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A HAEMATOLOGICAL STUDY OF HEALTHY ANGUILLA VULGARIS AND MUGIL CEPHALUS

by

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ABSTRACT

The present work reports results of examination of the blood of two eryhaline species. *Anguilla vulgaris* and *Mugil cephalus*, presenting the normal levels of the erythrocyte counts, size of the erythrocytes and the erythrocyte nuclei, the number of leucocytes and their differential counts, haematocrit values, haemoglobin content, erythrocyte sedimentation rate and specific gravity. It is to be noticed that, the cyclic nature of haematocrit variability is very similar to that observed in both, the erythrocyte counts and the haemoglobin content. It is concluded, therefore, that there is a clear correlation between the three haematological parameters, the red cell count, the haematorcrit value and the haemoglobin content.

Seasonal variations are also demonstrated in the total differential counts of leucocytes. An explanation for these changes is not yet available, since the exact function of each leucocyte type in teleosts is still obscure.

INTRODUCTION

Fishes represent the majority of vertebrate species, yet little is known of their haematology. Primary haematological techniques have been used including cell counts, haemoglobin determinations, microhaematocrits and differential counts, but reports are few and scattered. Blood parameters are not adequately known for wild specimens, consequently the detection of pathological deviations is often impossible. Among the reported studies is that by Katz.(1949) who determined cell number per unit volume with the haemocytometer for the silver salmon, *Oncorhynchus kisutch*. De wilede & Houston (1967) made a similar study of *Salmo gairdnerii*. Katz (1949) in addition, made differential cell counts as did Watson et al. (1962) for goldfish, *Carassius auratus*. Microhaematocrits for several salmonid species were reported by Snieszko (1961) and Normandeau (1962). Piper and Stephens (1962) reported additional microhaematocrit data and also haemoglobin determinations for lake trout *Salvelinus namaycush*. De wilde and Houston (1967) included haemoglobin determinations in their rainbow trout study. Barnhart (1969) measured haemoglobins, haematocrits and total blood cell count per unit volume in two strains of rainbow trout exposed to different diets.

MATERIAL AND METHODS

Two healthy euryhaline teleosts were sttdied : the grey mullet, *Mugil cephalus* Lin, and the Egyptian eel, *Anguilla vulgaris Lin*. They are known to be catadromus fishes, i.e. they feed in fresh-water and spawn in the sea. They were taken from the Nozha hydrodrome near Alexandria A.R.E., the

Nozha hydrodrome is not connected with the sea, hence the fish which reaches sexual maturity will not have the chance for spawning and resorption of sexual products takes place. Blood was obtained by venepuncture, arterial puncture or cardiac puncture. The method being varied to suit the requirement of the individual conditions. In some instances it was necessary to expose the heart and great vessels, before blood could be obtained. Coagulation was prevented by the use of heparin. The blood so obtained was used for red cells and leucocyte counts and for the determinations of haemoglobin, volume of packed red cells, erythrocyte sedimentation rate and specific gravity. In addition blood smears were prepared and stained with diluted Giemsa stain and fresh blood preparations were made. For the determination of erythrocyte sedimentation rate and the volume of packed red cells Kato's micro-haemopipette was used. After the determination of the sedimentation rate and the separation of the red cells from the white corpuscles and platelets, the haemopipette was centrifuged for 30 minutes at the rate of 3,000 r.p.m. in order to read the haematocrit. Centrifugation was carried out until complete packing occurred. Erythrocyte counts were carried out according to the procedure described by Hesser (1960) using the diluting solution recommended by Hendricks (1952). Hendricks diluting solution used to circument the problem of cell distortion and proved to be quite satisfactory in the present work. Gower's and Havem's diluting solution Hesser (1960) were unsatisfactory because the cells become distorted after few minutes.

The sizes of the erythrocyte and the erythrocyte nuclei were determined by means of an ocular micrometer. Fifty cells were chosen at randoms from a typical blood smear of each fish and ranges and means were computed. In human haematology, diluting fluids which lyse the erythrocytes are used. With fish blood this is not possible, as erythrocytes are also nucleated, and the nuclei remain without lysis thus making total erythrocyte counts difficult. Direct and indirect methods were used to determine the number of leucocytes. In the direct method, using Shaw's solution (1930) the differentiation between leucocytes and erythrocytes was readily made. The regular smeared preparations of blood stained with May-Giemsa's method were used for the microscopic observations (indirect method). This method was found to be very accurate in the present investigation. Because of the great similarity between leucocytes and immature erythrocytes as seen in the haemocytometer, it was not always possible to differentiate accurately between these cells. Lucas and Jamroz (1961) reported errors when using both techniques with avian blood, but recommended the indirect method.

