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Cryptobia acipenseris and *Haemogregarina acipenseris* infections in *Acipenser guldenstadti* and *A. persicus* in the Southern part of the Caspian Sea

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ABSTRACT

A parasitological investigation on the blood of acipenseridae fish in the southern part of the Caspian Sea (Iran) from 2000-2002 was undertaken. Altogether, 60 specimens were examined. The fishes included to this study are considered as *Acipenser persicus* (Persian Sturgeon, 20 specimens), Acipenser guldenstadti (Russian Sturgeon, 20 specimens) and *Acipenser stellatus* (20 specimens). During this study two blood protozoa were revealed: *Cryptobia acipenseris*, and *Haemogregarina acipenseris*.The specimens were collected by gill net from a fishing station located at Anzali. The characteristics of the fishes were recorded and the collected materials were examined in fresh and stained preparation. The prevalence of infection was also studied. This is the first record of *Cryptobia acipenseris* and *Heamogragarina acipenseris* in Russian and Persian sturgeons (New host) in the southern part of the Caspian Sea (Iran). All fish specimens examined during this study were collected from their natural environment. Heamoflagellates were found in different sturgeon without any pathological effects, but in the intensive farming of Sturgeons, *Haemogragarina acipenseris* may lead to severe infections and may cause anemia which decreases the productivity of the farm.

Keyword: *Acipenseridae*, *Cryptobia acipenseris*, *Haemogregarina acipenseris*, Iran.

INTRODUCTION

Study on the parasites of sturgeon began in the 17th century when Linnaeus reported *Argulus foliaceus* and *Piscicola geometra* in 1758 and 1761. Other researchers recorded several other parasites from sturgeons. According to Holcik (1989), around 137 parasites have been recorded from five acipenseridae species in the Caspian Sea. (*A. stellatus* 25, *A. nudiventeris* 32, *A. gueldenstadti* 47 and *Huso huso* 33).

In contrast to other fish families indigenous to Eurasia very few protozoa have been demonstrated in acipenserids. Research on the blood protozoan of acipenserids goes back to the description and study of the development of *Haemogregarina acipenseris* Nawrotzky in 1914 (Adeleida, Adeleidae) (Shulman, 1984). *Haemogregarina* are parasites of erythrocytes of turtles, snakes, crocodiles and fish. The genus *Haemogregarina* is considered to be the only blood sporozoan found in fishes (Becker, 1970). *Haemogregarina acipenseris* was found only in erythrocytes of Sterlet in the Volga river basin and also in the Danube River (Baska, 1990; Masoumian and Pazooki, 2000). According to Perekrepov`s data (Perekrepov, 1930) *Haemogregarina acipenseris* causes severe anemia and cachexia in infested fish. It has a severe pathological effect on fish through mass infection. However, *Haemogregarina* are evidently harmless in marine fish, with

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no report existing on their pathogenecity (Lom, 1970).

Studies on heamoflagelates of *Acipenseridae* were carried out by Lwoff *et al*. in 1926 (Bykhovkaya *et al*., 1964). The authors described *Cryptobia acipenseris*. The vegetative and sexual stages of this protozoan were found in the blood of various sturgeons (Baur *et al*., 2003). *Cryptobia* are parasitic in invertebrates and fish. The majority of species live in blood but some of them are parasites of the intestines and gills as well. Blood sucking leeches serve as an intermediate host of flagellates which proliferate in their intestine.

Research on study of the parasites of sturgeons in Iran goes back to the work of Rostami in 1943 who recorded *Amphilina foliacea* from *Acipenser persicus*. Later on, Niak *et al* in 1970 studied different parasites in sturgeon fingerlings in the hatcheries of northern parts of Iran (Guilan Province). Mokhayer did the first intensive parasitological investigations on sturgeons in 1972 and published a checklist of acipenserid parasites and later on, in 1975, he studied ecological parasitology in Acipenseridae fish. These studies were continued by other researchers such as: Rahmani (1986), Pourgholam (1993), Shamsi and Jalali (1997), Jalali (1998), Satari (1999) and Hajimoradloo (2000). They recorded 24 different parasite species from acipenserids in the southern part of the Caspian Sea.

