

## Original Article

# Hematology and plasma chemistry reference intervals for wild population of *Alburnus chalcoides* (Guldenstadt, 1772): Influence of sex, habitat and seasonal variation

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**Abstract:** The study was aimed to establish reference interval for some blood biochemical (sodium, potassium, calcium, and magnesium) and hematological (total count of white blood cells, neutrophils, lymphocytes, monocytes, and eosinophils) indices in both sexes of *Alburnus chalcoides* in response to its habitats with different physiochemical conditions and to seasonal variation. Fish samples were collected from its seasonal habitats, including river, estuary, and Caspian Sea. The mean concentration of sodium, potassium, calcium, and magnesium in plasma of fish were  $134.69 \pm 13.61$  (mmol/l),  $3.26 \pm 0.83$  (mmol/l),  $11.65 \pm 1.82$  (mg/dl) and  $2.13 \pm 1.12$  (mmol/l), respectively. These electrolytes demonstrated significant differences between the male and female as well as between the fish collected from different habitats. Total WBCs count was  $6045.35 \pm 960.25$  (per  $\text{mm}^3$ ) and the mean percentage of neutrophils, lymphocytes, monocytes and eosinophils were  $26.55 \pm 3.37\%$ ,  $67.74 \pm 3.72\%$ ,  $3.04 \pm 0.70\%$  and  $0.98 \pm 0.04\%$ , respectively. WBCs generally showed considerable differences between male and female. Furthermore, a higher percentage of monocytes, neutrophils and eosinophils were observed in the fish collected from river and estuary during spring and summer. The study demonstrated that differences in sex, habitat and seasonal variation cause variation in hematology and plasma chemistry reference intervals for wild population of *A. chalcoides* when migrating between its natural habitats.

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## Introduction

*Alburnus chalcoides* is a commercially valuable anadromous and benthopelagic fish, inhabiting in rivers, coastal lakes, estuaries, and brackish waters (up to 14 ppt). This species is distributed in the Caspian Sea basin, and its spawning occurs in small rivers or streams (Kottelat and Freyhof, 2007). The adult populations migrate upstream for spawning, and the embryonic development lasts 2-3 days, and then the larvae (with the age of 8-11 days) actively migrate downstream (Kottelat, 1997). After spawning, the adults migrate back to lakes, estuaries, and seas (i.e., brackish water).

Recently, due to overfishing, expanding hydroelectric development, and strong ecological impacts, the population of *A. chalcoides* has considerably declined, especially in the southern and southwest coasts (Kottelat and Freyhof, 2007; Nikoo

et al., 2010; Rajabi Nezhad and Azari Takami, 2001), and hence this fish has been classified as a vulnerable in the IUCN Red List (Kiabi et al., 1999; Rahmani et al., 2007).

Knowledge of hematological or biochemical reference ranges is important in assessing, managing, and better understanding the health status animal populations (Dickinson et al., 2002). Diagnostic assessment of blood hematological and biochemical parameters have been used extensively for many terrestrial animal species (Hrubec and Smith, 2004). Whereas this type of hematological utilization is limited for fishes. Hence, it is necessary to utilize such an information to assess the health status of fish populations in their natural habitat as well as in the captive condition.

Owing to migration between three types of aquatic ecosystems (i.e., river, estuary, and Caspian Sea),

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Table 1. The mean value of air temperature as well as water physiochemical parameters at the sampling location in river (Lale River), estuary and the Caspian Sea.

Month	Location	Dissolved oxygen (mg/l)	pH	Salinity (ppt)	Water temperature (°C)	Air temperature (°C)
April	Lale River Estuary	7.40	8.10	4	13.16	15.75
May		7.50	8.30	1	19.08	20.13
June	Lale River	9.60	8.40	0.00	24.48	25.08
July		9.20	8.30	0.00	25.50	26.02
August	Lale River Estuary	9.00	8.20	0.00	27.39	28.04
September		8.10	7.90	3.50	24.55	26.16
October		9.00	8.00	9.50	19.56	22.49
November		8.10	8.20	10.00	17.84	19.28
December	Caspian Sea	8.40	8.00	9.00	11.78	14.21
January		8.60	8.00	9.10	9.00	9.75
February		8.22	8.20	10.50	10.27	11.65
March		8.22	8.30	10.24	10.79	12.41

