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Research Article

INVESTIGATION OF GYNOGENETIC REPRODUCTION POTENTIAL AND SOME BIOECOLOGICAL PROPERTIES OF *Cobitis simplicispina* Hankó, 1925 IN KARGIN STREAM (PORSUK STREAM)

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ABSTRACT. Kargın Stream is one of the important branches of Porsuk Stream. In this study, 21 samples examined in order to determine some bioecological characteristics and gynogenetic reproductive potential of *Cobitis simplicispina*, which distributed in the Kargın Stream. The length-weights of the samples examined calculated as b value 3.004. The regression analysis showed that the b value was in the range of 2.709-3.299, and the species showed isometric growth. *Alburnus escherichii, Alburnoides kosswigi, Barbus escherichii, Capoeta baliki, Gobio sakaryaensis, Oxynoemacheilus angorae, Rutilus rutilus, Squalius pursakensis* and *Vimba vimba* were observed at the sampling station. In order to determine the gynogenetic reproductive potential, erythrocyte nucleus areas were calculated. When the erythrocyte nucleus areas of 16 individuals of *C. simplicina* were calculated, the smallest erythrocyte nucleus area found to be 9.13 μm^2 and the largest erythrocyte nucleus area was 11.5 μm^2. When the erythrocyte nucleus areas of all individuals divided, the minimum 1.25. These results showed that *C. simplicispina* did not show gynogenetic growth in Porsuk Stream. *C. sipmlicispina* known to show gynogenetic reproductive properties in the literature. In this study, data obtained for the first time about gynogenetic reproductive ability *C. simplicispina* for Turkey.

Keywords: *Cobitis simplicispina, gynogenetic, Length-Weight, erythrocyte nucleus area*

INTRODUCTION

The genus Cobitis is widespread in the Palearctic, from northern Morocco and the entire European continent to China and Japan in the East, Vietnam and Central Iran in the south [1, 2].

According to recent studies, 30 species of Cobitis genus are known in the Middle East [3]. Turkey has the most Cobitis species diversity in the Mediterranean.

Phylogenetic reconstructions based on mitochondrial (cyt b) and nuclear (RAG1) sequences are divided into seven lineage groups of Western Palearctic Cobitis species, and *C. simplicispina C. sipahilerae, C. bilseli, C. battalgilae, C. turcica, C. dorademiri, C. phrygica* species in the same groups [4].

The natural hybridization of the progeny of the near species which are produced by gynogenesis or parthenogenesis leading to the formation of permanent hybrid forms.

Also, the first stage of the speciation is clonal diploid formation. This formation is confirmed in the first Cobitis genus [5].

Cobitis populations are hybrid-diploid, triploid and tetraploid types known throughout Europe as a result of contacts between species and subspecies. It is known that these hybrids do not breed bisexually, but the instead of produce via non-typical gynogenesis and hybridogenesis. This reproductive mechanism is still unknown [1].

The more nutrient content and bigger offspring in eggs provide certain ecological advantages for clonal-bisexual loach triploids in the early stages of their ontogenesis. While this provides high adaptability in a local environment, reducing the structure containing the bisexual and monoclonal form [6].

It is known that there is sperm parasite on diploid forms of polyploidy *C. simplicispina* forms which are spread in Europe [7].

Individuals belonging to the genus Cobitis be found in almost all freshwaters in Turkey [8-10]. Although numerous studies on various aspects of Turkey waters of fish populations inside information about the length-weight relationship is limited with the most studied fish species [11-13]. There are few studies the length-weight relationship of *C. simplicispina* in the literature [14, 15]. In this study, data obtained for the first time about the gynogenetic reproductive ability for Turkey. In addition, we reported the length-weight relationship of *C. simplicispina* in Kargın Stream.

MATERIALS AND METHODS

Kargın Stream is in the province of Eskişehir in the Central Anatolia Region. The distance to Eskişehir is 19 kilometres. 39.6460 latitude and 30.3658 longitudes [16].



