks in the wetland is declining and restoring stock status is pursued by stakeholders.

Many researchers (Anwar 1971; Mokhayer, 1980; Sattari, 1996; Eslami and Jalali, 1998; Jalali and Barzegar, 2004; Borji *et al.*, 2012) investigated parasites of common carp in Iran. Sattari (1996) reported the most recent report on parasites of common carp in the Anzali wetland. Yet, there is not much clear knowledge on the causes of common carp stock decline. Therefore, this investigation was undertaken to identify the parasite fauna on wild common carp and to record species spatial and temporal distribution, prevalence and intensity data in relation to fish age and sex in the Anzali Wetland.

2. Materials and Methods

A total of 54 specimens of common carp (*Cyprinus carpio*) were collected from Anzali International Wetland (37° 25' N, 49° 28' E) in the southwest of the Caspian Sea (Guilan Province, Iran) from August 2011 through July 2012. Fish were preyed with gill net and transported alive in a tank to the Parasitology Laboratory of National Inland Water Aquaculture Research Center in Anzali Port under a dissolved oxygen saturation of approximately 85-90%. Dissolved oxygen concentration and water temperature were measured at the collection site upon arrival. Biometric characteristics of fish were recorded and common necropsy and parasitological methods applied according to Stoskopf (1993). All organs, except blood, were examined. Live trematodes were relaxed in distilled water at 4°C for 1 h and fixed in 10% hot buffered formalin. Live nematodes were fixed in hot 70% ethanol and cleared in hot lactophenol. All specimens fixed in 10% formalin were stained with aqueous Acetocarmine, dehydrated and mounted in Canada balsam. The worms were identified using parasites identification keys (Yamaguti, 1961; Bykhovskaya-Pavlovskaya *et al.*, 1962; Bykhovskaya - Pavlovskaya, 1985 ; Moravec,

1994) and then, were labeled and deposited at the Laboratory of Fish Diseases, Faculty of Natural Resources, University of Guilan, Iran.

Classical epidemiological variables (prevalence, intensity and abundance) were calculated according to Bush *et al.* (1997). The relative dominance of a parasite species was calculated as $N/N \text{ sum}$ (where N = abundance of a parasite species and $N \text{ sum}$ = sum of the abundance of all parasite species found) and expressed as a percentage. The relative 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 infestation and abundances of parasite species (with prevalence > 10%) with respect to the seasons, age groups and sexes were tested by Kruskal-Wallis test and Mann-Whitney U test. Significance level were evaluated at the 95% level ($p < 0.05$). Data were analyzed using the SPSS programme (Ver, 17) software.

3. Results

In the present study, the average weight and total length of fish specimens were 365.82 ± 263.14 g and 29.82 ± 8.41 cm, respectively. A total of 3165 parasite specimens from 10 species were isolated and identified in common carp, including: two protozoans *Ichthyophthirius multifiliis* and *Trichodina* sp.; two digenean trematodes: *Diplostomum spathaceum* and *Posthodiplostomum* sp.; two monogenean trematodes: *Dactylogyru*s *extensus* and *Diplozoon nipponicum*; two crustaceans: *Lernaea cyprinacea* and *Argulus foliaceus*; one cestode: *Caryophyllaeus fimbriceps* and one nematode: *Raphidascaris acus* larvae. The prevalence (%), mean intensity of infestation, range, abundance and relative dominance of the parasites in common carp are presented in Table 1.

Based on the dominance (D) of parasites, the eudominant parasites (Table 1) were *D. extensus*, *Trichodina* sp. and *I. multifiliis* (46.07%, 15.61% and

15.48%, respectively). The dominant parasite was *L. cyprinacea* (copepodid stages) (7.77%). The subdominant parasites were *C. fimbriceps*, *D. nipponicum*, *R. acus larvae* and *D. spathaceum* (4.48%, 3.85%, 3.41% and 2.05%, respectively). The recedent parasite was *Posthodiplostomum* sp. (1.23%) and subrecedent parasite was *Argulus foliaceus* (0.03%).

L. cyprinacea (copepodid stage) showed significantly greater abundance in summer (KW, $\chi^2 = 17.003$, $df = 3$, $p < 0.05$), and *C. fimbriceps* in autumn ($\chi^2 = 10.372$, $p < 0.05$) (table 2).

As shown in Table 3, the abundances of *Trichodina* sp. and *C. fimbriceps* in the age class of > 2 years old were significantly greater than that in the younger age classes ($\chi^2 = 20.108$, $p < 0.05$ and $\chi^2 = 19.565$, $p < 0.05$, respectively).

