Parasitic infection among larvae and fingerlings of the Persian sturgeon (*Acipenser persicus*) in Vniro tanks and earthen ponds

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Abstract

This study was conducted in two phases on sturgeon larvae and fingerlings produced from artificial breeding of five pairs of the Persian sturgeon (*Acipenser persicus*) broodstocks in Shahid Beheshti Hatchery in spring and summer 2006. Initially, 600 larvae were collected on 3 post days hatching (pdh) and 5 days after the onset of exogenous feeding. The second phase was conducted with 450 specimens collected from five earthen ponds, which included 150 larvae collected one week after stocking, 150 fingerlings collected 20 days after stocking and 150 fingerlings collected at the time of their release into rivers. No parasite was observed in the sturgeon larvae on day 3 pdh and 5 days after the onset of exogenous feeding. However *Trichodina reticulata* were observed in the larvae in the first week (prevalence = 10 - 20%) and also in the fingerlings (prevalence = 10 - 46.67%) 20 days after they were transferred to the earthen ponds. At the time of their release into the river, in addition to *T. reticulata* (prevalence = 13.33 – 100%) a digenean trematode, *Diplostomum spathaceum,* (prevalence = 6.67 - 30%) was also observed in the sturgeon fingerlings. It is evident from the present study that *Trichodina* and *Diplostomum* infection occurs after fingerlings are released into the earthen ponds and gradually increases with the progress in the rearing period. Increase in prevalence of *Trichodina* infection through the rearing period can be explained by the increase in water temperature and increase in dissolved organic matter in the ponds which provide the desirable conditions for the propagation of this unicellular ciliate. Significant differences were observed in the mean intensity of *T. reticulata* infection in sturgeon fingerlings during different stages of rearing into earthen ponds ($P<0.05$).

Keywords: *Acipenser persicus,* Parasite, Larvae, Fingerling, *Trichodina reticulata*, *Diplostomum spathaceum*

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Introduction

By rearing fish in good environmental condition, optimum fish health can be achieved (Winton, 1996). The unfavorable environmental conditions have seen in fish farms (overcrowding, poor hygiene, temperature variations, etc.) greatly favour disease transmission (Aguilar et al., 2005). Various protozoan and metazoan parasites can reside either inside or on the surface of the host. Pathogens or parasites do not always cause disease in fish, but may be present in a subclinical or carrier state (Barber, 2007). Parasites are among the important factors responsible for weight loss, disruption of reproduction or impotency, blindness, abnormal behavior, epithelial lesions, deformities of gills and other symptoms that ultimately lead to economic loss in the fish industry. Moreover external parasites may cause secondary fungal, bacterial and viral infections or act as carriers of bacteria, virus and other pathogens (Azari Takami, 1997). Several researchers (Dogiel and Bykhovskiy, 1939; Rahmani, 1985; Bauer et al. 2002; Pazooki and Masoumian, 2004; Sattari and Mokhayer, 2005) studied parasitic infection in sturgeon spawners. Niyak et al. (1970) studied *Trichodina* sp. contamination in sturgeons. Investigations on parasitic fauna in sturgeon fingerlings conducted by Mokhayer (1981), Ghoroghi (1996) and Shenavar Masouleh et al. (2003) in Iran are also worth mentioning. In view of the importance of parasitical factors, the extent of parasite infection under hatchery conditions was studied in sturgeon larvae and fingerlings. Sturgeons are susceptible to invasions of parasites during different stages of rearing. This will affect their normal growth and development and also jeopardize their culture. Considering the significance of disease control, the present study was conducted to determine the parasitical fauna in sturgeon larvae in Vniro tanks and sturgeon fingerlings in earthen ponds and to estimate the percentage prevalence and intensity of parasites and to study the effects of parasite intensity on sturgeon growth.

