

Occurrence of *Clinostomum Complanatum* (Rudolphi, 1819) (Digenea: Clinostomatidae) in *Capoeta capoeta gracilis* (Osteichthys: Cyprinidae) from Shiroud River, Iran

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ABSTRACT

112 specimens of *Capoeta capoeta gracilis* from the Shiroud river were examined. The prevalence and abundance of parasites were significantly higher in the area between the mouth to behind the opercle. Susceptibility of infection was not significantly different between male and female fish. The prevalence of infection was the lowest in the largest length group. Histopathological study revealed blood feeding metacercaria cause myositis.

INTRODUCTION

Family *Clinostomatidae* has a widespread distribution. Members of this family have been recorded from the area where mean temperature is about 10°C (11). *Clinostomum complanatum* (Rudolphi, 1819) is a digenetic trematode which causes yellow grubs in the muscle of fish and make them unsuitable for human consumption. If a human accidentally consumes raw or semicooked infected fish, the fluke attaches to the mucus membrane of the human oesophagus and causes injury (26).

Infected cases have been reported from Japan and Korea (8,12,15). The metacercaria of *C. complanatum* have recorded from *Perca fluviatilis* (11), *Rutilus rutilus* (11), *Aphanius dispar* (17), *Plecoglossus altivelis* (18), *Salmo trutta gairdneri* (27), *Misgurnus anguillicaudatus* (16,19), *Carassius carassius*, *C. gibelio langsdorfi*, *C. cuvieri*, *Cobitis anguillicaudatus*, *Cyprinus carpio*, *Pseudogobio esocinus*, *Pseudorasbora parva*, *Rhodeus lanceolatus*, *R. ocellatus* and *Hypomesus transpacificus*(4,6) *Carassius* spp. (1), *Morone chrysops*(25), *Zacco temminckii*, *Acheilognathus rhombea*, *Microphysogobio yaluensis*, *Carassius auratus* (9), *Lateolabrax japonicus*, *Leuciscus hakonensis* (5) and *Eutycea neotenes* (Texas salamander)(24).

Adult stage of parasite has been reported from *Nycticorax nycticorax* (7, 16, 28), *Ardea cinerea* (7, 13, 14, 18), *Egretta alba* (2, 22), *Egretta garzetta*, and *Egretta intermedia* (7, 22), *N. caledonicus* and *A. novaehollandiae* (22). The cercaria of *C. complanatum* have been recorded from some snails including, *Lymnaea auricularia* (13, 14) but *L. japonica* and *L. ollula* were recorded only as experimental hosts (3).

Present study aimed to consider variation in the abundance and prevalence of *C. complanatum* in *Capoeta capoeta gracilis* with regard to host sex, length groups and microhabitats.

MATERIALS AND METHODS

A total of 112 *Capoeta capoeta gracilis* and some *Pseudoraspora parva*, *Carassius auratus*, *Leuciscus cephalus*, *Alburnoides bipunctatus* were examined. Fish caught by cast net with 1.5m diameter and 6mm mesh from the Shiroud river (50° 48'0"E, 36°51' 48"N) 15 Km east of Ramsar, Iran (in the vicinity of the Caspian Sea).

Collected fish and snails were transferred live to the laboratory of the Fisheries Research Centre of Mazandaran province. The standard length and weight of each fish were measured to the

nearest millimetre and milligram, respectively and their sex was determined.

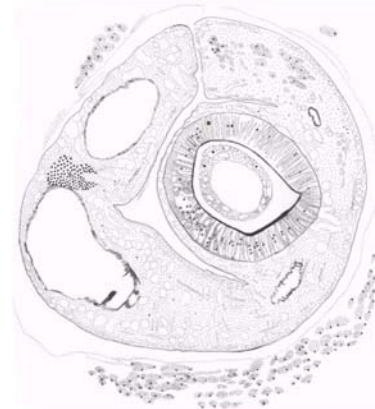
Fish were examined for the presence of external bumps (called yellow grubs) then dissected and studied internally for metacercarial cyst in the muscles and visceral organs. The locations of cyst and number of cyst were recorded.

