International Journal of Natural and Engineering Sciences
E-ISSN: 2146-0086 14 (1): 8-10, 2020

Gynogenetic Reproduction Potential of Invasive *Carassius gibelio* in Ula Pond

Sercan Başkurt[1*], Pinar Oztopcu-Vatan[1], Özgür Emiroğlu[1], Sadi Aksu[2]

[1]Eskişehir Osmangazi University, Faculty of Arts and Sciences, Department of Biology, Eskisehir/TURKEY.
[2]Eskişehir Osmangazi University, Vocational School of Health Services, Eskişehir, TURKEY.

*Corresponding Author
E-posta: sercanbaskurt@gmail.com

Received: 07 December 2019
Accepted: 16 January 2020

Abstract
Carassius gibelio originated in Central Asia and is an invasive species all over Europe and our country. *C. gibelio* gives significant damages to natural ichthyofauna and water quality in inland waters it enters. From Turkey, this species was reported first in 1988. Today, it has invaded many wetlands. The main reason for its success in invasion is gynogenetic reproductive ability. This species forms triploid individuals with 3n chromosomes using sperm from other Cyprinidae. There are three basic methods for determining the triploid population. These include determining the number of chromosomes, calculating the area of erythrocyte nuclei, and determining the amount of DNA by flow cytometry. In this study, smear preparations were made from blood samples of the ten *C. gibelio* samples caught from the Ula Pond. For each individual, erythrocyte nucleus area of at least 100 cells was calculated and arithmetic average calculated, and the gynogenetic reproduction potentials were investigated. The erythrocyte nucleus area of triploid (3n=156-164) individuals formed by gynogenetic reproduction is at least 1.31 percent larger than diploid (2n=100) individuals. Of the blood samples taken from individuals, the smallest of the erythrocyte nucleus area was found to be 10,89 μm^2 and the largest was 19.30 μm^2. According to these results it has been found to have gynogenetic reproductive potential.

Keywords: *Carassisus gibelio*, gynogenetic, invasive, erythrocyte nucleus area

INTRODUCTION

One of the most important factors threatening wetlands today is the inclusion of alien fish species in the fauna. The alien fish species entering the aquatic area can change the entire ecological balance. *C. gibelio* is one of the most important alien fish species for the freshwater area. *C. gibelio* can become an invader by produce dominant populations in a short time thanks to its successful reproduction ability and high tolerance to ecological conditions.

Depending upon the average heat of the globe, a large number of aquatic 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 [1].

Since alien fish 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 alien fish species are likely to bring along 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 [2].

First record of the Prussian carp, *Carassius gibelio*, from Turkey is from Lake Gala in Thrace Region [3]. Following this record several introduction records from Thrace and other regions were given respectively [4-7].

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 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 causes as 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 [1].

C. gibelio has a related parthenogenetic reproductive system (gynogenesis) that is base on sperm. Because of its gynogenetic reproductive system, this fish makes up populations with different genetic charecteristics in different locations [8].

The most important reason of *C. gibelio*'s success in invasion is gynogenetic reproductive ability. For this reason, gynogenetic reproductive potential should be monitored in the settled wetlands.

There are three basic methods for determining the triploid population. These include determining the number of chromosomes, calculating the area of erythrocyte nuclei, and determining the amount of DNA by flow cytometry [9-13]. In this study, it was aimed to determine the gynogenetic reproductive status by measuring the area and volume of the erythrocyte and erythrocyte nuclei of *C. gibelio* species obtained from Ula Pond.

MATERIALS AND METHODS

Ula Pond located in the province of Muğla in Turkey was made in 1987 for irrigation purposes. The deepest part of the pond is 20 m altitude and 645 m. [14].

This pond is fed by a stream called Akarca. When the water exceeds its holding capacity, this stream flows out of the pond. Ula pond was also used as a water reservoir for fire fighting helicopters [15].

Figure 1. Ula Pond satellite image

In this study, smear preparations were made from blood samples of the ten *C. gibelio* samples caught from the Ula Pond. For each individual, erythrocyte nucleus area of at least 100 cells was calculated and arithmetic average calculated, and the gynogenetic reproduction potentials were investigated.