Materials and methods

The study was coducted in two phases from May to July 2006 (in May in Vniro tanks and in June & July in earthen ponds), in Shahid Beheshti Sturgeon Hatchery located in northern Iran. In the first phase, 600 sturgeon larvae produced from five pairs of *Acipenser persicus* spawners were studied. A total of 120 larvae were collected from each batch; 60 larvae on 3 post days hatching (pdh) and 60 larvae were collected 5 days after the onset of exogenous feeding (from five Vniro tanks). In the second phase 450 sturgeon fingerlings collected from five earthen rearing ponds were studied. Fingerlings were collected randomly using bottom trawl (mesh size = 5 mm) one week after stocking, 20 days after stocking and at the time of their release into rivers (30 fingerlings from each earthen pond at each stage).

The experiments were conducted under identical conditions, following completely randomized design. The Vniro tanks (260 cm initial diameter, 30 cm height and 1600 l volume) and earthen ponds (200 m length, 100 m width and 2.30 m height) were supplied with the water from the Sefidroud River. Dissolved

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oxygen, pH and temperature in water ranged from 6.12 - 6.36 mg/l, 7.5 – 7.62 and 17.6 – 1.7.9 °C in May, 6.69 - 6.92 mg/l, 7.8 – 8.1 and 23.3 – 24.1 °C in June and 3.1 – 4.3 mg/l, 7.31 – 7.98 and 25 – 26.4 °c in July, respectively. The feeding regime for larvae in Vniro tanks is *Artemia salina* & *Daphnia* spp., however fingerlings in the earthen ponds mainly feed on Cladocera (including *Daphnia* spp.) and Chironomidae. Total length (to the nearest 0.1 cm) and weight (to the nearest 0.001 g) of the larvae and fingerlings were recorded at each stage. Wet slides from the external and internal surface of larvae and from skin, fins, gills, eyes and digestive tract of fingerlings under study were prepared and parasites were identified following standard methods (Stoskope, 1993). Standard statistical computations (prevalence, mean intensity, standard deviation) were calculated for all samples. Mean intensity was determined by dividing the total number of recovered parasites by the number of infected fish samples. Prevalence was also calculated by dividing the number of infected fish samples by the total number of examined ones and expressed as a percentage (Microsoft Excel, 2003). Kolmogorov-Smirnov test was used to normalize the data and histogram estabilished. In order to compare mean intensity of parasite infection in first week of stocking, 20 days after stocking and time of releasing to the river one – way ANOVA was used. Also, Tukey test applied to compare among groups. Chi-Square employed to compare the prevalence of parasites in the mentioned stages. The relationship between intensity of parasite infection with length and weight were compared with Pearson correlation coefficient (SPSS, version 14).

Results

No parasitic infection was observed in 3 dph larvae collected from Vniro tanks (mean weight = 22.5 ± 1.43 mg; n = 300) and in larvae collected five days after the onset of exogenous feeding (mean weight = 64.6 ± 21.37 mg; n = 300) (Tables 1 and 2).

Table 1: Parasitic infection in 3 dph larvae; n=300

Tank No.	n	Mean total length(mm) ± SD	Mean weight (mg) ± SD	Parasite
1	60	12.7 ± 1.15	23.3 ± 1.56	-
2	60	12.2 ± 0.96	21.5 ± 1.66	-
3	60	13.1 ± 0.79	20.6 ± 1.33	-
4	60	13.2 ± 0.65	24.1 ± 1.35	-
5	60	12.8 ± 0.89	23.1 ± 1.87	-

Table 2: Parasitic infection in larvae five days after the onset of exogenous feeding; n=300

Tank No.	n	Mean total length (mm)± SD	Mean weight (mg) ± SD	Parasite
1	60	20 ± 1.14	47.7 ± 7.64	-
2	60	21.2 ± 1.27	49.8 ± 4.34	-
3	60	21.3 ± 1.41	49.7 ± 9.37	-
4	60	24.1 ± 1.61	90.3 ± 21.61	-
5	60	23.5 ± 2.19	85.6 ± 20.29	-

