

Some Blood Parameters in Sea Bass (*Dicentrarchus labrax* L.) With Respect to Water Quality Parameters

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Abstract: This study was conducted to determine the seasonal values of some blood parameters (hematocrit, hemoglobin, the numbers of erythrocyte, leucocyte and trombocyte as well as Wintrobe erythrocyte indices) in clinically healthy sea bass reared at a commercial farm, Bodrum (Turkey) with respect to water quality parameters including water temperature, pH, dissolved oxygen and ammonia-nitrogen.

The results showed that water temperature and dissolved oxygen had an impact on hematocrit, hemoglobin and Mean Corpuscular Hemoglobin Concentration (MCHC) ($p < 0.01$). Erythrocyte, leucocyte and trombocyte counts were not statistically affected by water quality parameters detected. Whereas, there is a little statistically significant effect of ammonia-nitrogen on haematological parameters.

Key Words: Blood parameters, sea bass (*Dicentrarchus labrax* L.), water quality parameters

Deniz Levreğinde (*Dicentrarchus labrax* L.) Su Kalite Özelliklerine Bağlı Olarak Bazı Kan Parametreleri

Özet: Bu çalışma, ticari bir levrek işletmesinde (Bodrum, Türkiye), klinik olarak sağlıklı deniz levreklerinde bazı kan parametrelerinin su kalitesine bağlı olarak mevsimsel değerlerini belirlemek amacıyla yapılmıştır. Balıklarda kan parametrelerinden hematokrit, hemoglobin, eritrosit, lökosit ve trombosit sayıları ile Wintrobe eritrosit indisi, su kalite özelliklerinden ise su sıcaklığı, pH, çözünmüş oksijen ve amonyak azotu mevsimsel olarak izlenmiştir.

Bu çalışmada elde edilen bulgulara göre; hematokrit, hemoglobin ve Ortalama Alyuvar Hemoglobin Yoğunluğu (OAHbY), su kalitesinden en çok etkilenen parametrelerdir. Eritrosit, lökosit ve trombosit sayıları ise ölçülen su kalite parametrelerinden etkilenmemişlerdir. Ancak amonyak azotunun hematolojik parametreler üzerinde istatistiki olarak bir etkisi olduğu tespit edilmiştir.

Anahtar Kelimeler: Kan parametreleri, deniz levreği (*Dicentrarchus labrax* L.), su kalite özellikleri

Introduction

Production figures show a steady rise in aquaculture production worldwide. In particular there has been a steep rise in production of Mediterranean species, notably sea bass and sea bream. Production of sea bass has centred around the Mediterranean with full-scale production taking place principally in Turkey, Greece, Italy, Spain and France. Turkey produced a total of 5210 metric ton of sea bass in 1996, of which over 50 percent has been exported (Anonymous 1997).

There has been a recent growth of intensive sea bass and sea bream culture in the Mediterranean. Therefore more studies have been done on fish health. With this respect haematology can be routinely a valuable tool to control the fish health status. However, haematology is not commonly used in fish medicine part due to lack of reference intervals for various fish species.

The goals of this study were to determine if there were differences in the haematological profiles based on the seasonal water quality parameters of culture system in

which the fish were raised. These results provide a foundation for determining whether haematological parameters changes occur with changes in water quality parameters and helps to establish haematology as a diagnostic tool for sea bass.

Material and Methods

Sea bass (*Dicentrarchus labrax* L.), clinically healthy, weighing 44-110g from a commercial farm in Bodrum were taken in February, April, June and September 1997. Stocking rates were 8-10 kg/m³. Fish which were reared in rectangular floating cages were fed on a standard diet containing 46-52 % crude protein.

Blood samples was collected by heart puncture using disposable heparinized plastic syringe. Blood samples in capillaries were filled and centrifuged immediately at 12500 rpm (12500 rpm) and the hematocrit was determined directly after centrifugation (Siwicki and Anderson 1987).

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The hemoglobin concentration was determined by spectrophotometry using cyan-methemoglobin method (Blaxhall and Daisley 1973). Natt-Herrick solution was used for erythrocyte, leucocyte and trombocyte counts, as recommended by Stoskopf (1993). The Wintrobe erythrocyte indices were calculated from hematocrit, hemoglobin and erythrocyte numbers.

Water temperature, dissolved oxygen and pH determinations were measured at the sample locations. Water samples were collected for ammonia-nitrogen and stored in polyethylene containers, immediately were performed (Anonymous 1975).

Variance analysis (ANOVA) was used to determine whether water quality and haematological parameters differed among seasons. The relationships between water quality and haematological parameters were examined by an analysis of multiple regression (Neter et al. 1990).