Fish samples were caught in accordance with TS EN 14962 and TS EN 14011 standards. The samples were made on 21.08.2014. A total of ten individuals were caught, and blood samples were obtained from the obtained individuals via injectors via intracardiac area. Blood smear preparates were done immediately to prevent coagulation. Samples were air-dried and fixed in 96% ethanol for 2 minutes. After fixation, all samples dyed with haematoxylin for 1 minute and washed under tap water. Then, preparates dyed in eosin for 1 minute washed with distilled water and kept in xylene for 2 minutes as last step. All samples were examined under Nikon Eclipse TS100 inverted microscope and images were taken for diameter measurements of erythrocytes and nucleus with Kameram 21 software. Area and volume calculations were done with the formula [16;17].

The area and volumes of Erythrocyte and Erythrocyte nuclei were calculated using the following formula [16].

$$E_{Alan} = [(AxBx\pi)/4] \qquad N_{alan} = [(axbx\pi)/4]$$

$$(E_{Hacim} = 4/3x\ \pi x\ (A/2)x\ B/2)^2 \qquad N_{hacim} = 4/3x\ \pi x\ (a/2)x\ b/2)^2$$

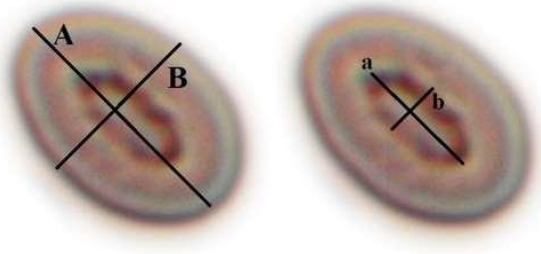

Figure 2. Measurement Template

RESULTS AND DISCUSSION

In this study, blood samples were taken from ten *C. gibelio* individuals caught from Ula Pond and smear preparations were prepared. Arithmetic mean of erythrocyte nucleus area of at least 100 cells was calculated for each individual. The results obtained are given Table 1.

Table 1. Calculated ENA, EA, ENV, EV values of *C. gibelio* samples in Ula Pond

	ENA µm²	EA µm²	ENV µm³	EV µm³
	Mean-std	**Mean**-std	**Mean**-std	**Mean**-std
1	**10,89**-2,13	**70,23**-4,8	**21,20**-6,76	**389,15**-41,06
2	**13,43**-1,49	**69,78**-4,59	**27,91**-5,02	**372,58**-37,43
3	**13,48**-1,44	**70,97**-3,86	**27,41**-5,17	**376,71**-35,01
4	**13,23**-1,80	**69,72**-4,07	**27,75**-6,34	**383,69**-38,53
5	**12,72**-1,82	**65,27**-4,93	**25,87**-6,33	**344,22**-42,65
6	**12,47**-1,29	**67,13**-4,02	**23,23**-4,03	**350,59**-35,23
7	**18,35**-1,15	**69,86**-5,11	**42,20**-5,23	**383,46**-41,93
8	**18,22**-1,34	**71,51**-5,29	**42,46**-5,55	**390,19**-47,19
9	**19,30**-1,82	**75,31**-4,78	**48,78**-6,46	**423,66**-39,53
10	**18,78**-1,24	**69,07**-4,95	**42,69**-5,39	**374,80**-44,83

ENA: Erythrocyte Nucleus Area, EA: Erythrocyte Area, ENV: Erythrocyte Nucleus Volume EV: Erythrocyte Volume

Table 1. The ratio of the calculated ENA values of *C. gibelio* samples in Ula Pond to each other