The specific gravity of the blood was determined by the standard copper sulphate Phillips et al. (1950).

The alkali haematin method Oser et al (1965) instead of acid haematin preparation for the determination of haemoglobin content was adopted in the present work since it gave more accurate results.

RESULTS AND DISCUSSION

The present work reports results of examination of the blood of two bony fishes, *Anguilla vulgaris* and *Mugil cephalus*, presenting the normal levels of the erythocyte counts, size of the erythrorytes and the erythrocite nuclei, the number of the leucocytes and their differential count, haematocrit values, haemoglobin content, erthrocyte sedimentation rate and specific gravity.

Erythrocyte counts

There is a lack of information concerning the morphologic and physiologic characteristics of the blood of both, *Anguilla vulgaris* and *Mugil cephalus*. No reports for both species from the Egyptian waters have been found.

The erythrocyte counts in the begining of the experimented period in autumn showed a range of 2.35 to 2.87 million per cubic millimeter (average 2.63 million/mm^3) for the eel, and a range of 2.50 to 3.20 million/mm^3 (average 2.80 million/mm^3) for the mullet, this average decreases in both species in winter after which it starts to increase gradually until it reaches its maximum value in summer (2.95 million/mm^3) for eel and (3.25 million/mm^3) for mullet.

The results of these determinations are given in table (1) and are shown graphically in Figs. (I, II).

As is evident from the tables and the graphs, erythrocyte counts of both *Anguilla vulgaris* and *Mugil cephalus*, varies with respect to time. Erythrocyte counts for the two species are higher in summer than in winter, this may be attributed to the high activity of fish due to the elevations of water temperature, low oxygen content and to the great amount of food found in water.

TABLE 1. ERYTHROCYTE COUNTS, HAEMATOCRIT VALUES AND HAEMOGLOBIN CONTENT OF HEALTHY *Anguilla vulgaris* AND *Mugil cephalus* MEASURED SEASONALY FROM AUTUMN 1972 TO SUMMER 1973. ALL MEASUREMENTS WERE MADE ON 10 FISHES TAKEN IN EACH SEASON.

	Species	Amplitude	Autumn	Winter	Spring	Summer
Erythrocytes $\times 10^6$	EEL	Range	2.35 2.87	2.45 2.67	2.65 2.91	2.88 2.99
		Aver.	2.63	2.55	2.80	2.95
		S.D.	0.05	0.02	0.10	0.05
	MULLET	Range	2.50 3.20	2.10 3.00	2.65 3.23	2.85 3.25
		Aver.	2.80	2.73	2.93	3.25
		S.D.	0.10	0.05	0.12	0.10
Haematocrit %	EEL	Range	25.0 33.9	25.0 29.7	27.0 32.5	29.0 33.0
		Aver.	29.5	27.5	30.2	31.5
		S.D.	2.0	2.2	1.5	1.0
	MULLET	Range	23.5 30.5	21.8 30.0	27.3 33.8	29.0 32.2
		Aver.	27.8	27.2	29.5	31.5
		S.D.	2 3	2.0	2.0	1.0
Haemoglobin g %	EEL	Range	7.6 7.9	6.0 7.3	7.8 8.6	8.3 9.0
		Aver.	7.7	6.8	8.0	8.7
		S.D.	0.6	0.3	1.0	0.08
	MULLET	Range	7.3 7.9	6.55 7 35	7.56 8.63	8.75 9.35
		Aver.	7.50	7.0	8.20	9.15
		S.D.	0.50	0.3	0.70	0.45

No reports concerning the erythrocyte counts for the eel and mullet except those of Kawamoto (1930) who reported a range of 2,546,000 million R.B. Cs/mm^3 for the Japanese eel, *Anguilla japonica*. McCay (1930) reported a range of 2,500,000 million R.B. Cs/mm^3 for the Congo eel. Kisch (1949) showed a range of 2.15 to 2.73 million R.B. Cs/mm^3 for *Anguilla bostoniensis*. Wintrobe (1934) reported a range of 2.12 to 2.83 million R.B. Cs/mm^3 for *Anguilla rostrata*.