Results

Water quality and haematological parameters measured in February, April, June and September 1997 are shown respectively in Figure 1 and in Table 1.

With respect to water quality parameters the results of ANOVA demonstrated significant differences in water temperature, dissolved oxygen and ammonia-nitrogen among seasons ($p < 0.05$). Water temperature varied between 14.0 - 24.6 °C during the research. It was the lowest in February (14.75 ± 0.14 °C) and the highest in June (24.02 ± 0.22 °C). The dissolved oxygen level in floating cages was never below 5.10 mg/l. The highest level of dissolved oxygen occurred in April (7.47 ± 0.19 mg/l) and its level decreased in June (5.70 ± 0.32 mg/l). Average pH values of water have shown insignificant fluctuations and it reached a maximum of 8.27 in April throughout our study ($p > 0.05$). The ammonia-nitrogen concentration in floating cages tends to be the highest during February (0.427 ± 0.09 mg/l). However, concentrations of ammonia-nitrogen were higher than recommended levels.

Significant differences were detected in hematocrit and hemoglobin levels ($p < 0.05$) with respect to seasons. At high temperatures sea bass showed increased hematocrit and hemoglobin content. The range of hematocrit and hemoglobin were 26.68 to 54.55 % and 8.17 to 16.18 g/dl, respectively.

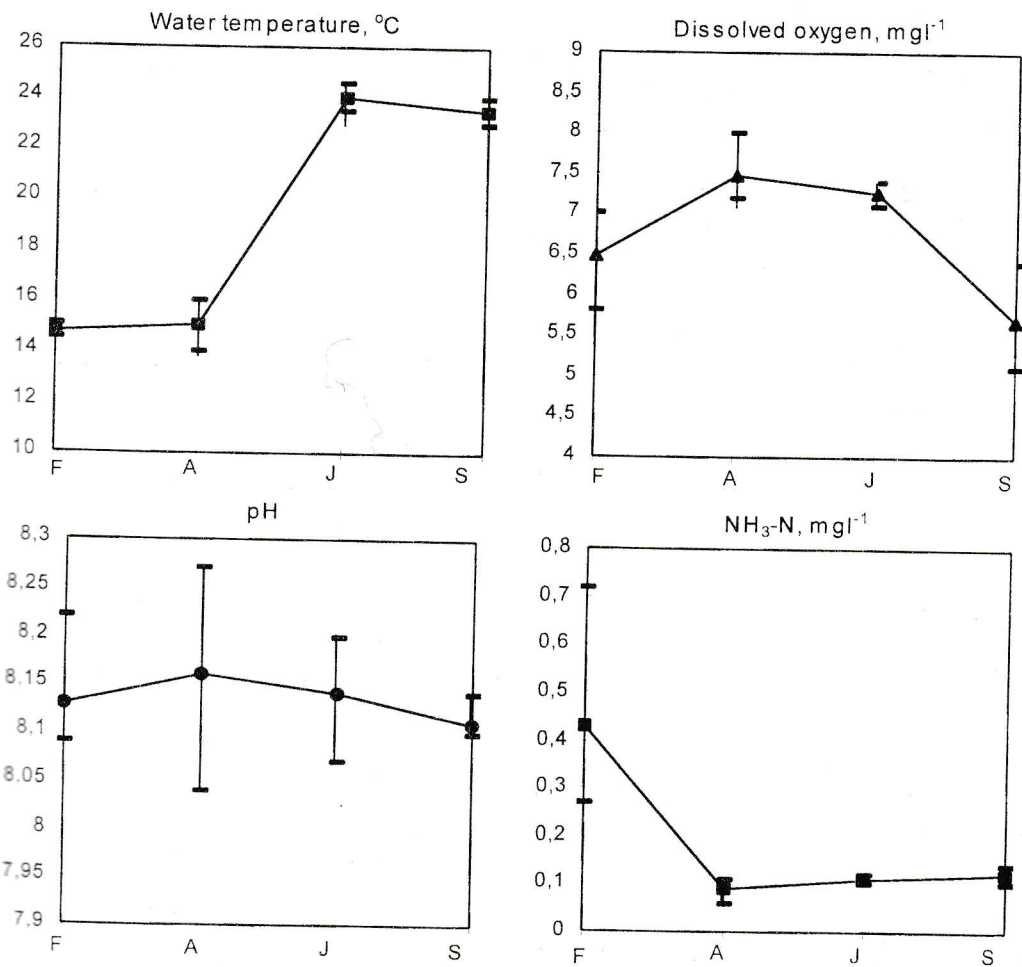


Figure 1. Seasonal (F, February; A, April; J, June; S, September) variation of water temperature, dissolved oxygen, pH and ammonia-nitrogen. Vertical lines indicate standard deviation.