Fig. 1. *Satellite image of The Kargın Stream*

Fish samples were caught in accordance with TS EN 14011 and TS EN 14962 standards by electrofishing from Kargın Stream in Eskisehir Province. Twenty-one individuals were caught by sampling. Length-weight data were obtained from the fish samples. Each sample was measured up to a total length of 0.1 cm (TL) and a total weight of 0.01 g (W).

Length and weight measurements were determined the growth characteristics of *C. simplicispina* population. Allometric growth equation was used according to Lagler (1966).

$W = a \times TL^b$

W= Weight of fish (gr)

TL= Total length of fish (cm)

a= In exponential equality, the Y-axis of the line is the

b= Explain the slope of the regression line [17].

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

The samples were made in May 2019. Blood samples were collected from the injected intracardiac area from fifteen fish samples. Blood smear preparations were done now to prevent coagulation. After drying in air, the samples were fixed in 96% ethanol for 2 minutes. Behind fixation, all samples dyed with haematoxylin for 1 minute and washed under tap water. Then, preparations dyed in eosin for 1 minute washed with distilled water and kept in xylene for 2 minutes as the last step. All samples were examined under Nikon Eclipse TS100 microscope, and images were obtained for erythrocyte and nucleus diameter measurements with Kameram 21 software. Area and volume calculations were done with the formula [18, 19].

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

Eqn. 1

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

Eqn. 2

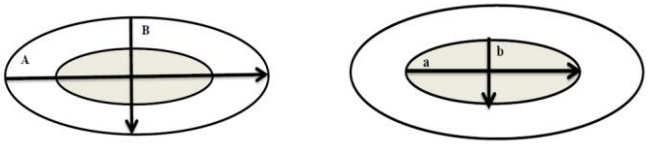


Fig. 2. *Measurement Template*

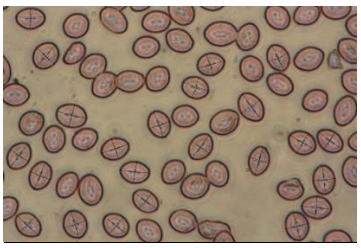


Fig. 3. *General view of stained erythrocytes*

RESULTS AND DISCUSSION

Weight of *Cobitis simplicispina* was examined ranged from 3.3 to 13.3 gr (Fig. 4).

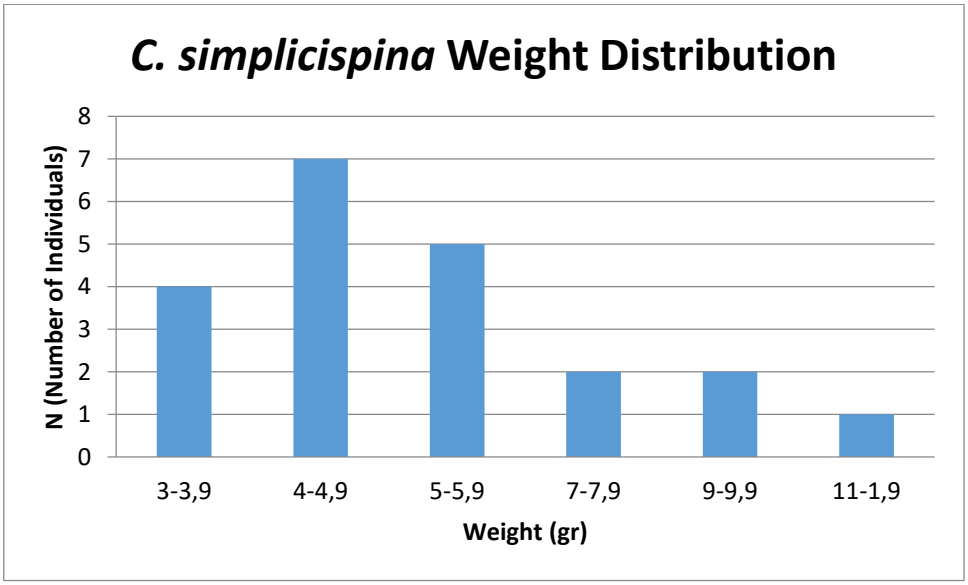


Fig. 4. *C. simplicispina weight distribution*

The total length of *Cobitis simplicispina* was examined ranged from 8.3 to 12.2 cm (Fig. 5).