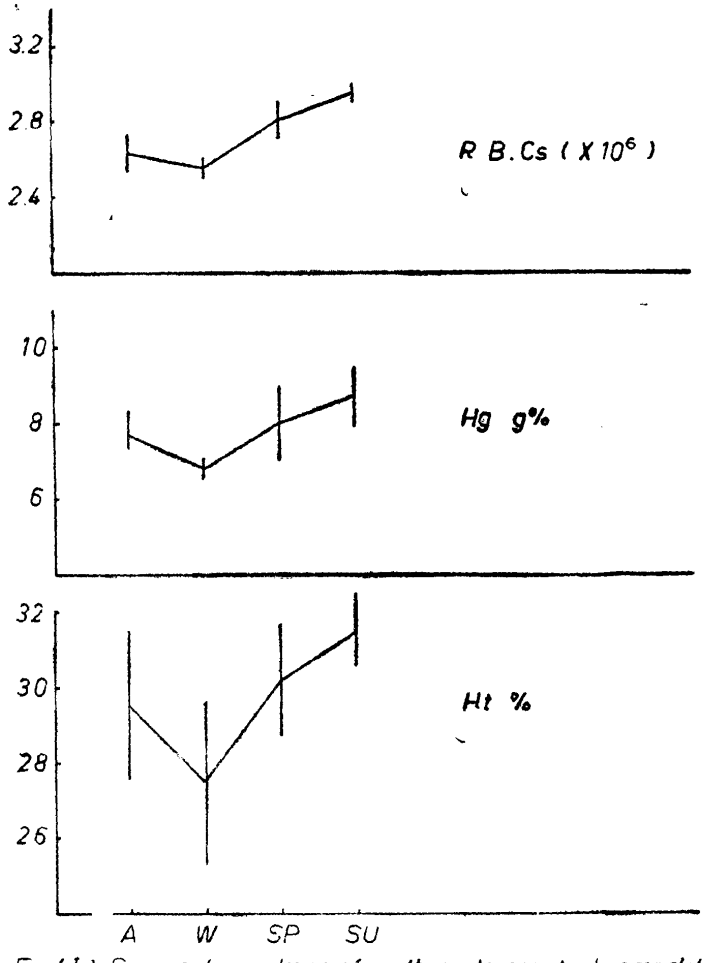

Fig (1) Seasonal variations of erythrocyte counts, haemoglobin content and haematocrit value of healthy Anguilla vulgaris

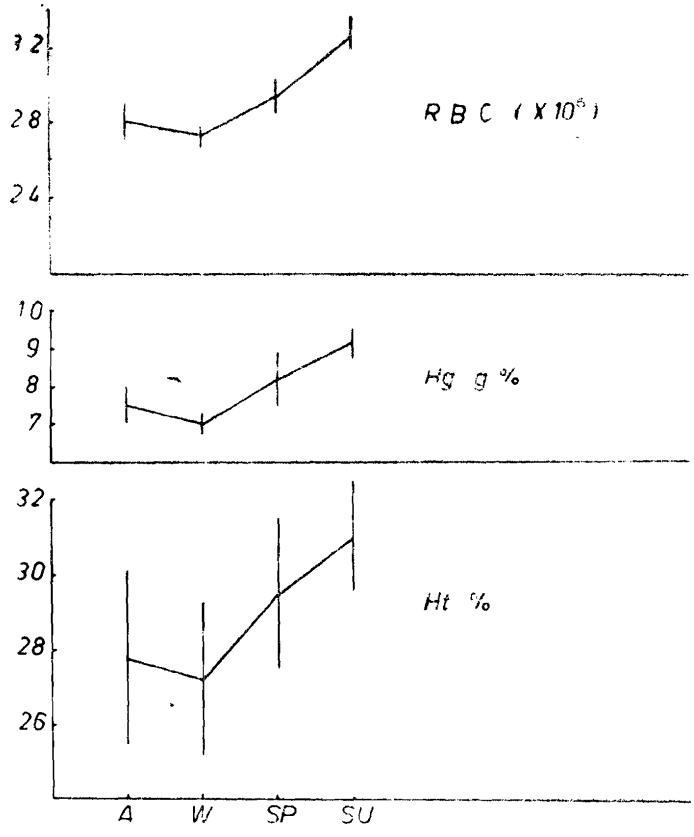

Fig (II) The seasonal variations of erythrocyte counts, haemoglobin content and hematocrit value of healthy Mugil cephalus.