Table 1: The prevalence, mean intensity, abundance and dominance of some parasites in *C. carpio* (N=54)

Parasite	Prevalence(%)	Mean ± SD	Range	Abundance±SD	Dominance(%)
<i>Diplostomum spathaceum</i> N=65	48.14	2.50±2.23	1-9	1.20 ± 1.98	2.05
<i>Trichodina</i> sp. N=494	48.14	19.00±29.36	3-150	9.15±22.32	15.61
<i>Ichthyophthirius multifiliis</i> N=490	40.74	22.27±58.68	1-241	9.07±38.55	15.48
<i>Lernaea cyprinacea</i> ^(CS) N=246	46.30	9.84±8.55	1-31	4.56±7.60	7.77
<i>Argulus foliaceus</i> N=1	1.85	1.00±-	1	0.019±0.14	0.03
<i>D. extensus</i> N=1458	92.59	29.16±38.37	1-191	27.02±37.67	46.07
<i>Raphidascaris acus larvae</i> N=108	37.04	5.40±5.34	1-42	2.00±4.14	3.41
<i>Caryophyllaeus fimbriceps</i> N=142	14.81	17.75±19.51	1-53	2.63±9.53	4.49
<i>Diplozoon nipponicum</i> N=122	38.89	5.81±11.95	1-28	2.26±7.88	3.85
<i>Posthodiplostomum</i> sp. (N=39)	11.11	6.5±4.81	2-12	0.72±2.54	1.23

CS= Copepodid stage

Table 2: The prevalence, mean density and abundance of some parasites of *C. carpio* in different seasons.

Parasite	<i>Diplostomum</i>	<i>Trichodina</i>	<i>Ichthyophthirius</i>	<i>Lernaea</i> ^(CS)	<i>D. extensus</i>	<i>Raphidascaris</i>	<i>Caryophyllaeus</i>	<i>Diplozoon</i>
	Prevalence (%)	Prevalence (%)	Prevalence (%)	Prevalence (%)	Prevalence (%)	Prevalence (%)	Prevalence (%)	Prevalence (%)
season	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
	Range	Range	Range	Range	Range	Range	Range	Range
	Abundance ±SD	Abundance ±SD	Abundance ±SD	Abundance ±SD	Abundance ±SD	Abundance ±SD	Abundance ±SD	Abundance ±SD
Spring (N=21)	42.86 4.11±3.18 1-9 1.76±2.90	57.14 22.00±40.40 4-150 12.57±31.97	42.86 48.00±88.03 1-241 20.57±60.76	28.57 6.33±4.27 1-13 1.81±3.63	85.71 45.33±47.87 3-191 38.86±47.03	28.57 2.33±1.21 1-4 0.67±1.24	14.29 11.67± 11.59 1-24 1.67±5.56	38.10 10.50 ±19.03 1-57 4.00±12.41
Summer (N=20)	52.17 1.83±0.72 1-3 0.96±1.07	39.13 17.33±14.92 5-50 6.78±12.48	43.48 5.40±4.70 1-15 2.35±4.06	73.91 12.12±9.16* 1-31 8.96±9.52	95.65 18.86±32.26 1-157 18.09±31.96	34.78 6.88±6.56 1-17 2.39±4.99	4.35 1.00± - 1 0.04±0.21	39.13 3.22 ±1.18 1-6 1.26±1.19
Autumn (N=13)	55.56 1.20±0.45 1-2 0.60±0.70	55.56 14.80±19.90 5-50 7.40±15.39	33.33 1.33±0.58 1-2 0.40±0.70	11.11 1.00± - 1-2 0.20±0.42	100 24.44±21.26 7-39 22.70±20.78	66.66 7.17±5.54 2-17 3.90±5.32	33.33 32.33±35.24* 1-5 10.60±19.39	44.44 2.25 ±1.89 1-5 0.90±1.60

CS= Copepodid stage * significant value

Table 3: The prevalence, mean density and abundance of some parasites of *C. carpio* in different age classes.