Cysts were opened using two sharp needles, metacercaria were transferred into saline (6.5‰), then fixed in 4% formalin. The metacercaria were stained by carmine-Alum, washed in acid alcohol, dehydrated in an alcohol series, cleared in xylol and mounted in Canada balsam. Data were analysed by SPSS statistical package version 6.1 (SPSS Inc.). The ecological terms used for parasite infection are derived from a previous study (21). Prevalence is the number of infected fish divided by the number of fish examined and abundance is the total number of parasite individuals in the fish population divided by total number of fish.

A camera lucida was used to draw the excysted and encysted metacercaria (Figures 1 and 2).

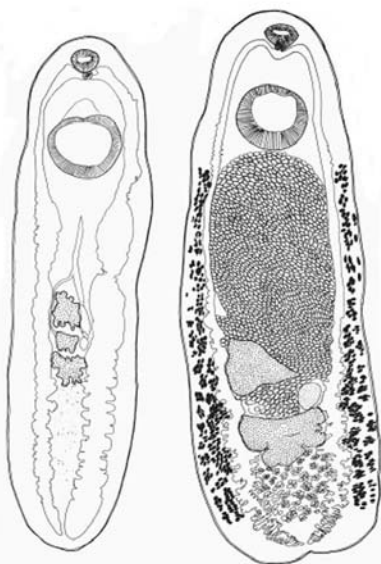
In order to find primary host in Shiroud river, snails were collected from two stations on this river 150 m apart.

Fig. 1. Metacercariae of *Clinostomum complanatum* (Rudolphi, 1819) from *Capoeta capoeta gracilis*



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Fig. 2. Metacercariae of *Clinostomum complanatum* (Rudolphi, 1819)(yellow grub) from the muscle of *Capoeta capoeta gracilis*($\times 100$)(left:Larvae; right:adult).



Collected snails were studied morphologically and after identification were crushed and studied under a dissecting microscope for cercaria. Some of them were placed in a Petry dish containing saline 6.5 ‰ and exposed to the direct light of a desk lamp. Then water content of Petry dish was examined under a high power microscope for the presence of swimming cercaria.

RESULTS

Except *Capoeta capoeta gracilis* and one specimen of *Pseudoraspora parva*, no other species caught in the Shiroud river (including *Carassius auratus*, *Leuciscus cephalus*, *Alburnoides bipunctatus*) were infected with metacercariae of *Clinostomum complanatum*.

The abundance and prevalence (expressed as percentage) of parasites in *Capoeta capoeta gracilis* were 3.77 ± 0.85 and 47.3, respectively. The number of parasites varied from one to a maximum of 60 per individual fish. The percentage frequency of grouped total parasite specimen numbers in *Capoeta capoeta gracilis* is shown in Fig. 3.

The abundance (Fig.4) and prevalence (Fig. 5) of parasites in different microhabitats were as follows: under the mouth to behind the opercle (2.12 ± 0.49 , 38.4), inner wall of opercle (0.28 ± 0.08 , 12.5), around the orbits (0.3 ± 0.09 , 12.5), in the muscle of different fins including pectoral (0.21 ± 0.12 , 6.3), dorsal (0.28 ± 0.10 , 12.5), anal (0.22 ± 0.06 , 13.4), pelvic (0.14 ± 0.09 , 4.5), caudal (0.14 ± 0.05 , 9.8) and the other area of body (0.07 ± 0.05 , 2.7).

