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Erythrocyte Sizes of *Carassius gibelio* Species in Porsuk Dam Lake (Eskisehir/Turkey)

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Abstract: *Carassius gibelio* (Bloch) is documented to have been moved from Asia across Europe in the 17th century since which time it has been reported to exist in fresh water across Europe. The first time this species was reported in Turkey was in 1988 and it has since been reported in fresh water occurring in various sites in Turkey. *C. gibelio* is an intrusive species that has a negative impact upon natural species occurring in the fresh water sites that it invades. It is known to end up achieving dominance in fresh water sites by not only increasing their total biomass through rapid growth but also competing with the indigenous species. The chief reason for the rapid invasion of this species is thought to be its gynogenetic reproductive ability. Various studies have demonstrated that this species makes up colonies with different genetic traits within the same medium of fresh water as a result of gynogenetic reproduction. A large number of colonies that are reproduced gynogenetically are made up of female individuals. Gynogenetic reproduction is determined upon either the number of chromosomes or the size of erythrocytes. The present study was carried out in seven samples made out of 381 individuals of *C. gibelio* (20 males and 361 females) cultivated from the dam on the Porsuk river between May and December 2009. About 95% of the population was made up of female individuals. Blood samples were taken off 4 female individuals in December and then preparations of blood smear were arranged. Afterwards, the area and volume of the erythrocyte cell of 50 individuals as well as nuclear area and volume of erythrocyte cells were calculated. These analyses showed the range of erythrocytes of the 4 individuals were 51.31, 58.70, 57.44 and 47.44 μm^2, respectively. Their range of erythrocyte nucleus were 8.50, 12.41, 8.54 and 7.26 μm^2, respectively. The volumes of erythrocyte cells were 221, 304, 271 and 195.6 μm^3, respectively while the volumes of erythrocyte nucleus were 14.66, 30.64, 16.24 and 11.72 μm^3, respectively. A comparison of nuclear area of the second individual which had the largest nucleus area with those of the remaining 3 individuals determined their areas to be 1.46, 1.45 and 1.71. Previous studies have revealed that nuclear area of erythrocytes of the individuals with triploid (3n) have a much bigger nucleus than individuals with diploid (2n). The population structure consisting of 95% females and the size difference between the erythrocyte nucleus areas displays the gynogenetic reproduction potential of *C. gibelio* population in Porsuk Dam lake.

Key words: *Carassius gibelio*, size of erythrocyte, gynogenetic reproduction, chromosome, invasion, rapid growth

INTRODUCTION

One of the basic characteristics of a marshy area is that the natural composition of the species occurring there does not change. If the composition of the species happens to change, so will the niche of the species occurring in this marshy area thus disrupting the whole food chain. Depending upon the average heat of the globe, a large number of hydrophilic organisms tend to migrate to and then inhabit the waters surrounding Turkey which is located on the northern latitudes. Efficiency of such marshy areas diminishes as a result of the increasingly deteriorating quality of the water occurring there. In attempt to minimize such side-effects, people generally fetch species with higher range of tolerance to these waters. However, these newcomers end up having fundamental but unfortunately generally negative impacts upon the ecological balance of the already existing environment.

Since exotic species are known for their high tolerance to new environments they have a tendency to dominate native species occurring in fresh water thus posing a menace for them in the long run in terms of biodiversity. As exotic species are likely to bring along

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exotic micro-organisms wherever they inhabit they tend to be considered serious threats in respect to having a part in development of new diseases as far as ihtiofauna is concerned (Ugurlu and Polat, 2007).

C. gibelio has been viewed as one of the invasive species infamous for its unfavourable impacts upon fresh water occurring in Europe. This species, known to have originated in Asia has recently been reported to inhabit fresh waters in West Asia, Siberia and the whole Europe (Kottelat, 1997). However, it is not one of the natural species occurring in Europe (Flajshans *et al*., 2008). Distribution of this species is thought by some to be a natural one despite insufficient verification (Kottelat, 1997). Mikelsar reported *C. gibelio* to have been transported to Europe from Asia in the 17th century (Vetemaa *et al*., 2005).

As for today, it is reported to occur in at least 12 countries in Europe as an invasive species (Ozcan, 2007). Among geographical locations in close proximity to Turkey where this fish has been reported to populate are Daget and Economidis as well as the river of Meric in West Thrace (Sari *et al*., 2008). *C. gibelio* is suspected either to have accessed Turkish waters over Greece and Bulgaria through. The Meric or to have been transported manually (Ozulug *et al*., 2004).