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Examination of fingerlings (mean weight = 297.4 ± 75.18 mg; n = 150) one week after they were stocked into earthen ponds showed that specimens from two of the earthen ponds were infected with *T. reticulata* (Fig. 3) with a prevalence of 10 - 20% and an intensity range of 6 - 8 per larvae. *T. reticulata* (prevalence = 10 - 46.6%; intensity range = 5 - 185) were also observed in fingerlings (mean weight = 1.22 ± 0.51 g; n = 150) collected from three of the earthen ponds 20 days after they were stocked. At the time of releasing fingerlings (mean weight = 3.22 ± 1.02 g; n = 150) into the river) after 40 - 45 days rearing in earthen ponds) in addition to *T. reticulata* (prevalence = 13.33 – 100%; intensity range = 5 - 510) a digenea trematode, *D. spathaceum* (Fig. 4) (prevalence = 6.67 - 30%; intensity range = 1 - 4) was also observed in the sturgeon fingerlings in all five ponds studied (Tables 3 to 5).

In this study according to

Table 3: Parasitic infection in sturgeon fingerlings one week after stocking in earthen ponds; n=150

Pond No.	*T. reticulata*			*D. spathaceum*		
	Prevalence (%)	Mean intensity ± SD	Intensity range	Prevalence (%)	Mean intensity ± SD	Intensity range
1	10	± ERR 6	6	-	-	-
2	-	-	-	-	-	-
3	20	1.41 ± 7	6 - 8	-	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-

Table 4: Parasitic infection in sturgeon fingerlings 20 days after stocking in earthen ponds; n=150

Pond No.	*T. reticulata*			*D. spathaceum*		
	Prevalence (%)	Mean intensity ± SD	Intensity range	Prevalence (%)	Mean intensity ± SD	Intensity range
1	26.67	39.25 ± 59.49	8 - 185	-	-	-
2	-	-	-	-	-	-
3	46.67	36.81 ± 23.17	5 - 80	-	-	-
4	10	80 ± 42.72	40 - 125	-	-	-
5	-	-	-	-	-	-

Table 5: Parasitic infection in sturgeon fingerlings at the time of their releasing into the river; n = 150

Pond No.	*T. reticulata*			*D. spathaceum*		
	Prevalence (%)	mean intensity ± SD	Intensity range	Prevalence (%)	mean intensity ± SD	Intensity range
1	63.33	111.05 ± 132.98	10 - 460	30	1.44 ± 1.01	1 - 4
2	100	144.73 ± 141.94	15 - 510	13.33	1.25 ± 0.5	1 - 2
3	93.33	77.25 ± 83.89	5 - 285	-	-	-
4	13.33	55.75 ± 19.12	35 - 80	-	-	-
5	66.67	43.25 ± 32.86	5 - 110	6.67	1.5 ± 0.71	1 - 2

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statistical results, significant differences were detected in the mean intensity of *T. reticulata* infection in sturgeon fingerlings during the different stages of rearing in earthen ponds (P<0.05). The results of study shows no significant differences in the intensity of *D. spathaceum* infection in sturgeon fingerlings during the different stages of rearing in earthen ponds (P>0.05). Also, results of Pearson correlation analysis indicated no significant correlation between weight (P>0.05, r=0.037) and length (P>0.05, r=0.031) in sturgeon fingerlings as a result of *Trichodina* infection. In addition, no significant positive correlation were obtained for Pearson correlation analysis between weight (P>0.05, r=0.108) and length (P>0.05, r=0.145) in sturgeon fingerlings due to *Diplostomum* infection.

According to Chi-Square test, the comparison of *Trichodina* prevalence in first week of stocking, 20 days after stocking and releasing time shows significant differences during three phases (Chi= 70.46, P= 0.00, df= 2). Figs 1 and 2 showed comparison of prevalence in *T. reticulata* and *D. spathaceum* in fingerlings during different stages of rearing in earthen ponds.