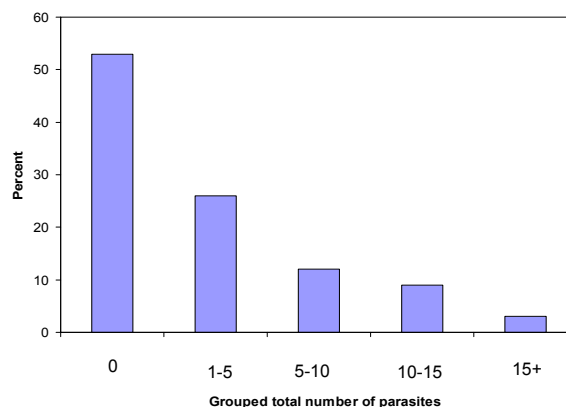
The prevalence ($\chi^2 = 89.95918$, $P < 0.000005$) and abundance ($F = 10.99$, $P < 0.00005$) of parasites in the area under the mouth to behind the opercle are significantly higher than the other microhabitats.

Sex ratios of fish were 3:5 in favour of female. Prevalence of parasite in male and female were 36.4, 54.4, respectively, but

there is not a significant difference between them ($\chi^2 = 3.49073$, $P = 0.062$). The abundance of parasites is higher in female (4.90 ± 1.35) than male (2.02 ± 0.50), but the differences is not significant ($t = 0.048$, $P = 1.438$).

The prevalence and abundance of parasites in different size group are summerised in Table 1.

Fig.3. The percentage frequency of grouped total parasite specimen numbers in *Capoeta capoeta gracilis*



The variation of abundance is not significantly different in length groups ($F = 2.1205$, $P = 0.02497$) but the prevalence shows a significant difference between the size groups ($\chi^2 = 9.3502$, $P = 0.02497$).

When infected snails are exposed to the direct light, the cercaria would leave the snails body as a result of positive phototactism. By using this phenomenon and crushing the snails, 209 snails (158 *Lymnaea gedrosiana*, 31 *Physa acuta*, 20 *Planorbis*) caught in the Shiroud river were examined, but no cercariae were found.

In the histopathological study of yellow grubs, in the caecum of trematodes blood were observed and metacercariae were surrounded with the connective tissue. Infiltration of inflammatory cells with mild haemorrhagy was observed in some muscular fibres. Hyaline degeneration was observed, indicating myositis.

DISCUSSION

During the course of present study, only *Capoeta capoeta gracilis* and one specimen of *Pseudoraspora parva* were found to be infected with metacercaria of *Clinostomum complanatum*. Four species namely; *Cyprinus carpio*, *Aphanius sophiae*, *Carrasius auratus*, *Barbus* sp. have been studied (13), but amongst them only *Aphanius sophiae* were infected with *Clinostomum complanatum*. It seems parasite has a high specificity to its host. In the Shiroud river, the parasites in infected fish observed to be causing cellular inflammatory reaction around metacercaria. This bumps easily can be seen by humans and normally they do not consume such fish, but in some fish from the Shiraz area many metacercaria were found in their muscles and viscera, causing no inflammatory reaction (personal observations). Two forms of occurrence of metacercaria in fish (encysted and excysted) possibly indicate

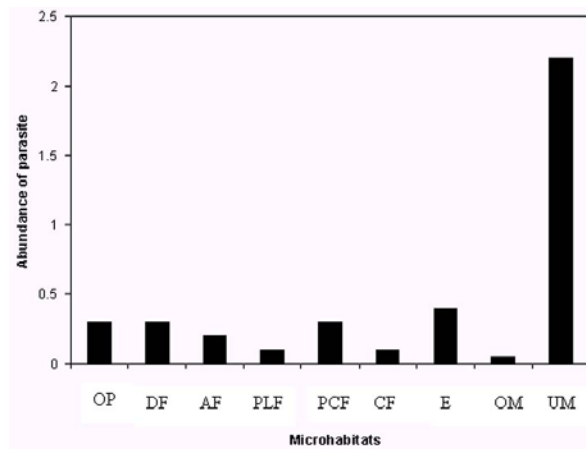
some fish develop resistance to the metacercarial stage of *C. complanatum*. The later form (excysted metacercaria in the muscle) could be more harmful to human health when they use semi-cooked fish. Furthermore, some people use raw fish traditionally in treating the jaundice which cause infection in humans.