Parasite	<i>Diplostomum</i>	<i>Trichodina</i>	<i>Ichthyophthirius</i>	<i>Lernaea</i> ^(CS)	<i>D. extensus</i>	<i>Raphidascaris</i>	<i>Caryophyllaeus</i>	<i>Diplozoon</i>
Age/year	Prevalence (%) Mean ±SD Range Abundance e±SD	Prevalence (%) Mean ±SD Range Abundance ±SD	Prevalence (%) Mean ±SD Range Abundance ±SD	Prevalence (%) Mean ±SD Range Abundance ±SD	Prevalence (%) Mean ±SD Range Abundance ±SD	Prevalence (%) Mean ±SD Range Abundance ±SD	Prevalence (%) Mean ±SD Range Abundance ±SD	Prevalence (%) Mean ±SD Range Abundance ±SD
< 2 (N=6)	50 1.33±0.58 1-2 0.67±0.82	0	16.67 2.00± 2 0.33±0.82	0	100 12.33±10.61 1-26 12.33±10.61	50 1.67±0.58 1-2 0.83±0.98	0	16.67 1.00± - 1 0.17±0.41
2 (N=20)	48.39 3.2±2.65 1-9 1.55±2.43	35.48 11.82±8.16 4-30 4.19±7.43	35.48 4.73±5.14 1-15 1.68±3.75	61.29 10.68±8.46 1-28 6.55±8.42	90.32 26.75±35.82 1-157 24.19±35.05	35.45 6.00±5.85 1-17 2.13±4.46	3.22 1.00± - 1 0.03±0.18	48.38 3.07±1.79 1-6 1.48±1.98
> 2 (N=13)	40 1.83±1.17 1-4 0.76±1.09	86.67 26.85±40.04* 3-150 21.41±36.17	53.33 52.13±93.19 1-241 25.65±66.86	33.33 7.60± 10.04 1-25 2.53±6.17	93.33 42.36±49.99 6-191 37.35±46.63	40 6.17±5.49 2-17 2.18±4.32	46.67 20.14±19.76* 1-53 8.29±15.84	33.33 15.00 ±23.74 1-57 4.41±13.80

CS= Copepodid stage * significant value

Abundance of *Trichodina* sp. (Table 4) was significantly greater in the eastern parts of the wetland compared with other stations ($\chi^2 = 7.954$, $p < 0.05$ and $\chi^2 = 8.011$, $p < 0.05$, respectively).

Prevalence and abundance of *D. spathaceum*, *I. multifiliis*, *D. extensus* and *C. fimbriceps* in females (Table 5) were not-significantly greater than in males (*Z* test, $p > 0.05$ and KW, $p > 0.05$, respectively).

Table 4: The prevalence, mean intensity and abundance of some parasites of *C. carpio* in different localities

Parasite	<i>Diplostomum</i>	<i>Trichodina</i>	<i>Ichthyophthirius</i>	<i>Lernaea</i> ^(CS)	<i>D. extensus</i>	<i>Raphidascaris</i>	<i>Caryophyllaeus</i>	<i>Diplozoon</i>
locality	Prevalence (%) Mean±SD Range Abundance±SD	Prevalence (%) Mean±SD Range Abundance ±SD	Prevalence (%) Mean±SD Range Abundance ±SD	Prevalence (%) Mean±SD Range Abundance ±SD	Prevalence (%) Mean±SD Range Abundance±SD	Prevalence (%) Mean±SD Range Abundance±SD	Prevalence (%) Mean±SD Range Abundance ±SD	Prevalence (%) Mean±SD Range Abundance ±SD
Central Part (N=21)	28.57 3.33±2.80 2-9 0.95±2.09	28.57 10.00±0.00 10 2.86±4.63	38.10 53.25±92.58 1-241 20.29±60.84	57.14 10.17±8.67 1-18 5.81±8.24	90.48 40.42±54.45 1-191 36.62±53.18	28.57 8.17±7.11 1-17 2.33±5.19	4.76 24.00± - 24 1.14±5.24	42.86 2.67±1.58 1-6 1.14±1.68
East Part (N=20)	70 2.64±2.34 1-9 1.85±2.30	70 25.00±38.03 * 4-150 17.50±33.58	55 5.45±4.59 1-15 3.00±4.34	55 11.09±8.68 1-28 6.10±8.47	85.71 25.06±24.08 3-78 22.55±24.04	35 2.71±1.50 1-5 0.95±1.57	15 4.00±5.20 1-10 0.60±2.23	40 11.13 ±18.75 1-57 4.45±12.68
West Part (N=13)	46.15 1.33±0.52 1-2 0.62±0.77	46.15 14.00±17.91 3-50 6.46±13.65	23.08 1.33±0.58 1-2 0.31±0.63	15.38 1.00± - 1-2 0.15±0.38	100 18.38±19.81 4-191 18.38±19.81	53.85 5.71±5.49 1-17 3.08±4.87	30.77 26.50±23.78 4-53 8.15±16.74	30.77 2.25±1.89 1-5 0.69±1.44

CS= Copepodid stage * significant value

Table 5: The prevalence, mean intensity and abundance of some parasites of *C. carpio* in males and females.