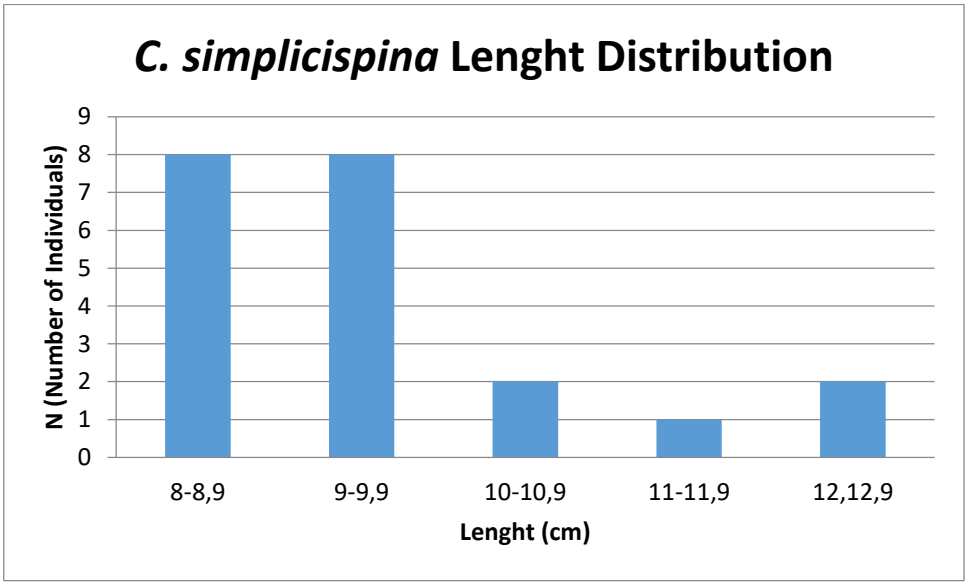


Fig. 5. *C. simplicispina length distribution*

Table 1. *The length and weight values of C. simplicispina*

	Minimum	**Maximum**	**Mean**	**Standard Error**
Weight (gr)	3.3	11.3	5.63	2.29
Length (cm)	8.3	12.2	9.60	1.18

The b value of the population was calculated to be 3,004. This value calculated in 95% confidence interval of b (2.709-3.299) showed that the population has isometric growth (Table 2).

Table 2. *b value, 95% confidence interval of b, a value, R^2*

b	%95 CI of b	a	R^2
3,004	2,709-3,299	0,006	0,979

Our data was showed that the length-weight relationship of the population depending on the total length and weight values of the examined *Cobitis simplicispina* in Figure 6.

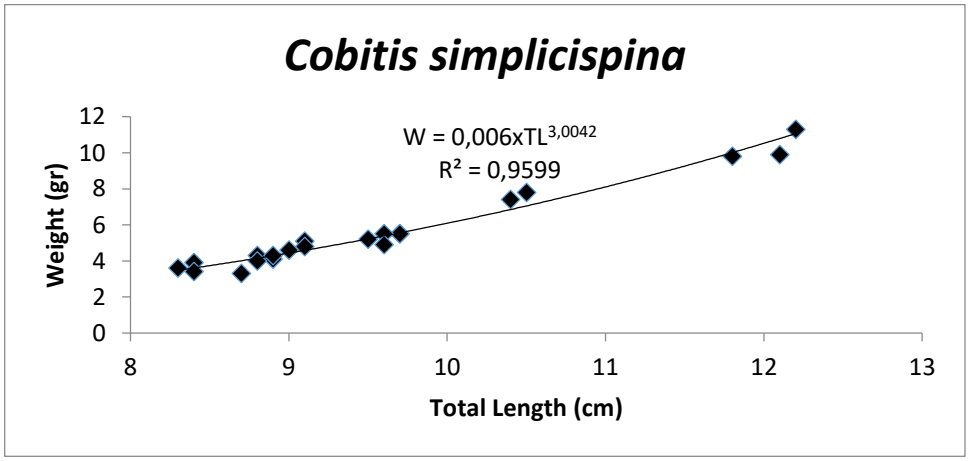


Fig. 6. *C. simplicispina length-weight relationship*

We make smear preparations from fifteen *C. simplicispina* blood. The Erythrocyte Nucleus Area, Erythrocyte Area, Erythrocyte Nucleus Volume, Erythrocyte Volume were calculated at least 100 cells then its arithmetic means were found. The results obtained are given in the table below Table 3.