C. gibelio was first reported in Turkey by Baran ve Ongan in Lake Gala in Thrace (Ozulug, 1999). Around 10-15 years after this discovery, *C. gibelio* was reported in another 46 locations across Thrace and Anatolia (Ozcan, 2007). The number of marshy areas invaded by this species has been increasing ever since. One of these areas is the dam built on the river of Porsuk. The study is the first to report presence of *C. gibelio* in the Porsuk Dam.

Now a days, a large number of sources of fresh water in Turkey are at risk of invasion by *C. gibelio.* One of the chief reasons for the overgrowth of the population of *C. gibelio* appears to be ecological disruption of the marshy areas overrun by this fish which is known to be capable of surviving in severely contaminated areas with dense vegetation and low levels of oxygen via its amazing adaptation ability (Gudkov, 1985).

Recent studies have emphasized that *C. gibelio* makes up 20-30% of the fish that have been caught in the river of Volga, a severely contaminated lake, since 1977 (Gudkov, 1985) whereas this species was reported to occur in only small numbers previous to 1977. Gudkov (1985) reported the number of *C. gibelio* to have been continually increasing at a remarkable pace across all marshy areas occurring in Europe, apart from reservoirs in the Black sea region for over 5 decades, 1960s and 1970s in particular (Gudkov, 1985). Surprisingly enough, this incredible increase coincides with the period during which marshy areas were exposed to the highest levels of contamination due to industrial revolution which makes a lot of sense in terms of ecological disruption.

C. gibelio experiences amazing growth during the early years of its life. It also lays too many eggs that can stay intact for a long time. Furthermore, this fish is able to reproduce even with the sperms of males belonging to other Cyprinidae families. All these factors result in its dominance over the other fish that co-exist in the same environment apart from the fact that it causes a serious problem of poor quality water by helping speed up mixture of the substances accumulated at the bottom with the water above thus disrupting the benthos of the water.

C. gibelio has a related partenogenetic reproductive system (gynogenesis) that is based on sperm. Because of its gynogenetic reproductive system, this fish makes up populations with different genetic characteristics in different locations (Jia *et al*., 2008). This gynogenetic reproductive system of this species results in diploid-polyploid individuals in natural settings, most of them born females (Kalous, 2002). It is thought to be this gynogenetic trait of *C. gibelio* that enables it to dominate marshy areas so rapidly and successfully. Thus, the majority of the population created by C. *gibelio* which is infamous for stealing the sperms of the males belonging in other species, turns out to be females with 3n chromosomes and so leads to a destructive competition for reproduction among fish.

This being the case, it so appears that it is of vital importance to know reproductive ability of this species and take necessary precautions accordingly in coping with it. In order to confirm that *C. gibelio* reproduces gynogenetically, it has been suggested that either erythrocyte size or chromosome numbers of this fish be determined (Nakanishi and Onozato, 1987).

The present study aims to determine gynogenetic reproductive ability of *C. gibelio* which is being reported to occur in the dam built on the Porsuk river for the first time with the help of measuring areas and volumes of erythrocytes themselves as well as cellular areas and volumes of erythrocytes.

MATERIALS AND METHODS

With its 824 km lenght Sakarya river is one of the longest rivers in Turkey. Whereas Porsuk creek is one of the most important sources that carry water to Sakarya river. Porsuk creek is one of the most