*A. chalcoides* is exposed to different cyclical external conditions which in turn change its internal biochemical status (Naderi and Abdoli, 2004). The external cyclical conditions may influence blood biochemical and hematological parameters, and understanding normal health status of *A. chalcoides* during migration between these different aquatic ecosystems is ecologically of high importance. Therefore, the present study was aimed to determine reference ranges of some hematological and plasma biochemical parameters of both sexes of *A. chalcoides* when migrating between its annual habitats (river, estuary, and the Caspian Sea).

## Materials and Methods

**Sampling location:** For this study, 84 healthy individuals of *A. chalcoides* (average total length:  $16.5 \pm 4$  cm; average weight:  $45.69 \pm 0.27$  gr; male=42 and female=42) were collected from three locations across its annual migration way (i.e., river, estuary, and Caspian Sea, with salinity of 0.4, 3.75, and 9.71 ppt, respectively). These locations are in the southern coast of the Caspian Sea and Lale River in the Chamkhaleh, Guilan Province (Fig. 1). The sampling was done during 12 month in 2014; from the river, in May, June, July and August; from the estuary, in April and September; and from the Caspian Sea, in October, November, December, January, February, and March.



Figure 1. Sampling location in the southern coast of the Caspian Sea.

Some physicochemical parameters of water, including salinity, dissolved oxygen, pH, and temperature, at the sampling sites (Table 1).

**Sex and age determination:** The collected fish were dissected and the sex determination was done according to gonadal characteristics. Age was determined by scale reading from the fish. Briefly, scale samples (8-15 scales) were removed from below

Table 2. Reference interval of four main blood plasma electrolytes (calcium, potassium, sodium, and magnesium) in both male and female *Alburnus chalcoides* in different season.

Plasma ion	Season							
	Spring		Summer		Autumn		Winter	
	Sex							
	M	F	M	F	M	F	M	F
Calcium (mg/dl)	10.30	10.55	10.25	11.89	12.68	13.65	11.83	12.61
Potassium (mmol/l)	1.63	2.69	3.18	3.31	3.06	3.73	3.48	4.53
Sodium (mmol/l)	122.50	124.47	129.21	130.29	146.65	147.36	139.13	137.90
Magnesium (mmol/l)	1.40	1.15	2.83	3.03	2.47	2.46	1.47	2.08

M= male, F= female

the lateral line and between the anal and caudal fins and cleaned in 5% sodium peroxide, and the scale radiuses were counted based on the von Bertalanffy equation (Biradar, 1989). Fish with 3-4 years old were used for the study.

**Blood collection and hematological and biochemical analysis:** Fish blood was drawn from the caudal vein using heparinized and unheparinized syringes. The heparinized samples were used for white blood cell counting (total count of white blood cells (WBC), neutrophils, lymphocytes, monocytes, and eosinophils) and the for plasma biochemical evaluation (sodium, potassium, calcium, and magnesium ions) was used unheparinized samples. After centrifugation at 2000 g, blood plasma was collected and stored at -20°C until further assay. Total calcium and magnesium concentrations were determined with colorimetric assay kit and Unico UV-2100 Spectrophotometer. Sodium and potassium were analyzed via a flame photometer (Model PFP7, Jenway, Essex, UK) (Wong et al., 2001). To establish the percentage of leukocytes, WBCs were counted in each treatment with Sysmex K-1000 Analyzer (Ballarin et al., 2004; Clark and Kruse, 1990).

**Statistical analysis:** Data analysis was performed using SPSS software (SPSS, version. 22). All results are presented as the mean  $\pm$  standard deviation (SD). Significant differences were determined using one-way ANOVA, followed by Tukey test to compare the differences between the fish groups collected from the river (Lale River), Lale River estuary, and Sea ( $P < 0.05$ ). T-test student was applied to elucidate the effect of sex and seasonal variation on plasma ions. In addition, Pearson correlation coefficient was used to

determine the relationship between the biometric indices (length and weight) and blood WBCs.