Parasite	<i>Diplostomum</i>	<i>Trichodina</i>	<i>Ichthyophthirius</i>	<i>Lernaea</i> ^(CS)	<i>D. extensus</i>	<i>Raphidascaris</i>	<i>Caryophyllaeus</i>	<i>Diplozoon</i>
Sex	Prevalence (%) Mean±SD Range Abundance ±SD	Prevalence (%) Mean±SD Range Abundance±SD	Prevalence (%) Mean±SD Range Abundance ±SD	Prevalence (%) Mean±SD Range Abundance ±SD	Prevalence (%) Mean±SD Range Abundance±SD	Prevalence (%) Mean±SD Range Abundance ±SD	Prevalence (%) Mean±SD Range Abundance ±SD	Prevalence (%) Mean±SD Range Abundance±SD
Male (N=29)	41.38 2.67±2.53 1-9 1.10±2.08	44.83 23.31±40.06 3-150 10.45±28.76	37.93 18.00±45.94 1-156 6.83±28.86	44.83 9.00±8.97 1-28 4.03±7.43	89.66 21.92±24.53 1-105 19.66±24.16	41.38 5.00±4.31 1-16 2.07±3.68	17.24 20.80±24.13 1-53 3.59±12.13	44.83 3.15±2.70 1-10 1.41±2.38
Female (N=18)	61.11 2.45±2.30 1-9 1.5±2.15	55.56 14.50±12.79 5-50 8.06±11.90	50 31.56±78.70 1-241 15.78±56.37	55.65 10.40±9.19 1-31 5.78±8.54	94.44 44.18±53.59 4-191 41.78±53.15	94.44 8.20±8.04 2-17 2.78±5.43	27.78 12.67±10.26 4-24 2.11±6.00	16.67 33.33 1-10 4.00±13.33

CS= Copepodid stage

4. Discussion

Forty two species of parasites of common carp (*C. carpio*) of Iran, including 12 protozoans (*Chilodonella* sp., *Cryptobia* sp., *Dermocystidium* sp., *Ichthyobodo* sp., *Goussia carpeli*, *Ichthyophthirius multifiliis*, *Myxobolus lobatus*, *Myxobolus* sp., *Tetrahymena* sp., *Trichodina dumerguei*, *Trichodina* sp. and *Trichodinella* sp.); 14 monogeneans (*D. achmerowi*, *D. anchoratus*, *D. dulceity*, *D. extensus*, *D. sahuensis*, *D. vastator*, *Diplozoon* sp., *D. paradoxum*, *G. cyprini*, *G. elegans*, *G. shulmani*, *G. sporostoniae*, *G. stankovici* and *Tetraonchus monenteron*); 4 digeneans (*Ascocotyle* sp., *Diplostomum spathaceum*, *Echinochasmus* sp. and *Ichtyocotylorus* sp.); 4 cestodes (*Bothriocephalus gowkongensis*, *Caryophyllaeus brachycollis*, *C. fimbriceps* and *C. laticeps*); 1 nematode (*Contraecaecum* sp.); 2 acanthocephalans (*Acanthocephalorhynchoides cholodkowski* and *Neoechinorhynchus rutili*); and 3 crustaceans (*Argulus foliaceus*, *Ergasilus peregrines* and *Lernaea* sp.) have been reported by many authors Jalai & Barzegar, 2004; Ghorbanzadeh, 1995; Moradi, 1993; Mokhayer, 1980; Jalai; 1998; Fadaei Fard et al., 2001).

In this study, 10 parasite species were found in common carp, of which, two species, *Raphidascaris acus larvae* and *Posthodiplostomum* sp. are reported for the first time. Vilizzi et al., (2015) reported occurrence of *Posthodiplostomum cuticola* from feral common carps in Eber Lake, a natural lake in Turkey.

Many piscivorous fishes have been reported as host of adult *R. acus*. But, the main definitive host of *R. acus* is pike, *Esox lucius* (Moravec, 1970). Although, oligochaetes and chironomid larvae (natural paratenic hosts) are the most frequent hosts of *R. acus*, but the larval stage of this nematode occurs in a number of fish species of various fish families, serving either as intermediate or paratenic hosts (Moravec, 1994). *R. acus* has been previously reported from *E. lucius* and *S. glanis* in the southern part of the Caspian Sea (Daghigh Roohi et al., 2014a and Sattari, 1996). The occurrence of its larvae has

also has been reported from *Tinca tinca*, *Carassius auratus gibelio* and *Abramis brama orientalis* (Daghigh Roohi et al., 2014b and Sattari, 1996) and *S. erythrophthalmus* (Khara et al., 2004). Finding the larval stages of the nematode in *C. carpio* here is reported for the first time (a new host record).