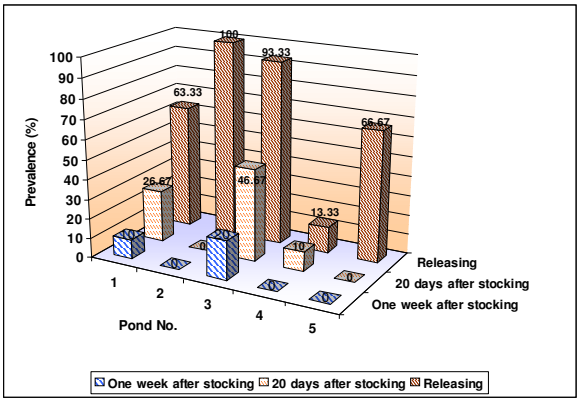

Figure 1: Comparison of prevalence in *T. reticulata* in fingerlings during different stages of rearing into earthen ponds

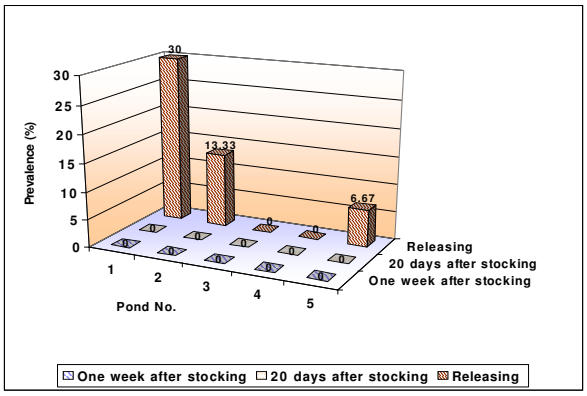

Figure 2: Comparison of prevalence in *D. spathaceum* in fingerlings during different stages of rearing into earthen ponds

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Figure 3: *T. reticulata* (X746), scale bar 10 µm

Figure 4: *D. spathaceum* (X373), scale bar 50 µm

Discussion

No parasitic infection was observed in larvae (3 pdh and 20 days after the onset of exogenous feeding) in the Vniro tanks. This is probably because the rearing period in Vniro tanks is very short and also due to the absence of intermediate hosts at this stage. Other reasons could be lower water temperature in this period compared to earthen ponds. The micro and macro conjugants of *T. reticulata* were found, not differing markedly in size. In the macroconjugants, a new ring of denticles is formed outside the resorbed old one. In some species of *Trichodina*, the center of the disc reveals a special pattern. It is reticulated in *T. reticulata* Hirschman and Partsch, 1955 an almost strictly host–specific ectoparasite of *Carassius carassius* and *C. auratus* all over their distribution range with diameter of attaching disk (AD) 44-57 µm and number of dentil (ND) 21 to 34. In

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Japanese fish farms, it caused considerable damage and mortalities in *C. carassius* and *C. auratus* and also in *Cyprinus carpio* (Lom and Dykova, 1992). It is to be noted that after the harvest of sturgeon fingerlings, the rearing ponds studied in the present study are also used to rear carp species, which could be a possible explanation for the occurrence of *T. reticulata* in sturgeon fingerlings studied in the present study. Investigations by Shenavar Masouleh et al. (2003) revealed that parasitic infection in sturgeon fingerlings in rearing ponds is limited to a narrow range of species. This is because the short period that the fingerlings are stocked in rearing ponds does not allow for the complete development of most parasites. The absence of intermediate hosts, unfavorable conditions in temperature for the reproduction and development of most parasites during the maintenance of fingerlings in earthen ponds are among the reasons for the low diversity of parasites in farmed fish (Jalali Jafari, 1998). *T. reticulata* was observed in 40% of the earthen ponds under study one week after stocking the sturgeon larvae in the ponds. This percentage increased to 60%, 20 days after stocking and to 100% at the time of release of the fingerlings into the river. Percentage of prevalence and mean and range of intensity also increased through the rearing period in earthen ponds. Significant differences were detected in the mean intensity of *T. reticulata* infection in sturgeon fingerlings during the different stages of rearing in earthen ponds. These results conform to the findings of Shenavar Masouleh et al. (2003).