The range reported by the previous authors for eel species lies between 2.12 — 2.855 million R.B. Cs/mm^3 and this coincides nearly with the range we obtained (2.55 — 2.9) for the same species in the present investigation. On the other hand, Cameron (1970) was the only auther we could find who reported a range of 2.843 to 3.131 million R.B. Cs/mm^3 for *Mugil cephalus* other than that of our Egyptian water which is nearly in agreement with our study on the same species.

Erythrocyte size

Erythrocyte size and nucleus size showed a range of 14.4 x 6.9 to 15.4 x 7.6 u and a range of 7.2 x 3.5 u to 7.6 x 3.9 u for *Anguilla vulgaris*, while *Mugil cephalus* showed a range of 14.0 x 9.0 u to 14.9 x 15.5 u and a range of 7.0 x 4.0 u to 7.7 x 4.8 u [Table, (2) Figs. III, IV.

TABLE 2. ERYTHROCYTE AND NUCLEUS SIZE OF HEALTHY *Anguilla vulgaris* AND *Mugil cephalus*. MEASURED SEASONALY FROM AUTUMN 1972 TO SUMMER 1973.

	Species	Amplitude	Autumn	Winter	Spring	Summer
Erythrocyte size u Width Length	EEL	Range	14.2 15.0	14.5 15 3	14.0 15.7	15.0 15.6
		Aver.	14.4	14.8	15.2	15.4
		S.D.	0.35	0.4	0.4	0.5
	MULLET	Range	6.5 7.4	6.8 7.5	6.2 7.8	7.1 7.9
		Aver.	6.9	7.1	7.0	7.6
		S.D.	0.4	0.35	0 55	0.25
Nucleus size u Width length	EEL	Range	7.2 7.8	7.4 7.8	7.1 7.2	7.0 7.4
		Aver.	7.6	7.5	7.2	7.2
		S.D.	0.4	0.5	0.38	0.4
	MULLET	Range	3.9 4.0	3.7 4.2	3.5 3.6	3.6 3.8
		Aver.	3.9	3.9	3.5	3.7
		S.D.	0.31	0.22	0.35	0.41
Erythrocyte size u Width Length	EEL	Range	13.8 14.8	14.3 15.0	13.8 14.5	14.0 14.9
		Aver.	14.5	14.7	14.0	14.9
		S.D.	0.25	0.65	0.8	0.35
	MULLET	Range	8.60 11.2	8.5 11.2	8.5 10.0	9.0 11.1
		Aver.	10.3	10.0	9.0	10.5
		S.D.	0.58	0.60	0.63	0.35

(Count.) TABLEr 2.

	Species	Amplitude	Autumn	Winter	Spring	Summer
Nucleus size u Width Length	EEL	Range	6.8 8.5	6.9 7.8	7.1 7.9	6.7 7.6
		Aver.	7.7	7.3	7.5	7.0
		S.D.	0.35	0.43	0.38	0.53
	MULLET	Range	4.70 4.10	3.7 4.3	4.4 5 0	4.0 4.2
		Ave .	4.80	+.0	4.8	4.0
		S.D	0.52	0.4	0.3	0.31