Table 3. *Calculated ENA, EA, ENV, EV values of C. simplicispina samples in Kargın Stream*

	ENA µm²	EA µm²	ENV µm³	EV µm³
	Mean-std	**Mean**-std	**Mean**-std	**Mean**-std
1	**10,04**-1,44	**58,60**-3,48	**18,48**-4,54	**294,25**-30,13
2	**10,33**-1,33	**60,55**-4,79	**18,98**-4,25	**307,51**-38,03
3	**9,47**-1,31	**59,47**-4,46	**15,97**-3,48	**295,98**-34,27
4	**10,59**-1,37	**58,04**-4,43	**18,42**-3,78	**278,07**-33,61
5	**10,42**-1,33	**62,91**-3,96	**18,19**-3,69	**318,18**-29,69
6	**10,85**-1,45	**60,50**-4,83	**19,27**-3,83	**297,65**-36,93
7	**10,59**-1,43	**60,34**-4,54	**18,57**-4,15	**295,96**-38,24
8	**11,44**-1,32	**62,70**-4,20	**20,31**-3,61	**310,49**-35,49
9	**9,79**-1,45	**60,63**-4,50	**17,20**-4,15	**308,89**-35,18
10	**11,54**-1,71	**65,08**-4,34	**21,55**-5,20	**337,65**-36,83
11	**9,88**-1,10	**55,25**-2,68	**16,37**-2,84	**257,67**-22,32
12	**10,12**-0,98	**56,70**-3,20	**17,38**-2,59	**269,98**-21,77
13	**11,06**-1,51	**60,94**-4,52	**19,50**-4,23	**302,15**-36,57
14	**10,84**-1,03	**61,45**-3,34	**18,74**-3,18	**305,92**-27,52
15	**9,83**-1,10	**55,56**-3,37	**16,10**-2,97	**256,57**-26,35
16	**9,13**-1,23	**54,35**-3,71	**14,18**-2,99	**247,81**-26,19

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

Table 4. *Minimum, maximum, mean and standard error values of ENA, EA, ENV, EV values all samples*

	ENA µm²	**EA µm²**	**ENV µm³**	**EV µm³**
Minimum	9,13	54,35	14,18	247,81
Maximum	11,54	65,08	21,55	337,65
Mean	10,37	59,57	18,08	292,80
Standard Error	0,68	2,99	1,83	24,57

Table 5. *The ratio of the calculated ENA values of C. simplicispina samples to each other*