## Results

Figure 2 shows the mean reference level of potassium, sodium, calcium, and magnesium in plasma of adult male and female *A. chalcoides*. The values of potassium and calcium in the female were significantly higher than those of males, whereas magnesium showed higher level in the males than females ( $P < 0.05$ ). No significant sex related difference was found in the mean plasma sodium level.

The effect of seasonal variation and subsequent cyclical condition on the above mentioned plasma electrolytes in the male and female *A. chalcoides* is shown in Table 2. The highest and lowest amount of calcium and sodium was measured in the fish collected from the Caspian Sea during autumn and spring, respectively. Plasma potassium concentration was measured with the highest level in winter, but the lowest in spring. Furthermore, these electrolytes (except magnesium) illustrated significant differences between the fish collected from the sampling habitats (i.e., river, estuary, and Caspian Sea).

During sampling period, white blood cell count demonstrated no significant difference in the percentage of lymphocytes, monocytes and eosinophils between the male and female *A. chalcoides* (Fig. 3), but the number of neutrophils in the female animals was higher than the males ( $P < 0.05$ ).

However, significant differences were observed when compared to their respective ones collected from

Table 3. The correlation between the biometric indices (length and weight) and blood WBC of *Alburnus chalcoides*.

Parameter	Total weight	Gonadal weight	Total length
WBC	0.251*	0.195	0.305**
Neutrophils	0.089	0.197	0.018
Lymphocytes	0.028	-0.112*	-0.099*
Monocytes	-0.101*	-0.142*	-0.001**
Eosinophil	0.156	0.205	0.127

Asterisk (\*) and double asterisk (\*\*) show significant difference at  $P < 0.05$  and  $P < 0.01$ , respectively.