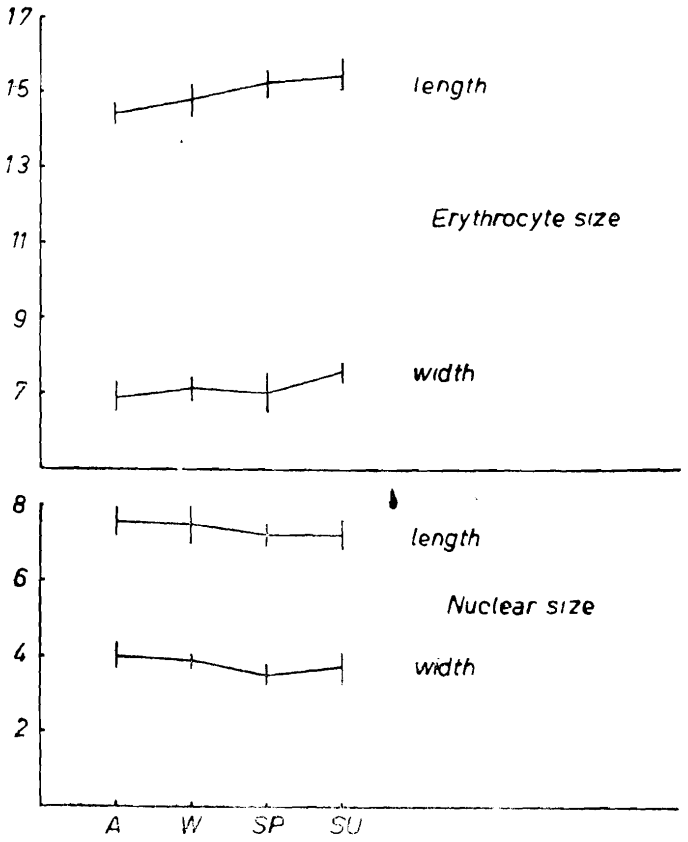

Fig (III) Seasonal variations of erythrocyte and nuclear size of *Angiulla valgaris*

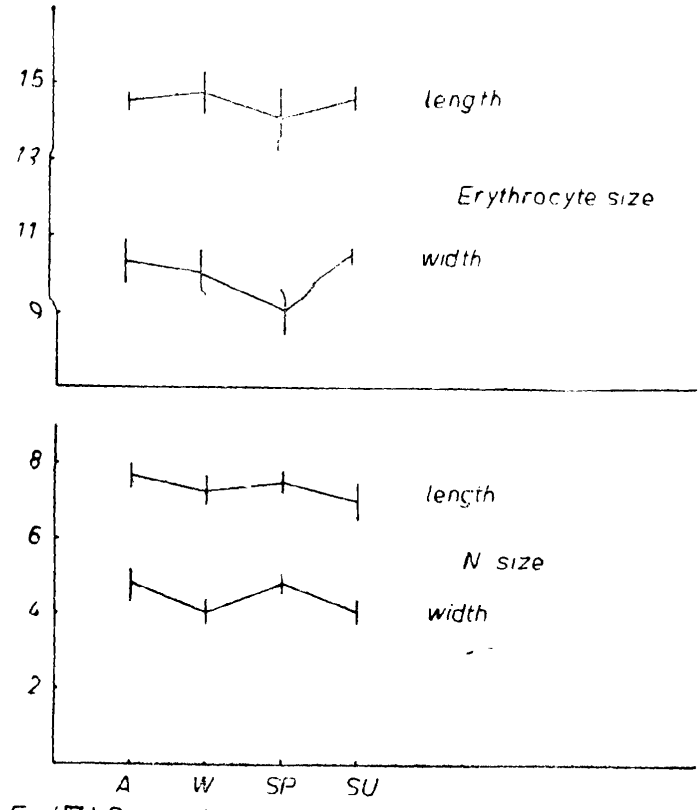

Fig (IV) Seasonal variations of erythrocyte and nuclear size of *Mugil cephalus*

Kawamoto (1930) reported a range of 13.16 x 6.8 u in diameter and 2-3 u in thickness of *Anguilla japonica*. Kisch (1949) reported a range of 11.7 x 8.2 u to 13.2 x 7.6 u for *Anguilla bostoniensis*. Downey (1930) reported that the erythrocyte size other than that of Egyptian water for *Anguilla vulgaris* was 14.6 × 9.0 u and the nucleus size was 3.4 × 2.5 u. Kisch (1949) reported a range of 13.3 × 8.1 u for *Anguilla bostoniensis*. These findings are not in agreement with our findings. To our knowledge, no reports for *Mugil cephalus* with respect to both erythrocyte and nuclear size were found.

Leucocyte counts

Apart from the erythrocytes with their respiratory pigments, fish blood contains various types of colourless or white cells, the leucocytes in the majority of fishes considerably exceeds that in mammals especially man,

and amounting in some species of bony fishes, to more than 100,000 leucocytes per mm^3 of blood (Pavlovskii 1962).