			Individuals Samples															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
			10.04	10.33	9.47	10.59	10.42	10.85	10.59	11.44	9.79	11.54	9.88	10.12	11.06	10.84	9.83	9.13
Individuals Samples	1	10.04	1.00	0.97	1.06	0.95	0.96	0.93	0.95	0.88	1.03	0.87	1.02	0.99	0.91	0.93	1.02	1.10
	2	10.33	1.03	1.00	1.09	0.98	0.99	0.95	0.98	0.90	1.06	0.90	1.05	1.02	0.93	0.95	1.05	1.13
	3	9.47	0.94	0.92	1.00	0.89	0.91	0.87	0.89	0.83	0.97	0.82	0.96	0.94	0.86	0.87	0.96	1.04
	4	10.59	1.05	1.02	1.12	1.00	1.02	0.98	1.00	0.93	1.08	0.92	1.07	1.05	0.96	0.98	1.08	1.16
	5	10.42	1.04	1.01	1.10	0.98	1.00	0.96	0.98	0.91	1.06	0.90	1.05	1.03	0.94	0.96	1.06	1.14
	6	10.85	1.08	1.05	1.15	1.03	1.04	1.00	1.02	0.95	1.11	0.94	1.10	1.07	0.98	1.00	1.10	1.19
	7	10.59	1.05	1.02	1.12	1.00	1.02	0.98	1.00	0.93	1.08	0.92	1.07	1.05	0.96	0.98	1.08	1.16
	8	11.44	1.14	1.11	1.21	1.08	1.10	1.05	1.08	1.00	1.17	0.99	1.16	1.13	1.03	1.06	1.16	1.25
	9	9,79	0.97	0.95	1.03	0.92	0.94	0.90	0.92	0.86	1.00	0.85	0.99	0.97	0.88	0.90	1.00	1.07
	10	11.54	1.15	1.12	1.22	1.09	1.11	1.06	1.09	1.01	1.18	1.00	1.17	1.14	1.04	1.06	1.17	1.26
	11	9.88	0.98	0.96	1.04	0.93	0.95	0.91	0.93	0.86	1.01	0.86	1.00	0.98	0.89	0.91	1.01	1.08
	12	10.12	1.01	0.98	1.07	0.96	0.97	0.93	0.96	0.88	1.03	0.88	1.02	1.00	0.91	0.93	1.03	1.11
	13	11.06	1.10	1.07	1.17	1.04	1.06	1.02	1.04	0.97	1.13	0.96	1.12	1.09	1.00	1.02	1.13	1.21
	14	10.84	1.08	1.05	1.14	1.02	1.04	1.00	1.02	0.95	1.11	0.94	1.10	1.07	0.98	1.00	1.10	1.19
	15	9.83	0.98	0.95	1.04	0.93	0.94	0.91	0.93	0.86	1.00	0.85	1.00	0.97	0.89	0.91	1.00	1.08
	16	9.13	0.91	0.88	0.96	0.86	0.88	0.84	0.86	0.80	0.93	0.79	0.92	0.90	0.83	0.84	0.93	1.00

CONCLUSION

In this study, we investigated the length-weight relationship and the gynogenetic reproduction potential of the *C. simplicispina* in Kargın Stream.

The b value is used to monitor the health of the fish population. The slope b value of the regression line in the length-weight relationship provides useful information about fish growth. The confidence interval (95% CI) of the calculated b value shows negative allometric if b <3, isometric 2.5-3.5, and positive allometric growth ≥3. *C. simplicispina* b value was calculated in the Kargın stream, and the population showed isometric growth. Cobitis species live in water benthos. Cobitis species are one of the fishes, react quickly to any ecological changes (pollution, change of flow regime, etc.) that occur in freshwater. Due to these properties, Cobitis species can be used as bioindicators in inland waters.

Our study, b value was found to be 3.004, standard error of b value was 0.141 and 95% CI of b 2.709-3.299. The correlation coefficient value of the regression was $R^2 = 0.959$. It was determined that *Cobitis simplicispina* showed isometric growth in Kargın stream.

Abremenko reported that there is 1.35 times the difference between the nucleus squares of 2n (bisexual) and 3n (gynogenetic) individuals [18]. In order to determine the gynogenetic reproduction potential of *Cobitis simplicispina*, we calculated ENA, EBV, EA and EV values.

Our results demonstrated *Cobitis simplicispina* population in Kargın stream did not reproductive by gynogenetic.

Although gynogenetic reproductive ability is not seen in this population, Cobitis may show gynogenetic reproductive ability when they are moved from their natural habitats to another habitat. For this reason, it is recommended to monitor the gynogenetic reproduction status of the Cobitis populations.

Gynogenetic reproduction populations are more tolerant of environmental conditions. Therefore, gynogenetic reproduction populations are not recommended as bioindicator species. Furthermore, it is recommended that gynogenetic reproduction fish populations could be evaluated intolerant species group in biological indices

As a result, it was determined that *C. simplicispina* is not gynogenetic reproduction. These fish species was suitable for biological monitoring in Kargın Stream population.

REFERENCES

[1] Arai, K., Boroñ, A. (2003). Proceedıngs of the Second Internatıonal Conference On Loaches Of The Genus Cobıtıs And Related Genera, (51). Olsztyn, Poland: Folia biologica (Kraków).