	CG1	CG2	CG3	CG4	CG5	CG6	CG7	CG8	CG9	CG10
	10,89	13,43	13,48	13,23	12,72	12,47	18,35	18,22	19,3	18,78
10,89	1,00	1,23	1,24	1,21	1,17	1,15	**1,69***	**1,67***	**1,77***	**1,72***
13,43	0,81	1,00	1,00	0,99	0,95	0,93	**1,37***	**1,36***	**1,44***	**1,40***
13,48	0,81	1,00	1,00	0,98	0,94	0,93	**1,36***	**1,35***	**1,43***	**1,39***
13,23	0,82	1,02	1,02	1,00	0,96	0,94	**1,39***	**1,38***	**1,46***	**1,42***
12,72	0,86	1,06	1,06	1,04	1,00	0,98	**1,44***	**1,43***	**1,52***	**1,48***
12,47	0,87	1,08	1,08	1,06	1,02	1,00	**1,47***	**1,46***	**1,55***	**1,51***
18,35	0,59	0,73	0,73	0,72	0,69	0,68	1,00	0,99	1,05	1,02
18,22	0,60	0,74	0,74	0,73	0,70	0,68	1,01	1,00	1,06	1,03
19,3	0,56	0,70	0,70	0,69	0,66	0,65	0,95	0,94	1,00	0,97
18,78	0,58	0,72	0,72	0,70	0,68	0,66	0,98	0,97	1,03	1,00

According to the obtained results (see table 2), the ENA ratios of CG7, CG8, CG9 and CG10 individuals were found to be greater than 1.35.

Because of the benefit particularly provided in agricultural fishing, the populations contain of triploid individuals are preferred. Thanks to the developing technology, the studies for suppression of the chromosomes concerned with sex formation and for creating individuals with more or larger chromosomes have been very successful [17]. Triploid individuals were formed by biotechnological methods in order to provide more production. [18]. "*Carassius auratus gibelio*'s method for increasing the incubation rate and survival rate of larvae" in the patent study (World Intellectual Property Organization, patent number CN105638519); In China, *C. gibelio* meat is found to be delicious by the local people and therefore population productivity and resistance have been increased by improvement and genetic studies. [19]. However, when these triploid individuals are transported to fresh waters outside their natural environment, they pose a threat to natural fish species due to their high reproductive ability. In addition, due to their high nutritional properties, they make a dominant food competition with natural fish species. For these reasons, the gynogenetic reproductive potentials of the newly introduced aquatic environment must be monitored for the balance of the ecosystem.

In our study, gynogenetic reproduction potential of *C. gibelio* species, which formed a population in Ula pond, was measured by erythrocyte nucleus length and width and mathematical formulas were used to calculate ENA. Abremenko reported that ENA is greater than 1.35 in individuals with chromosome 3n [16].

According to our results demonstrated that ENA is greater than 1.35 in CG7, CG8 CG9 and CG10 individuals. (Table 2). According to these results, it was found for the first time that there were individuals with gynogenetic growth in the *C gibelio* population in Ula Pond. It is recommended to control the population size of the invasive species by monitoring the gynogenetic reproductive potential of *C. gibelio* population in Ula Pond for the protection of natural fish species and maintaining of water quality.

REFERENCES

[1] Emiroğlu, Ö. (2011). Alien fish species in upper Sakarya River and their distribution. African Journal of Biotechnology, 10(73), 16674-16681.

[2] Uğurlu S, Polat N (2007). Exotic fish species inhabiting in freshwater sources within the province of Samsun. J. Fish. Sci. Com. 1(3): 139-151.

[3] Baran, I, Ongan, T. (1988). Gala Gölü'nün Limnolojik Özellikleri Balıkçılık Sorunları ve Öneriler [Limnologycal Features of Lake Gala, Fisheries Problems and Suggestions]. – Gala Gölü ve Sorunları Sempozyumu, Dogal Hayatı Koruma Dernegi Bilimsel Yayınlar Serisi, Istanbul, p. 46–54.

[4] Özuluğ M. (1999). A taxonomic study on the fish in the Büyükçekmece dam lake. Turk. J. Zool. 23: 439-451.

[5] İlhan A, Balık S, Sarı H.M, Ustaoğlu M.R (2005). Carassius (Cyprinidae, Pisces) species in inland waters of Western and Middle Anatolia, Southern Marmara, Thrace and Western Black Sea Regions and their distributions. E.U. J. Fish. Aquat. Sci. 22: 3-4, 343-345.