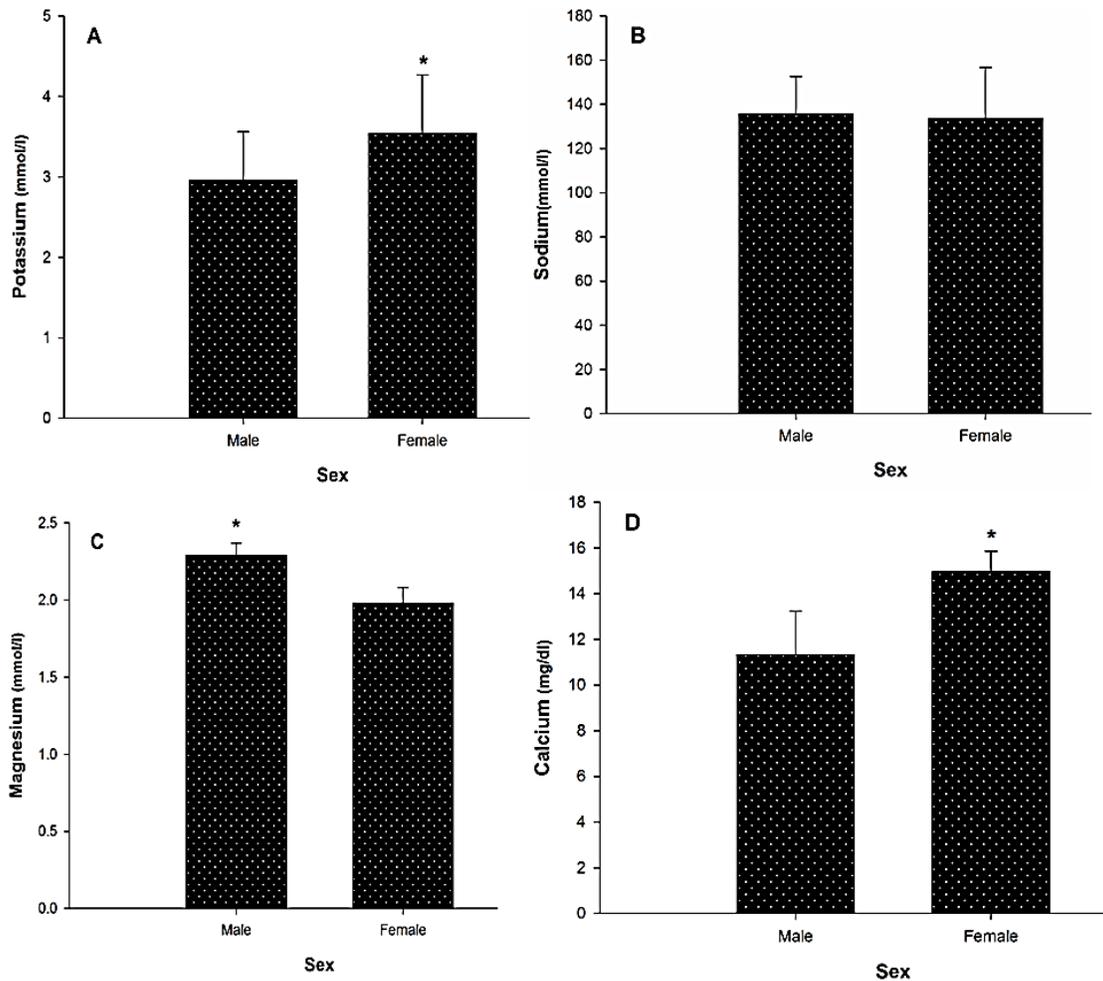


Figure 2. The mean reference level of potassium (A), sodium (B), magnesium (C), and calcium (D) in plasma of adult male and female *Alburnus chalcoides* (data expressed as mean  $\pm$  SD).

other locations (Fig. 4). The highest percentage of monocytes, neutrophils and eosinophils was found in the blood of the animals collected from the estuary (i.e., Lale River Estuary) ( $P < 0.05$ ), and the percentages in the fish sampled from different locations showed an order of estuary > river > Caspian Sea. The mean percentage of lymphocytes showed no significant difference between three habitats ( $P < 0.05$ ).

The correlation between biometric indices (length

and weight) and blood WBC is shown in Table 3. Pearson analysis showed a strong positive correlation between WBC, neutrophils, lymphocytes, and eosinophil with weight and length of the collected fish, but monocytes showed a negative correlation with weight and length ( $P < 0.05$  and  $P < 0.01$ ).