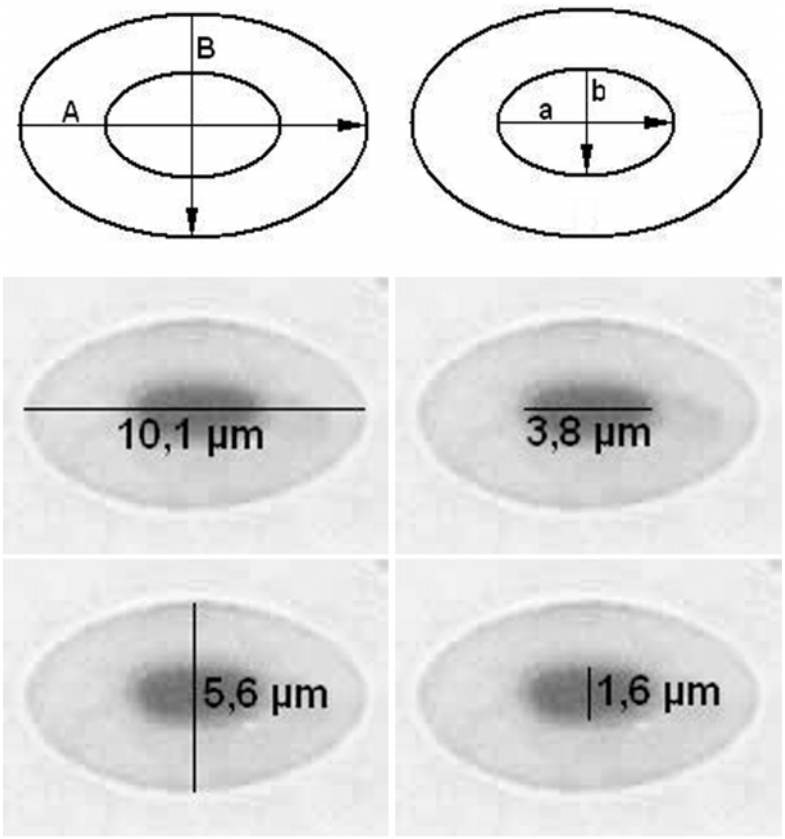

Fig. 1: Measurement template

significant economical and social areas of middle Anatolia. There is heavy industrial and agricultural activity and dense human settlement within the area that the creek passes through. As a result of these activities Porsuk creek is heavily polluted (Canbek *et al*., 2007). The Porsuk Dam was constructed between Kutahya and Eskisehir provincial borders to protect Eskisehir city center from floods and to make use of water for domestic and agricultural means.

Porsuk Dam is also used extensively for fishing purposes. In this study 7 samples were made between May-December 2009 and 381 individuals were collected. Fresh blood samples were collected by intracardiac means with the help of an injector from 4 individuals that were caught in December. Smear spreads were carried out on clean glass slides without permitting coagulation of blood samples.

Air-dried blood preparates were kept in Harris Haematoxylin cytological dye for 3 min after metanol fixation and then washed. All of the prepared blood preparates was examined under Olympus CH40 light microscope photographed with the help of Spot Insight 3.2.0. digital camera and Spot advanced version 4.0.6 software and diameter measurements of erythrocyte cells and nucleus were carried out. The areas and volumes of Erythrocyte nucleus and erythrocyte cells were calculated by using the following formula (Fig. 1).

$$E_{Area} = [(AxBx\pi)/4] \quad N_{area} = [(axbx\pi)/4]$$

$$(E_{Volume} = 4/3x\ \pi x\ (A/2)\times(B/2)^2$$

$$N_{volume} = 4/3x\ \pi x\ (a/2)\times(b/2)^2$$

RESULTS AND DISCUSSION

The present study was carried out in seven samples made out of 381 individuals of *C. gibelio* (20 males and 361 females) cultivated from the dam on the Porsuk river between May and December 2009.

About 95% of the population was made up of female individuals. Blood samples were taken off 4 individuals in December and then preparations of blood smear were arranged and erythrocyte areas, erythrocyte volumes, areas and volumes of erythrocyte nucleuss of 50 erythrocyte cells from each individual were calculated (Table 1). In the studies on *C. gibelio* in Turkey the

Table 1: The area and volume of the erythrocyte cell, erythrocyte nucleus size in freshwater fish *C. gibelio*

Samples	EA (μm^2)		NA (μm^2)		EV (μm^3)		NV (μm^3)	
no	Mean (Min.-Max.) SD	Rate	Mean (Min.-Max.) SD	Rate	Mean (Min.-Max.) SD	Rate	Mean (Min.-Max.) SD	Rate
1	51.3	2/1	8.5	2/1	221	2/1	14.6	2/1
	(38.4-63.8)	1.1	(7.4-16.2)	1.46*	(128-350)	1.37	(10-46.3)	2.09*
	6.5	-	2.0	-	55.3	-	8.1	-
2	58.7	-	12.4	-	304	-	30.6	-
	(44.7-73.4)	-	(8.6-20.5)	-	(202-443)	-	(13.8-63.0)	-
	6.6	-	2.7	-	57.9	-	10.6	-
3	57.4	2/3	8.5	2/3	271	2/3	16.2	2/3
	(35.4-90.7)	1.0	(7.3-12.0)	1.45*	(110-567)	1.12	(10.8-28.4)	-
	11.4	-	1.2	-	95.7	-	4.7	1.88*
4	47.4	2/4	7.2	2/4	195	2/4	11.7	2/4
	(32.3-62.1)	1.2	(6.1-9.5)	1.70*	(118-359)	1.55	(8.1-23.6)	2.61*
	6.3	-	0.9	-	51.4	-	3.8	-