Table 1. Seasonal values of selected haematological parameters of healthy sea bass in 1997.

Parameter	February			April			June			September		
	Average value	Min	Max	Average value	Min	Max	Average value	Min	Max	Average value	Min	Max
Hematocrit (%)	30.08±0.59	26.68	31.45	30.32±0.98	28.20	32.35	36.86±1.72	32.00	40.00	44.40±3.84	38.46	54.55
Hemoglobin (g/dl)	9.11±0.17	8.17	9.51	9.18±0.28	8.57	9.77	11.07±0.49	9.67	11.98	13.25±1.11	11.53	16.18
Erythrocyte (106 /µl)	3.65±0.11	3.45	3.94	3.61±0.16	3.23	3.96	4.12±0.18	3.73	4.51	3.93±0.11	3.77	4.23
Leucocyte (103 /µl)	22.25±1.93	17.00	26.00	24.25±0.85	22.00	26.00	24.00±0.71	23.00	26.00	23.75±1.03	22.00	26.00
Trombocyte (103 /µl)	1.00±0.40	0	2.00	1.75±0.25	1.00	2.00	3.25±0.45	2.00	4.00	2.00±0.91	0	4.00
MVC (fl)	82.19±1.45	79.00	86.02	84.69±6.28	71.21	97.23	90.11±6.10	73.90	102.49	113.30±10.60	97.60	144.30
MCH (pg)	24.96±0.41	24.13	26.08	25.64±1.86	21.64	29.53	26.36±1.73	22.33	30.75	33.79±3.08	29.26	42.80
MCHC (g/dl)	30.29±0.03	30.23	30.36	30.23±0.05	30.20	30.39	30.04±0.06	29.95	30.21	29.85±0.07	29.66	29.97

Table 2. Multiple regression analysis results.

Variables	Dependent variable; hematocrit			Dependent variable; hemoglobin			Dependent variable; MCHC			
	Coef.	St. dev.	t-ratio	Coef.	St. dev.	t-ratio	Coef.	St. dev.	t-ratio	p
(Constant)	96.154	148.442	0.648	28.186	42.906	0.657	27.040	3.637	7.435	0.00
Water temperature	0.804	0.330	2.432	0.232	0.096	2.432	-2.712E-02	0.008	-3.350	*0.006
Dissolved oxygen	-3.749	1.613	-2.324	-1.081	0.466	-2.319	9.679E-02	0.040	2.449	*0.032
pH	-6.106	18.068	-0.338	-1.764	5.222	-0.338	0.357	0.443	0.806	0.437
NH ₃ -N	-7.226	8.780	-0.823	-2.081	2.538	-0.820	0.253	0.215	1.175	0.265
					R ² =%67.8	*p<0.05			R ² =%77.9	*p<0.05

There were no change in erythrocyte, leucocyte and trombocyte counts ($p > 0.05$). The erythrocyte ranged from 3230000/ μ l to 4510000/ μ l. Mean leucocyte counts varied from 22250 \pm 1931/ μ l to 24250 \pm 854/ μ l. Trombocyte counts ranged between 0-4000/ μ l.

The Wintrobe erythrocyte indices (MVC, MCH, MCHC) showed seasonal fluctuations ($p < 0.05$).

All regressions involving water temperature and dissolved oxygen (independent variables) in terms of haematological parameters selected showed significant correlations. However, especially hematocrit, hemoglobin and MCHC values mostly were affected by water temperature and dissolved oxygen. (Table 2) There were no other clear relationship between pH, ammonia-nitrogen and haematological parameters measured.

Discussion and Conclusion

A normogram for the determination of the different haematological parameters needs to be developed for the different species for fish health status. The failure to establish a normogram may be attributed to various factors such as environment, age, sex activity, feeding regime etc. Thus, this study focuses on evaluating possible changes in some haematological (hematocrit, hemoglobin, erythrocyte, leucocyte and trombocyte counts, Wintrobe erythrocyte indices) characteristics of cage-cultured European sea bass (*Dicentrarchus labrax* L.) under different seasonal conditions (water temperature, dissolved oxygen, pH and ammonia-nitrogen).

In general, the haematological parameters measured in this study are within the normal range of other teleost fishes (Stoskopf 1993). However, seasonal fluctuations in haematological parameters were reported by several authors (Ezzat et al. 1973, Castritsi and Kavaidas 1993, Rajnovic et al. 1991, Mishra et al. 1977) and the definition of 'normal range' is more difficult for parameters that have seasonal and sexual fluctuations (Warner and Williams 1977).