## Discussion

Some environmental conditions such as temperature,

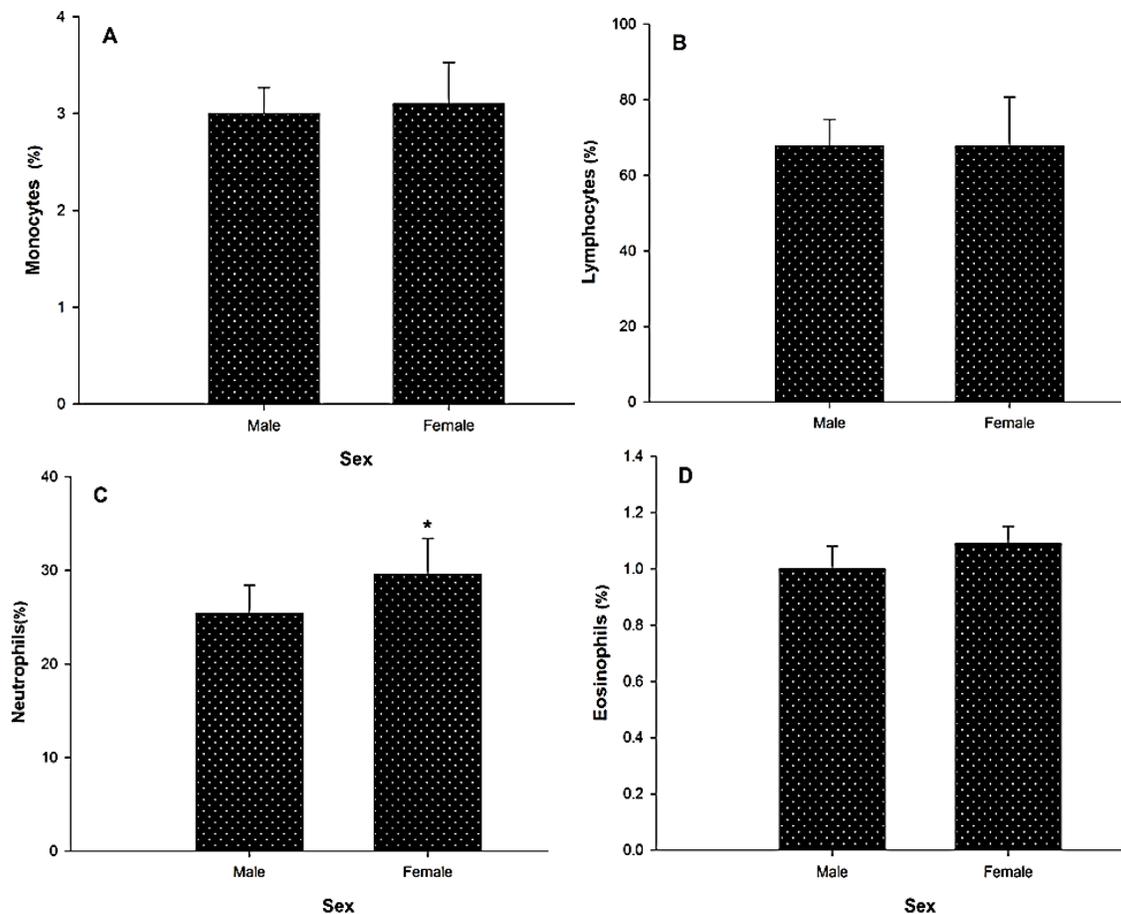


Figure 3. The mean percentage of monocytes (A), lymphocytes (B), neutrophils (C), and eosinophils (D) in the blood of male and female *Alburnus chalcoides* (data expressed as mean  $\pm$  SD).

seasonality, oxygen, and salinity as well as internal factors (age, sex, reproductive state, genetic variation, and stage of development) have been documented to affect hematologic values (Knowles et al., 2006). Due to lack of information concerning some hematological and biochemical parameters of *A. chalcoides*, the present study aimed to determine a reference value for WBCs count and some plasma electrolytes predominantly involve in osmoregulation during presence in its environmentally different habitats. Higher amount of potassium and calcium were observed in the female; the female fish collected during the autumn showed the highest concentrations of plasma calcium and the lowest level in the spring (breeding season). Owing to a wide fluctuation in plasma calcium level in female fish during the breeding cycle, i.e., their gonadal maturation (Webb et al., 2002), the observed difference in the plasma calcium concentration in the female fish can be an

evidence for higher amount of plasma calcium in the non-breeding seasons (autumn and winter) of *A. chalcoides*. Hence, plasma calcium level is a reliable indicator for oocyte maturation (involving in vitellogenesis) and its level gradually increases during the pre-spawning period and reaches to a peak value just before spawning, and then illustrates a sharp decline after the spawning (Bromage et al., 1993). Support for this result has come from previous reports in which the amount of plasma calcium in female Atlantic flatfish *Hippoglossus hippoglossus* were 4.5 and 2.5 mmol/L, respectively, before and after spawning period (Björnsson et al., 1998; Bromage et al., 1993).

Magnesium participates in many biological processes such as modulating a large number of enzymes as well as regulating energy metabolism and protein synthesis (Davis and Gatlin III, 1996). Magnesium deficiency is associated with deficiency