EA: Erythrocyte Area, EV: Erythrocyte Volume, NA: Erythrocyte Nucleus Area, NV: Erythrocyte Nucleus Volume *p<0.05

male/female ratios were found as follows by Balik *et al.* (2004) in Egirdir lake as 1.14/1 by Izci (2004) in Egirdir lake for *Carassius auratus* as 2.05/1 by Tarkan in Omerli Dam as 0.07/1 in Iznik lake as 0.63/1; by Bostanci *et al.* (2007 a, b) in Bafra fish lake as 0.02/1; in Egirdir lake as 1.46/1; by Cinar in Beysehir lake as 0.92/1; by Ozkok *et al.* (2007) in Egirdir lake as 1.08/1 by Sasi (2008) in Topcam Dam lake as 0.01/1; by Sari *et al.* (2008) in Buldan Dam lake as 0.005/1; by Simovic and Jovanovic in Begej channel as 0.06/1 in studies carried out in fresh water in Estonia by Vetemaa *et al.* (2005) in Kahala as 0.06/1; in Jalase as 1.12/1; in Piusa as 0.07/1; all populations in Vortsjarv, Ilmatsalu, Tammiku, Kingi, Karna, Mehikoorma and Maardu localities consist of females for salty water in Leigo as 0.06/1; in Matsalu as 0.84/1 in Haademeeste as 0.7/1; in Kasmu as 0.66/1 and in Saunja localities as 0.79/1; Leonardos *et al.* (2008) in Chimaditis lake (Greece) has reported a population structure dominated by females.

In the study the male/female ratio of *C. gibelio* population in Porsuk Dam was determined as 0.05/1. It was reported that the individuals which arise when *C. gibelio* eggs are stimulated with *Cyrinus carpio* consist of 98% female and 2% male and 15% of the bisexual generation which arises as a result of crossbreeding of male and female individuals is male individuals (Fan and Shen, 1990). Pipoyan and Rukhyan (1995) reported that *Carassius auratus gibelio* gynogenetically reproduces and Paschos reported that the female individuals in Pamvotis lake in Greece which gynogenetically reproduce and have 150 chromosomes are sperm parasites and they gynogenetically reproduce with the sperms of other Cyprinidae species and therefore 97.7% of the population consists of female individuals. According to Erytrochyte diameters, the first population which includes triploid individuals as a result of gynogenetic reproduction characteristic in Turkey was reported from Kayalikoy dam lake (Kalous, 2004). Thereafter, the female intensive population structures which is an indicator of gynogenetic reproduction were reported from Omerli dam lake, Bafra fish lake (Bostanci *et al.*, 2007 a), Topcam Dam lake (Sasi, 2008), Buldan Dam lake (Sari *et al.*, 2008).

Male-female ratios demonstrate that there is a population which gynogenetically reproduces in Porsuk Dam lake as well. When we look at the square and capacity measures of Erythrochyte nuclei as well as male-female ratio and compare square and capacity measures of individual no. 2, it is seen that its square measures are 1.4-1.7 times more and its capacity measures are 1.8-2.6 times more than the other individuals.

This difference in nucleus square and capacity measures demonstrates that the individuals having 3n chromosome formed in Porsuk Dam lake as a result of gynogenetic reproductions.

The first and only study for determination of gynogenetic reproduction characteristic of *C. gibelio* in Turkey was performed by Kalous (2004) on 2 fishes that were obtained from Istanbul fish market. In this study they measured the erythrocyte nucleus averages as 19.85 and 18.27 μm^2 and used the results of the studies which were performed in the Czech Republic as comparison material.

According to the study in the Czech Republic, it is determined that nucleus squares of 2n individuals are 10.76 μm^2 and nucleus squares of 3n individuals are 18.5 μm^2 and according to these results it is determined that *C. gibelio* population in Kayalikoy Dam gynogenetically reproduces. Abramenko in their study, reported that there is 1.35 times difference between the nucleus squares of 2n and 3n individuals. Since the difference between the nucleus squares is minimum 1.45 in the study, we can say that there are 3n individuals in *C. gibelio* population in Porsuk Dam.

CONCLUSION

With this study, existence of *C. gibelio* species in Porsuk Dam which is one of the significant marshy lands of Turkey and which provides great amounts of fish production and the population structure with 3n chromosome of which big part consists of females as a result of gynogenetic reproduction were pointed out. It is an unavoidable truth that this species will be the dominant species in Porsuk Dam because of its high ecological tolerance ability and gynogenetic reproduction characteristic.

In accordance with this information, determination of the best struggle way against this species and putting it into effect immediately are necessary for sustainable fish production in the region.

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