Some cases of halzoun, laryngopharyngitis, reported from Japan (12, 15, 20) and Korea(8). In humans, as in herons, the parasites firmly attaches to the mucus membrane of the pharynx.

Excystment of metacercaria were observed in *Misgurnus anguillicaudatus* but in this fish excysted metacercaria caused serious damage to host (19).

In *Aphanius sophiae* prevalence of infection in male and female were observed to be 53.5 and 32.9, respectively (13).

Fig. 4. Variations in the abundance of *Clinostomum complanatum* in different microhabitats of *Capoeta capoeta gracilis*



(AF=anal fin, CF=caudal fin, DF=dorsal fin, E=eye, Om=other microhabitats, OP=opercle, PCF=pectoral fin, PLF=pelvic fin, UM=Under the mouth to behind the opercle)

In *Chana punctatus* prevalence was found to be 50 among females and 33 among the male (10). In *Aphanius dispar* 47.5% male and 55.9% female fish were infected (17).The result of present study showed there is no significant difference in susceptibility of infection between male and female.

Usually one expects larger fish have more parasites as they have been exposed to infection for the longer time but, in length group 4 (fish above 16 cm length), the prevalence of parasites significantly decrease and no significant difference were

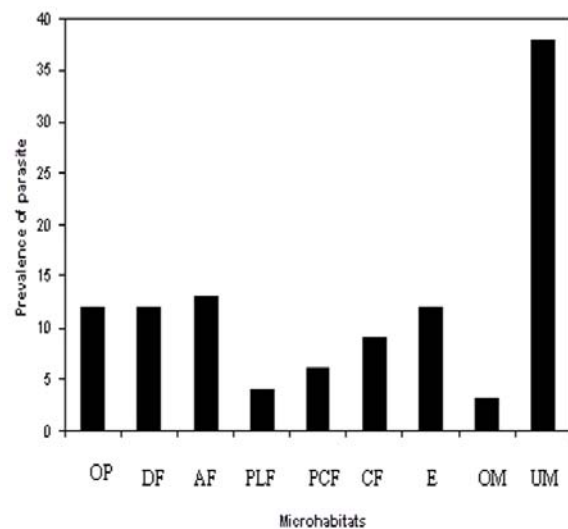
Table 1. Variations in the abundance and prevalence of *Clinostomum complanatum* in different length groups of *Capoeta capoeta gracilis* from Shiroud river

Length groups	N	Abundance ±SE	Prevalence
Less than 7cm	27	0.93±0.35	37
7-11cm	50	4.77±1.30	60
11-15cm	26	6.04±2.55	42.3
Above 15cm	9	0.44±0.44	11.1

observed in abundance. Decrease in the prevalence of infection in the larger fish (above 16 cm) could be as a result of increase in the mortality of infected fish, increase in the resistance of the host with increase in length, change of feeding habit of the host.

The higher prevalence and abundance of parasites in the area under the mouth to behind the opercle is probably due to the presence of *Clinostomum complanatum* which feed on blood, feeding in the very vasculated area. It has been found that the main habitat of *C. complanatum* in *Carassius spp.* was the muscles around the gills (1).

Fig. 5. Variations in the prevalence of *Clinostomum complanatum* in different microhabitats of *Capoeta capoeta gracilis*



(AF=anal fin, CF=caudal fin, DF=dorsal fin, E=eye, OM=other microhabitats, OP=opercle, CF=pectoral fin, PLF=pelvic fin, UM=under the mouth to behind the opercle).

In the histopathological sections infiltration of inflammatory cells was observed which happen as a result of immune reaction. This has also been recorded in *A. Dispar* (17). Also degenerative changes in the muscle tissues around the cyst, pressure atrophy and degeneration of hepatic cords as well as cellular infiltration in the kidney was found. It was observed in *Misgurnus anguillicaudatus* that metacercaria caused muscle atrophy and serious damage to fish tissue and body wall, which resulted in fish mortality (19).

As snails were not infected, at the present stage of knowledge the source of infection is known.

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