Trichodina infestation (prevalence = 67.33%, mean intensity = 86.4 ± 41.52, intensity range = 5 - 510) in the present study was higher than that reported by Shenavar Masouleh et al. (2003). Severe incidences of contamination by symbiotic ciliates such as *T. reticulata* is normally reported in weak fishes or in fishes maintained under bad conditions. Increase in dissolved organic matter in water, over growth of aquatic vegetation in rearing ponds and over feeding of fish are among the factors that disturb the physical and chemical conditions of water and thus facilitate the reproduction and growth of this ciliate (Peyghan, 2001). Increase in prevalence of *Trichodina* infection through the rearing period can be explained by the increase in water temperature and increase in dissolved organic matter in the ponds which provide the desirable conditions for the propagation of this unicellular ciliate. However it was reported in the eyes of the fingerlings examined in 60% of the ponds studied at the end of the rearing period. The possible explanation for this could be that the parasite completes its life cycle after about 40 days which coincides with the end of the rearing period of fingerlings in the earthen ponds and the time of their release into rivers. No significant differences were detected in the intensity of *D. spathaceum* infection in sturgeon fingerlings during the different stages of rearing in earthen ponds. These results conform to the findings reported by Shenavar Masouleh et al. (2003). It has also been suggested that the infection affects the growth of fish by impairing their feeding efficiency. For example, Buchmann and Uldal (1994) studied the

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growth of rainbow trout (*Oncorhynchus mykiss*) exposed to the parasite at fish farming conditions and observed that larger fish had fewer parasites in the least infected eye. This suggests that fish may be able to feed sufficiently if the infection affects only one of the eyes. Furthermore, Owen et al. (1993) observed that even a low-level infection may affect the vision of threespined sticklebacks. However, effects of the parasite on fish growth have not been studied using experimental exposure of fish in a treatment-control setup. Furthermore, studies have not quantified cataract coverage in relation to fish growth although cataracts are likely to be the key factor affecting the vision of fish. Thus, generality of the effect of the parasite on fish growth, or its variation in relation to host species specific factors, has not been established in detail (Karvonen and Seppala, 2008). Results of Pearson correlation analysis indicated no significant correlation between weight and length in sturgeon fingerlings as a result of *Trichodina* infection which were similar to results obtained by Shenavar Masouleh et al. (2003). Also no significant positive correlations were obtained for Pearson correlation analysis between weight and length in sturgeon fingerlings as a result of *Diplostomum* infection. These results are in agreement with findings of Shenavar et al. (2003). In another investigation conducted on rainbow trout, Momin (1995) reported that there was no relationship between parasite intensity reported that and weight and length of larvae ($P>0.05$). However infections of the eye fluke *D. spathaceum* in cultured fish have received considerable attention because of their deleterious effects on fish (Buchmann and Uldal, 1994). According to Suomalainen et al. (2006) *D. spathaceum* infection in *Thymallus thymallus* make them more susceptible to *Aeromonas* infection. Considering *A. salmonicida* infection is pathogenic in fish (Austin et al, 1998), it is possible that a synergism occurs between this bacteria and *D. spathaceum* resulting in disease and mortality of fingerlings infected with *D. spathaceum.* All these studies indicate that although *Diplostomum* may not directly affect fish health it can indirectly cause severe losses in fish populations. It is for this reason that we have to take parasitic infection in hatcheries seriously. In order to prevent parasitic infection of fingerlings, pond preparation (complete drying of ponds at the end of each rearing period and liming), disinfection of ponds to prevent the presence of intermediate hosts, and measures include controlling water quality are strongly recommended.

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