As shown in Table 2, the abundance of *Lernaea cyprinacea* in summer season was significantly greater than the other seasons. This parasite is one of the most well-known parasitic crustaceans that reproduces continuously in warm seasons when the temperature is suitable for growth and reproduction of this parasite, i.e., 22-30 °C (Sharifian, 2015). At this temperature, *Lernaea* completes the life cycle within 17-22 days and its adult type sets on the skin of new hosts. So significantly greater incidence of *L. cyprinacea* in this season in Anzali Wetland seems logical.

The monozoic tapeworm *Caryophyllaeus laticeps* (Pallas, 1781) is a common parasite of cyprinids (Karanis and Taraschewski, 1993). According to Amlacher (1992) bream, *Abramis brama* (L.), and white bream (*B. bjoerkna*) are the major hosts for *C. laticeps*, while the congeneric species *Caryophyllaeus fimbriceps* is mainly found in *C. carpio*. In the southern part of the Caspian Sea, *C. laticeps* has been reported from *C. carpio*, and *C. fimbriceps* from *C. carpio* and *A. brama orientalis* and *T. tinca* (Sattari, 1996).

Abundance of *Caryophyllaeus laticeps* was significantly greater in autumn. Bertasso and Avenant-Oldewage (2005) reported that temperature has a significant effect on cestodes with a heteroxenous life cycle. In Table 3, the abundance of *Trichodina* sp. and *Caryophyllaeus fimbriceps* in fishes by age older than 2 years were significantly greater than the younger ones. Since, *Trichodina* is an external parasite. its infestation is directly related to the size of the host. Tubifex is the intermediate host for *Caryophyllaeus fimbriceps*, any increase in age and feeding enhances chance of infestation.

The special diet of the host fish specimens, preferring potential parasite intermediate hosts like, amphipods and aquatic insect larvae, seems to be the

main reason for the low or high diversity of their helminth parasite community (Poulin, 2007).

As shown in Table 4, the abundance of *Trichodina* sp. in Eastern parts of Anzali Wetland was significantly greater than the western and central areas. This seems to be related to the greater amounts of organic matters of the discharged wastewaters.

High prevalence of monogenean in the present study (92.65%) coincides with some other studies carried out on common carp (95.4%) in Syrian fish farm (Al-Samman et al., 2006). Previous studies in Iran, show that feral form of common carp living in the Caspian Sea basin were infested with only two species of Dactylogyrus, *D. extensus* and *D. anchoratus* (Jalali 1998). Among Dactylogyrus species found on common carp of Iran, *D. anchoratus* and *D. extensus* need special attention due to their low range of host specificity and high tolerance to a wide range of temperature and salinity. These characteristics make them the most successful among invading parasites of fishes. Dactylogyrus *extensus* infestation is also common in Turkey. Feral common carp infestation to *D. extensus* has been reported over eleven natural lakes and nine man-made reservoirs of Turkey (Vilizzi et al., 2015).

The occurrence of *D. spathaceum* metacercariae, a digenean trematode, has been reported from several fish species in the southern part of the Caspian Sea consisting of *T. tinca*, *C. auratus* gibelio, *C. carpio*, *A. brama* orientalis, *E. lucius*, *P. fluviatilis*, *S. glanis* *Vimba vimba persa*, *Alburnus chalcoides*, *Rutilus rutilus caspius* and *H. molitrix* (Sattari, 1996; Daghigh Roohi, 1997). Eye fluke *D. spathaceum* is a common parasite between the Anzali Wetland fishes. It seems that the reason is the availability of intermediate hosts in this Wetland.

In this study, the parasite fauna on common carp in Anzali wetland was determined and also variance of infestation rates were investigated in relation to location, seasons, sex and age of host. It can thus be suggested that probably feeding activity of fish host in different seasons and age classes play a role in

infestation to *C. laticeps*. On the other hand, infestation to *L. cyprinacea* depends on the season and infection is more common in summer. It seems none of the identified parasites in fish is considered a serious threat to this species. Possibly restoring the ecological condition of Anzali wetland and preventing overfishing will help in rebuilding of this fish stocks in Anzali wetland.

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