The results of this study indicate that hematocrit, hemoglobin and MCHC were mostly affected by water temperature and dissolved oxygen. Hematocrit and hemoglobin content of sea bass were elevated at high temperature of June and September than those of February and April. In contrast with this findings, Castritsi and Kavaidas (1993) observed that hematocrit and hemoglobin of sea bass reared in Greece were higher in February ($T=12.5$ °C, $DO=8.4$ mg/l) and lower in August ($T=24.5$ °C, $DO=6.4$ mg/l). This contrast might be due to several factors such as different levels of dissolved oxygen, nutritional status and stocking rate.

According to this study clinically healthy sea bass reared in floating cages did not demonstrate the marked changes in the erythrocyte, leucocyte and trombocyte numbers. These characteristics were independent of the water quality.

The MCV, MCH and MCHC are corpuscular indices that are particularly useful in most to describe anemias and can be used in diagnosis and therapy. In fish haematology, the MCH and MCV values should be interpreted with caution since the red blood cells from which both are calculated may have a large amount of error (Stoskopf, 1993). In this study, the water quality parameters had a little effect on MVC and MCH although Wintrobe erythrocyte indices fluctuation depending on water quality was significant. Considering the wide variability that is seen in erythrocyte count it is possible that MVC and MCH were less affected by water quality parameters.

Water temperature and pH levels in floating cages were close to the optimal levels for the culture of sea bass. In the present study, ammonia-nitrogen concentration was above the safe limit (Lawson 1995). It is to be expected that blood parameters of sea bass would change related to high level of ammonia-nitrogen. But this concentration did not appear to adversely affect blood parameters measured in healthy sea bass. Besides, this study shows a small statistically significant effect of ammonia-nitrogen on haematological parameters.

It can be concluded that when determining ranges of haematological characteristics water quality parameters such as water temperature, dissolved oxygen and ammonia must be taken into consideration. We hope that haematological values reported in this study represent a normal range of values that will be useful in monitoring the health of cultured sea bass.

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Water temperature and pH levels in floating cages were close to the optimal levels for the culture of sea bass in the present study. Ammonia-nitrogen concentration was above the safe limit (Lawson 1995). It is to be expected that high level of ammonia-nitrogen in the water would affect the health of sea bass. But the concentration did not appear to adversely affect blood parameters measured in healthy sea bass. Besides this study shows a small statistically significant effect of ammonia-nitrogen on haematological parameters.

It can be concluded that when determining ranges of haematological characteristics water quality parameters such as water temperature, dissolved oxygen and ammonia must be taken into consideration. We note that haematological values reported in this study represent a normal range of values that will be useful in monitoring the health of cultured sea bass.

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A program for the determination of the optimal haematological parameters needs to be developed for the cultured species for fish health status. The future to establish a program may be limited to various factors such as environment, age, sex, activity, feeding regime etc. Thus, the study focuses on evaluating possible changes in some haematological (haematocrit, erythrocyte, leucocyte and thrombocyte counts, Wintre erythrocyte indices), characteristics of cultured European sea bass (*Dicentrarchus labrax*) under different seasonal conditions (water temperature, dissolved oxygen, pH and ammonia-nitrogen).

In general, the haematological parameters measured in this study are within the normal range of other local studies (Stoskopf 1993). However, seasonal fluctuations in haematological parameters were reported by several authors (Ezal et al. 1973, Castro and Kavares 1993, Castro et al. 1991, Minna et al. 1975) and the definition of normal range is more difficult for parameters that have seasonal and sexual fluctuations (Ezal and Williams 1977).

The results of this study indicate that haematocrit, erythrocyte and leucocyte were mostly affected by water temperature and dissolved oxygen. Haematocrit and erythrocyte count of sea bass were elevated at high temperature of June and September, than those of February and April, in contrast with the findings of Castro and Kavares (1993) observed that haematocrit and erythrocyte count of sea bass (both in pond and in floating cages) were higher in August and lower in April.

The contrast might be due to several factors such as different levels of dissolved oxygen, nutritional status and stocking rate.

Table 2. Haematological parameters of sea bass (*Dicentrarchus labrax* L.) reared in floating cages under different seasonal conditions (water temperature, dissolved oxygen, pH and ammonia-nitrogen).

Parameters	February	April	June	September
Haematocrit (%)	18.5	19.2	21.5	22.1
Erythrocytes (x10 ¹² /L)	1.2	1.3	1.5	1.6
Leucocytes (x10 ⁹ /L)	1.5	1.6	1.8	1.9
WBC/C (x10 ⁹ /L)	1.5	1.6	1.8	1.9
Platelets (x10 ⁹ /L)	1.5	1.6	1.8	1.9
WBC/C (x10 ⁹ /L)	1.5	1.6	1.8	1.9