[2] Kottelat, M. (2012). Conspectus Cobıtıdum*: An Inventory Of The Loaches Of The World (Teleostei: Cyprınıformes: Cobıtoıdei), The Raffles Bulletin of Zoology, (26), 1–199.

[3] Freyhof, J., Baycelebi, E., Geiger, M. (2018). Review of the genus Cobitis in the Middle East, with the description of eight new species (Teleostei: Cobitidae), In Zootaxa (Vol. 4535).

[4] Perdices, A., Ozeren, C. S., Erkakan, F., Freyhof, J. (2018). Diversity of spined loaches from Asia Minor in a phylogenetic context (Teleostei : Cobitidae), 1–16.

[5] Vasil'eva, E. D., Vasil'ev, V. P. (2019). Natural Hybridization in Spined Loaches of the Genera Cobitis and Sabanejewia (Cobitidae), Journal of Ichthyology, 59(5), 776–785.

[6] Vasil'ev, V. P., Lebedeva, E. B., Vasil'eva, E. D. (2011). Evolutionary ecology of clonal-bisexual complexes in spined loaches from genus Cobitis (Pisces, Cobitidae), Journal of Ichthyology, 51(10), 932–940.

[7] Kotusz, J. (2008). Morphological Relationships Between Polyploid Hybrid Spined Loaches of the Genus Cobitis (Teleostei: Cobitidae) and their Parental Species, Annales Zoologici, 58(4), 891–905.

[8] Fricke, R.; Bilecenoglu, M.; Sarı, H. M. (2007). Annotated checklist of fish and lamprey species (Gnathostomata and Petromyzontomorphi) of Turkey, including a Red List of threatened and declining species, Stuttgarter Beitr. Naturk. Ser. A Nr. 706 169 S., 3 Abb., 8 Tab. Stuttgart, 10. IV. 2007.

[9] Kottelat, M.; Freyhof, J. (2007). Handbook of European freshwater fishes. Kottelat, Cornol, Switzerland and Freyhof, Berlin, Germany, pp. 646.

[10] Kottelat, M. (2012). Conspectus Cobitidum: an inventory of the loaches of the world (Teleostei: Cypriniformes: Cobitoidei), Raffles Bull. Zool. Suppl. 26, 1–199.

[11] Emiroğlu, Ö., Giannetto, D., Aksu, S., Başkurt, S., Çiçek, A., Tarkan, A. S. (2019). Length-weight, length-length and empirical standard weight equations for Capoeta baliki, Capoeta sieboldii and Chondrostoma angorense, three endemic cyprinid species of northwestern Anatolia, Iranian Journal of Fisheries Sciences.

[12] Gaygusuz, Ö., Aydın, H., Emiroğlu, Ö., Top, N., Dorak, Z., Gaygusuz, Ç. G., Başkurt, S., Tarkan, A. S. (2013). Length–weight relationships of freshwater fishes from the western part of Anatolia, Turkey, Journal of Applied Ichthyology, 29(1), 285-287.
[13] Tarkan, A. S., Gaygusuz, Ö., Acıpınar, H., Gürsoy, Ç., Özuluğ, M. (2006). Length–weight relationship of fishes from the Marmara region (NW-Turkey), Journal of Applied Ichthyology, 22(4), 271-273.
[14] Ekmekci, F., Erk'akan, G. (2003). Preliminary data on growth and reproduction of Cobitis cimplicispina from Turkey, Folia Biologica, 51(Suppl.).;
[15] Erk'akan, F., Innal, D., Ozdemir, F. (2014). Technical contribution Length–weight relationships for some endemic stone and spine loach species in Anatolia, J. Appl. Ichthyol, 30, 244-245.
[16] Türkiye Haritası, Eskişehir, Nehirleri Irmakları, Erişim Adresi https://www.haritatr.com/harita/Kargin-Cayi/17835
[17] Le Cren, E.D. (1951). The Length-Weight Relationships and Seasonal Cycle in Gonad Weight and Condition in Perch (Perca fluviatilis), J. Anim. Ecol., 20, 210-219 pp.
[18] 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.
[19] 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.