During this study two blood protozoan -*Cryptobia acipenseris* and *Haemogregarina acipenseris*- from *Acipenser persicus* Borodin 1897 (Persian Sturgeon) and *Acipenser guldenstadti* Brandt 1833 (Russian Stugeon) were recorded.

MATERIAL AND METHODS

During the present study, the protozoan parasite examination on the blood of *Acipenser guldenstaedti persicus* Bordin 1897 (Persian Sturgeon, 20 specimens), *Acipenser guldenstaedti* Brandt 1833 (Russian Sturgeon, 20 specimens) and *Acipenser stellatus* Pallas 1811 (20 specimens) were carried out. Fishes were caught from the southern part of the Caspian Sea. The specimens were collected from a fishing station located at Anzali. A fisherman caught the fish with a gill net. The characteristics of the fishes, including length, weight, sex and age were recorded and summarized in Table 1. The blood smears were taken and studied under a light microscope. From each specimen three blood smears were taken and fixed in 100% methanol for 30 seconds and stained with Gimsa solution. Before examining them, they were coated with immersion oil in order to make them better visible for the light microscopy. The parasites were identified and measured according to Lom and Dykova (1992) and Molnar (2003) (personal communication).

Table 1. Characteristics of the examined fishes.

Fish species	Length(cm)	Weight(Kg)	Age
Acipenser guldenstadti	131-183(154.5)	10.5-36.5(21.8)	10-20(15.5)
A. persicus	146-219(172.5)	12-42.5(29.9)	12-28(17.1)
A. stellatus	138-195(168.5)	11.2-38.5(25.2)	11-25(16.3)

RESULTS

Examination of the blood in fresh preparations and stained smears showed *Cryptobia acipenseris* Lwoff *et al*. 1926 and *Haemogregarina acipenseris* Nawrotzky

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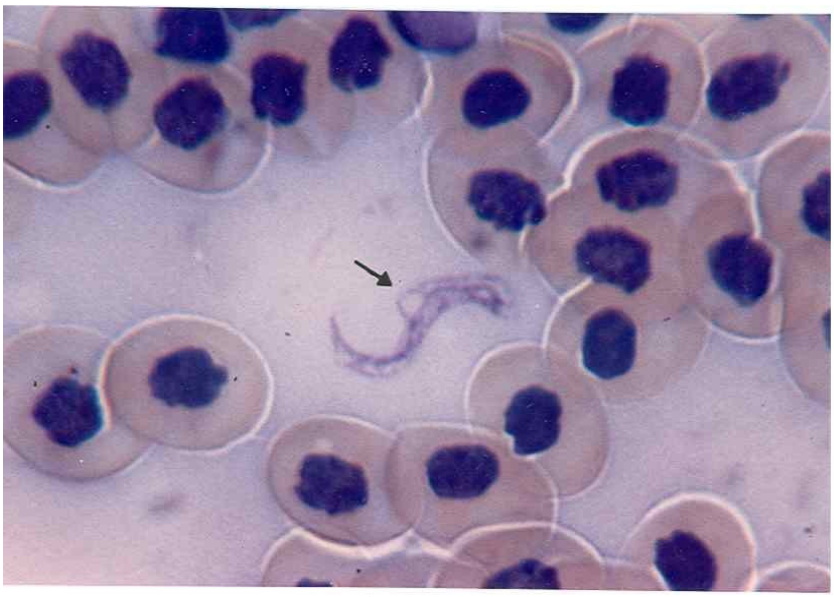

Figure 1. *Cryptobia acipenseris* (↑) in the blood plasma among the red blood cells. Gimsa, magn. 1900X.

1914 infections in the Russian and Persian sturgeon populations of the Caspian Sea.

The size of the body of the *Cryptobia acipenseris* was 21-24 µm long and 3.1-9.3 µm wide. The body of this species is armed with two flagella, which is an important feature of the genus *Cryptobia*. (Figure1). The incidence of the *Cryptobia acipenseris* infection in fresh preparates was 45% (9 fish) for *Acipenser persicus* and 55% (11 fish) for *Acipenser guldenstaedti*, but in the case of stained smears it was only 15% (3 fish) and 25% (5 fish) respectively. The fresh microscopically examination showed a higher intensity.