[6] Tarkan, A.S., Copp, G.H., Top, N., Özdemir, N., Önsoy, B., Bilge, G., Filiz H., Yapıcı, S., Ekmekçi, F. G., Kırankaya, Ş. G., Emiroğlu, Ö., Gaygusuz, Ö., Gürsoy Gaygusuz, Ç., Oymak A., Özcan, G., Saç, G. 2012. Are introduced Gibel Carp Carassius gibelio in Turkey more invasive in artificial than in natural waters?. Fisheries Management and Ecology, 19(2):178- 187

[7] Yerli, S. V., Mangıt, F., Emiroğlu, Ö., Yeğen, V., Uysal, R., Ünlü, E., Alp, A., Buhan, E. & Zengin, M. (2014). Distribution of Invasive Carassius gibelio (Bloch, 1782)(Teleostei: Cyprinidae) in Turkey. Turkish Journal of Fisheries and Aquatic Sciences, 14(2), 581-590.

[8] Jia, Z. Y., Shi, L. Y., Sun, X. W., Lei, Q. Q. (2008). Inheritance of microsatellite DNA in bisexual gynogenesis complex of fangzheng silver crucian carp, Carassius auratus gibelio (Bloch). Journal of Fish Biology, 73(5), 1161-1169.

[9] Nakanishi, T. and H. Onozato, 1987. Variability in the growth of isogeneic crucian carp *Carassius gibelio langsdorfii*. Nippon Suisan Gakkaishi, 53: 2099-2014.

[10] Zhang, Q., Arai, K. (1999). Distribution and reproductive capacity of natural triploid individuals and occurrence of unreduced eggs as a cause of polyploidization in the loach, Misgurnus anguillicaudatus. Ichthyological Research, 46(2), 153-161.

[11] Gao, Z., Wang, W., Abbas, K., Zhou, X., Yang, Y., Diana, J. S., Wang, H., Wang, H., Yang, L., Sun, Y. (2007). Haematological characterization of loach Misgurnus anguillicaudatus: comparison among diploid, triploid and tetraploid specimens. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 147(4), 1001-1008.

[12] Xiao, J., Zou, T., Chen, Y., Chen, L., Liu, S., Tao, M., Zhang, C., Zhao, R., Zhou, Y., Long, Y., You, C., Yan, J., Liu, Y. (2011). Coexistence of diploid, triploid and tetraploid crucian carp (Carassius auratus) in natural waters. BMC genetics, 12(1), 20.

[13] Hulak, M., Kaspar, V., Psenicka, M., Gela, D., Li, P., Linhart, O. (2010). Does triploidization produce functional sterility of triploid males of tench Tinca tinca (L.)?. Reviews in fish biology and fisheries, 20(3), 307-315.

[14] Küçükçe, A. (1999). Ula Göleti'nin (Muğla) Fiziko- Kimyasal ve Biyolojik (Balık Faunası) Yönden İncelenmesi. Muğla Sıtkı Koçman Üniversitesi Fen Edebiyat Fakültesi araştırma tezi, 42 sayfa.

[15] Önsoy, B., Filiz, H., Tarkan, A.S., Bilge, G. & Tarkan, A. N. (2011). Occurrence of nonnative fishes in a small man-made lake (Lake Ula, Muğla): past, present, future perspectives. Turkish Journal of Fisheries and Aquatic Sciences, 11, 209-215.

[16] Emiroğlu, Ö., Uyanoğlu, M., Canbek, M., Başkurt, S. (2010). Erythrocyte sizes of *Carassius gibelio* species in Porsuk Dam Lake (Eskisehir/Turkey). Journal of Animal and Veterinary Advances, 9(24), 3077-3082.

[17] Emiroğlu, Ö., Uyanoğlu, M., Başkurt, S. (2012). Comparison of the Erythrocyte Sizes of Carassius gibelio and Carassius carassius Species Living Together in Akgol (Adapazari/Turkey). Asian Journal of Animal and Veterinary Advances, 7(9), 876-883.

[18] Kerby, J.H. and R.M. Harell, 1990. Hybridization Genetic Manipulation and Gene Pool Conservation of Striped Bass. In: Culture and Propagation of Striped Bass and its Hybrids, Harrell, R.M., J.H. Kerby and R.V. Minton (Eds.). American Fisheries Society, Grosvenor Lane Bethesda, M.D., pp: 159-190.

[19] Yao, J., Qin, G., Xiong, W., Wu, Y., Zhu, Z., Song, J., Xiong, H. (2016). Method for raising hatching rate of carassius auratus gibelio and survival rate of larvas. China Patent No CN105638519.