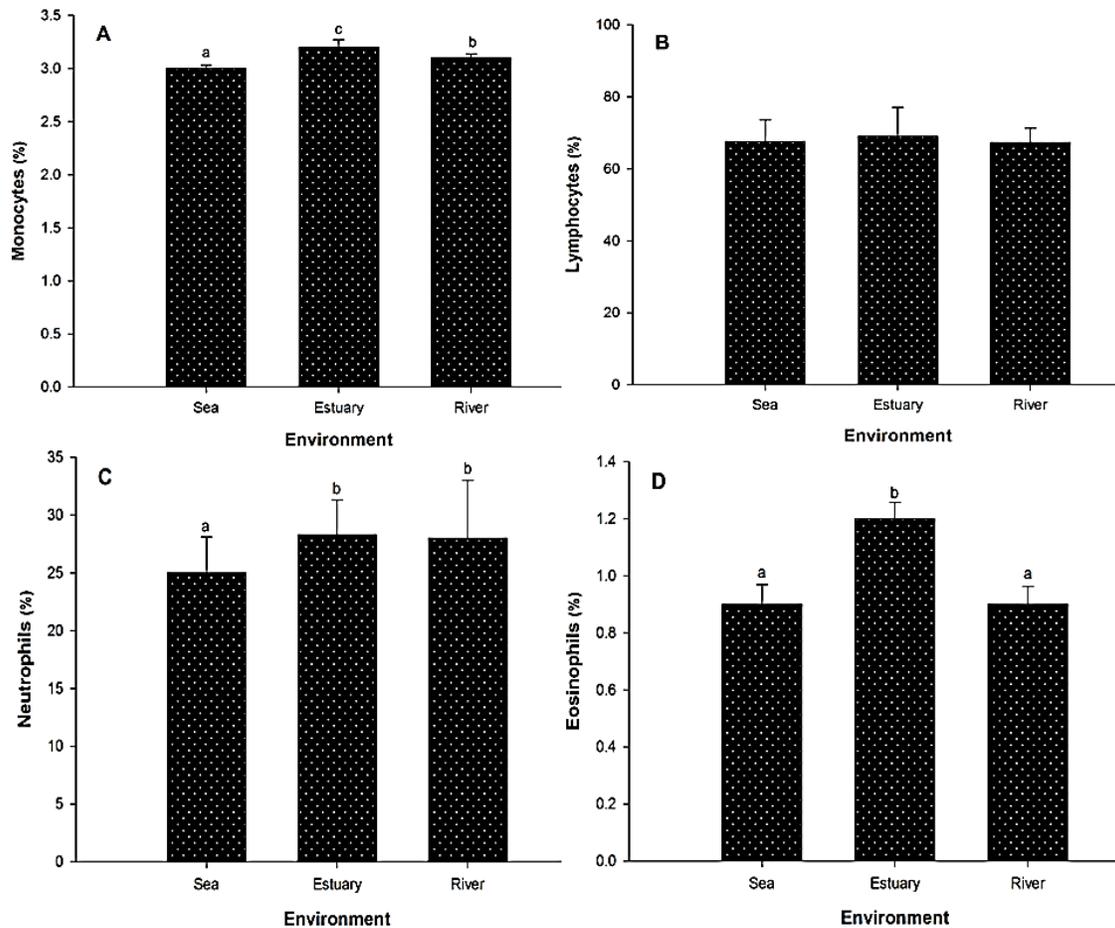


Figure 4. The mean percentage of monocytes (A), lymphocytes (B), neutrophils (C), and eosinophils (D) in the blood of *Alburnus chalcoides* collected from the Caspian Sea (sea), estuary (Lale River Estuary), and river (Lale River) (data expressed as mean  $\pm$  SD).

of other important minerals such as calcium, sodium and potassium which reflects the  $Mg^{2+}$ -dependence of ion exchange mechanisms such as sodium/potassium ATPase pumps and positive canals (Ellis et al., 2002). The present study demonstrated higher plasma magnesium concentration in the male *A. chalcoides*; however, no significant difference was observed associated with season and sampling location. Hence, the acquired data regarding the difference in plasma magnesium level between male and female *A. chalcoides* can be considered as new physiological information about the fish. This data corroborate the findings of Bagheri et al. (2007) who reported that both plasma calcium and magnesium are influenced by gender, growth phase and stage of gonadal maturity. Plasma sodium and potassium increase in fish migrate from hyposmotic to hyperosmotic environments (Mancera et al., 2017); therefore, the

observed higher plasma sodium level in *A. chalcoides* collected from sea compared to those of river and estuary is an acceptable osmoregulatory and physiological phenomenon. This value agrees with that observed in young green sturgeon (*Acipenser medirostris*), an anadromous species, when migrating from freshwater to sea water (Poletto et al., 2017). In addition, no sex dependent difference was observed in plasma sodium concentration, suggesting that plasma sodium concentration is independent to gonadal difference in males and females. Similar results also have been reported by Khodadadi (2009) who found no sex dependent difference in plasma sodium level of *Barbus sharpeyi*.