Study of the stained smears showed *Haemogregarina acipenseris* infection of the red blood cells by light microscope. Only two types of the developmental stages of *Haemogregarina* were detected in all fish species. One of them was observed in the cytoplasm of red blood cells in unicellular and uninuclear form, the second form circulated free in the plasm of the blood. The incidence was: 10% (2 fish) of *Acipenser persicus* and 30% (6 fish) of *Acipenser guldenstaedti*. The nucleus of the infected red blood cells was peripherally located and in some cases it was picnotic and stained dark blue with Gimsa solution. The parasite was 4-5 µm long and 1.5-3 µm wide. (Figure 2). In the case of *Acipenser stellatus*, no infection was found among the examined fishes.

DISCUSSION

This is the first record of *Cryptobia acipenseris* and *Haemogregarina acipenseris* in Persian and Russian sturgeons in the southern part of the Caspian Sea (Iran) and, according to the results of this study, *A. persicus* is shown as a new host. Study of the blood in fresh and stained preparations revealed that fish derived from the natural environment can often have infection by *Cryptobia acipenseris* and *Haemogregarina acipensers*. Experimental studies showed cryptobia and haemogregarina invasion to be accompanied by a strong anemia (Grabda, 1991).

Compared to the results of Baska (1990) and Masoumian and Pazooki (2000), the density of infections is higher than in the present study. Those studies were carried out

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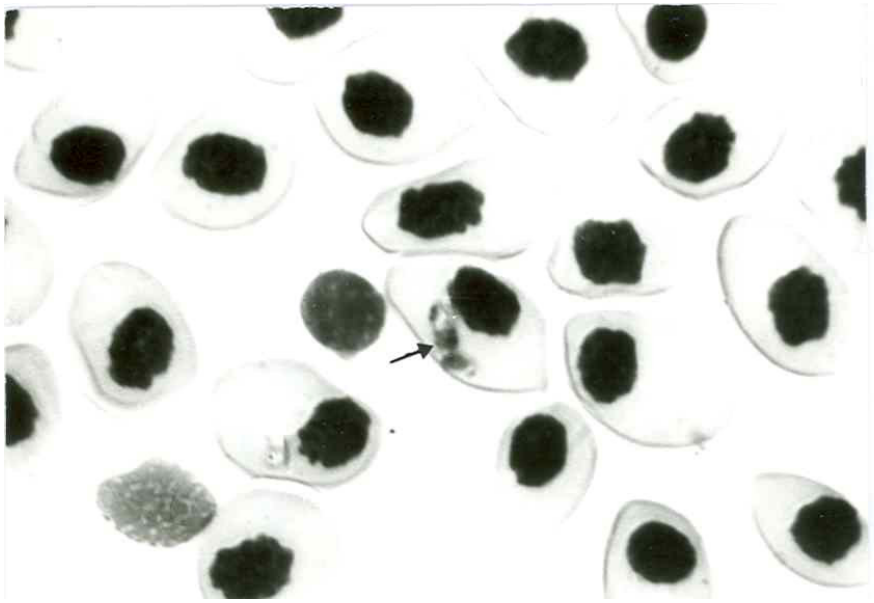

Figure 2. *Haemogregarina acipenseris* trophozoit (↑) in the cytoplasm of a red blood cell. Gimsa, magn. 2100X.

in the Danube River on the Sterlet. It is assumed that the higher prevalence of *Cryptobia acipenseris* in Sterlet is related, on the one hand, to the younger age of the examined population and, on the other hand, it is probably connected to the greater intensity of pollution in environment of the Danube, which can influence the resistance of fish against parasitic infections (Lom, 1970).

Both fish were infected by the same parasites. The prevalence of the infection with *Cryptobia acipenseris* in fixed and stained smears was lower than in fresh smears. It was 45% for Persian sturgeon and 55% for Russian sturgeon. The fresh microscopic examinations showed higher intensity, because the parasites had an intensive movment among the blood cells making it easier to spot them. The lower prevalence observed in fixed and stained smears showed that fresh examination is more effective. So this method is suggested for obtaining a correct and quick diagnosis.

In 1994, Woo divided the *Cryptobia* into two subgenera. The haematozoic and normally digenetic species (i. e. indirect life cycle) belong to the subgenus *Trypanoplasma* while the subgenus *Cryptobia* contains the non-haematozoic (i. e. one host) parasites.

Woo in 1995 also emphasised the Cryptobia parasites are family and tissue especific, both haematozoic and non-haematozoic species. It is assumed that *Cryptobia (Trypanoplasma) acipenseris* is specific to the Acipenseridae fishes.

According to the results of this study two types of developmental stages in *Haemogregarina* were identified in all fish species. One of them was observed in the cytoplasm of red blood cells in unicellular and uninuclear form (intracellular), the second form circulated free in the plasm of the blood (extracellular). However, the hypothesis of Perekrepov (1930) that all stages of *Haemogregarina acipenseris* develop in fish is improbable because during a long period only two very similar developmental stages had been detected. It means the acipenserids are temporary hosts (or intermediate hosts) of *Haemogragarina*

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and the other stages can be found in one of the blood sucking parasites.

All fish specimens examined during this study were collected from their natural environment. Heamoflagellates were found in different sturgeon species without any pathological effects, but in the intensive farming of sturgeons, *Haemogregarina acipenseris* may lead to severe infections and cause anemia which decrease the productivity of the farm.

The above mentioned parasites severe low pathogenic effects in the natural water, but in certain conditions (e.g. in the intensive culture of sturgeons) their effect of them must be kept in mind. In aquacalture the prevention of diseases is cheaper and easier than medical treatment. Fish disease is an important component of the aquaculture industry.

With increased interest in sturgeon farming, there has been an increased interest in parasites of these fishes and the diseases associated with them (Kozlov, 1993). Therefore, any attempt to increase the productivity of pond farms or to improve the stocks of acipenseridae in the natural waters requires detailed knowledge of the parasites inhabiting the localities involved (Woo, 1995). In order to be able to deal with the fish parasites encountered, one has to recognize and identify them, know their biology, life cycle, invasion pathway, relationships within food chains, and many other biological phenomena. Differences in the environmental factors, physical conditions, genetic resistance, physical stresses, as well as in host age and sex, also play a part in determining the susceptibility of the fishes to diseases.

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آلودگی ماهیان قره برون و چالباش حوزه جنوبی دریای خزر به انگلهای *Cryptobia* *acipenseris* و *Haemogregarina acipenseris*

ج. پازوکی و م. معصومیان

چکیده

در بررسیهای انگل شناسی انجام شده در سالهای ۱۳۷۹ لغایت ۱۳۸۱ برروی ماهیان قره برون و چالباش (*A. guldenstadti* و *Acipenser persicus*) در حوزه جنوبی دریای خزر برای اولین بار انگلهای *Haemogregarina acipenseris* و *Cryptobia acipenseris* شناسائی و جداسازی گردیدند. نمونه‌های مطالعه شده ازیک صیدگاه ماهیان خاویاری در منطقه انزلی بوسیله تور گوشگیر صید شدند. پس از صید، مشخصات ماهیان ثبت شد و گسترش مرطوب و رنگ‌آمیزی شده خون آنها با میکرسکوپ نوری مطالعه و درصد آلودگی محاسبه گردید. درطی این مطالعه برای اولین بار ماهی قره برون بعنوان میزبان جدید این انگلها معرفی میشود. تمامی نمونه‌ها از محیط طبیعی جمع آوری و مطالعه شده‌اند. انگلهای خونی جدا

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شده بدون علائم پاتولوژیک در ماهیان آلوده مشاهده گردیدند، ولی در پرورش متراکم ماهیان خاویاری انگل *Haemogregarina acipenseris* میتواند با ایجاد کم خونی درتولید مزارع تاثیرگذار باشد.

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