Evaluating the number of is an important hematological index to evaluate immune system and also a biomarker for terrestrial and aquatic studies (Modesto and Martinez, 2010; Sancho et al., 2000). In

the present study, WBC count demonstrated the order of percentage as follows lymphocytes> neutrophils> monocytes> eosinophils. However, the fish collected from river and estuary during spring and summer illustrated significantly higher percentage of WBC (except lymphocytes) compared to those collected from sea in the autumn and winter. In addition, the percentage of neutrophils in the females was significantly higher. Support for this results has come from previous studies in which significantly higher percentage of lymphocytes was recorded in the spawning season, and also the level of WBCs number in the females were higher than those in males; i.e., some sex-related differences in hematological indices was observed (Adel et al., 2017; Marijani et al., 2017; Örün and Erdemll, 2002).

### Conclusion

The study demonstrated that differences in sex, habitat and seasonal variation cause variation in hematology and plasma chemistry reference intervals for wild population of *A. chalcoides* when migrating between its natural habitats, and knowledge about health status of this fish species is better to be considered just according to its habitat environment and further investigation are need to determine precise other hematological and biochemical reference intervals for the fish in river, estuary, and Caspian sea.

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## چکیده فارسی

### میزان مرجع پارامترهای خون‌شناسی و شیمیایی پلاسمای جمعیت وحشی ماهی شاه کولی (*Alburnus chalcoides*): تأثیر جنسیت، زیستگاه و تغییرات فصلی

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#### چکیده:

مطالعه حاضر سعی دارد که میزان طبیعی و مرجعی از برخی پارامترهای بیوشیمیایی (سدیم، پتاسیم، کلسیم و منیزیم) و خون‌شناسی (تعداد کل گلبول‌های سفید خون، نوتروفیل، لنفوسیت، مونوسیت و ائوزینوفیل) در دو جنس نر و ماده ماهی شاه کولی را در زیستگاه‌های طبیعی با خصوصیات فیزیکی‌وشیمیایی متفاوت و همچنین در ارتباط با تغییرات فصلی گزارش نماید. نمونه‌های ماهی از سه زیستگاه (رودخانه، مصب و دریای خزر صید شدند. میانگین غلظت سدیم، پتاسیم، کلسیم و منیزیم در پلاسمای ماهیان به ترتیب  $۱۳۴/۶۹ \pm ۱۳/۶۱$ ،  $۳/۲۶ \pm ۰/۸۳$ ،  $۱۱/۶۵ \pm ۱/۸۲$  و  $۲/۱۳ \pm ۱/۱۲$  میلی‌مول بر لیتر بود. این الکترولیت‌ها تفاوت معنی‌داری بین جنس‌های نر و ماده و همچنین بین ماهیانی که از زیستگاه‌های مختلف جمع‌آوری شده بودند، نشان دادند. کل گلبول‌های سفید خون  $۶۰۴۵/۳۵ \pm ۹۶۰/۲۵$  عدد در میلی‌متر مکعب و همچنین درصد نوتروفیل‌ها، لیمفوسیت‌ها، مونوسیت‌ها و ائوزینوفیل‌ها به ترتیب  $۲۶/۵۵ \pm ۳/۳۷$ ،  $۶۷/۷۴ \pm ۳/۷۲$ ،  $۳/۰۴ \pm ۰/۷۴$  و  $۰/۹۸ \pm ۰/۰۴$  بودند. گلبول‌های سفید تفاوت معنی‌داری بین جنس نر و ماده داشتند. مونوسیت‌ها، نوتروفیل‌ها و ائوزینوفیل‌های ماهیانی که از رودخانه و مصب رودخانه در بهار و تابستان جمع‌آوری شده بود، درصد بالاتری نشان دادند. تحقیق حاضر بیانگر آن است که جنسیت، زیستگاه و همچنین تغییرات فصلی می‌توانند تغییرات و تفاوت‌هایی در میزان طبیعی پارامترهای خون‌شناسی و شیمی پلاسمای خون بین جمعیت‌های وحشی ماهی شاه کولی در هنگام مهاجرت بین زیستگاه‌های طبیعی آن ایجاد نمایند.

کلمات کلیدی: پارامترهای بیوشیمیایی، دریای خزر، شاخص‌های خونی، زیستگاه فصلی.