Leucocyte counts showed a range of 2.50—4.20 thousand/mm^3 in *Anguilla vulgaris*, and a range of 2.69—4.99 thousand/mm^3 in *Mugil cephalus*, Table (3) Figs. (V, VI). No significant differences could be noticed between the two species. The lowest average for leucocyte counts was found in winter. It is a characteristic feature of both *Anguilla vulgaris* and *Mugil cephalus* blood that they possess a considerably lower leucocyte counts of values recorded for some other species of teleosts. Yokayama (1947) gave a mean of 43, 400/mm^3 for the blood of yellow perch, *Perca flavescens*. Lagler et al (1967) reported a range from 20,000/mm^3-150,000/mm^3 for a number of fish species. Badawi (1968) showed a range of 2.705-4.993 tho./mm^3 for males rainbow trout and a range of 2.730-4.995/mm^3 for Females of Oriental Yeast Co., and a range of 2.900-5.063 thousand/mm^3 for the males and a range of 2.852-4.850 thousand/mm^3 for females of Nikko. However, some authors reported leucocyte counts, for certain fishes, which are lower than those of both *Anguilla vulgaris* and *Mugil cephalus*. Field et al (1943) gave an average of 3,675 and 2,105 W.B. Cs/mm^3 for the carp *Cyprinus carpio* and brook trout, *Salvelinus fontenalis* respectively.

Leucocyte differential counts

In stained preparations, granular (the neutrophil, acidophil, eosinophil and basophil) and non-granular (Lymphocytes; the small and the large) types were observed. No polymorphonuclear leucocytes as described by Pavloviskii (1962) were observed in the blood of both *Anguilla vulgaris* and *Mugil cephalus*.

In the eel the lymphocytes showed a range of 78.0-89.8% and the mullet showed a range of 78.6-92.2% while granulocytes showed a range of 14.8-17:2% for the eel. and a range of 14.5-22.3% for the mullet, Table (3), Figs. (V, VI).

Comparing the differential counts of both species studied with those of other fish species, we find that there are considerable differences. Counts for both trout, *Salmo manaycush* Lieb et al (1953) showed 91.2% lymphocytes, 8.2% monocytes and 0.6% granulocytes. The yellow perch, *Perca flavecens* had 67.7% lymphocytes and 32.4% neutrophilic granulocytes which the auther names "heterophils" (Yokayama, 1947). Onoda (1934) reported 34.9% lymphocytes, 65.1%. neutrophilis for the common carp, *Cyprinus carpio*, 22.0% lymphocytes and 78.0% granulocytes for the carp, *Cyprinus*

cyprinus, and 40.0% lymphocytes, 60.0% granulocytes for the Loach *Miscurnus fossillis*. Sano (1957) reported 2.5% monocytes, 77.5% lymphocytes and 20.0% neutrophilis for the Japanese eel, *Anguilla japonica*.

TABLE 3. LEUCOCYTES AND THEIR DIFFERENTIAL COUNTS (LYMPHOCYTES AND GRANULOCYTES) OF HEALTHY *Anguilla vulgaris* AND *Mugil cephalus*, MEASURED SEASONALY FROM AUTUMN 1972 TO SUMMER 1973.

	Species	Amplitude	Autumn	Winter	Spring	Species
Leucocyte counts $\times 10^3$	EEL	Range	3.52 3.81	2.50 3.82	3.75 4.10	3.61 4.20
		Aver.	3.65	3.35	3.98	3.88
		S.D.	0.30	0.19	0.25	0.23
	MULLET	Range	3.46 4.11	2.69 2.998	4.33 4.86	4.33 4.99
		Aver.	3.79	2.880	4.56	4.81
		S.D.	0.33	0.21	0.27	0.19
Lymphocytes %	EEL	Range	85.1 89.7	78.0 81.0	78.0 80.5	81.8 89.8
		Aver.	87.3	79.2	79.5	85.9
		S.D.	1.30	1.95	2.05	2.0
	MULLET	Range	88.6 90.3	87.3 92.2	78.6 82.5	88.7 90.3
		Aver.	89.7	89.2	80.3	88.2
		S.D.	1.33	1.35	1.2	1.72
Granulocytes %	EEL	Range	15.1 17.2	15.3 16.8	14.8 16.4	15.5 16.9
		Aveer.	16.3	16.1	15.5	16.2
		S.D.	0.70	0.30	0.69	0.25
	MULLET	Range	15.5 18.0	14.6 20.3	14.5 22.3	14.6 20.4
		Aver.	16.7	17.2	18.2	17.5
		S.D.	0.65	0.73	0.70	0.65

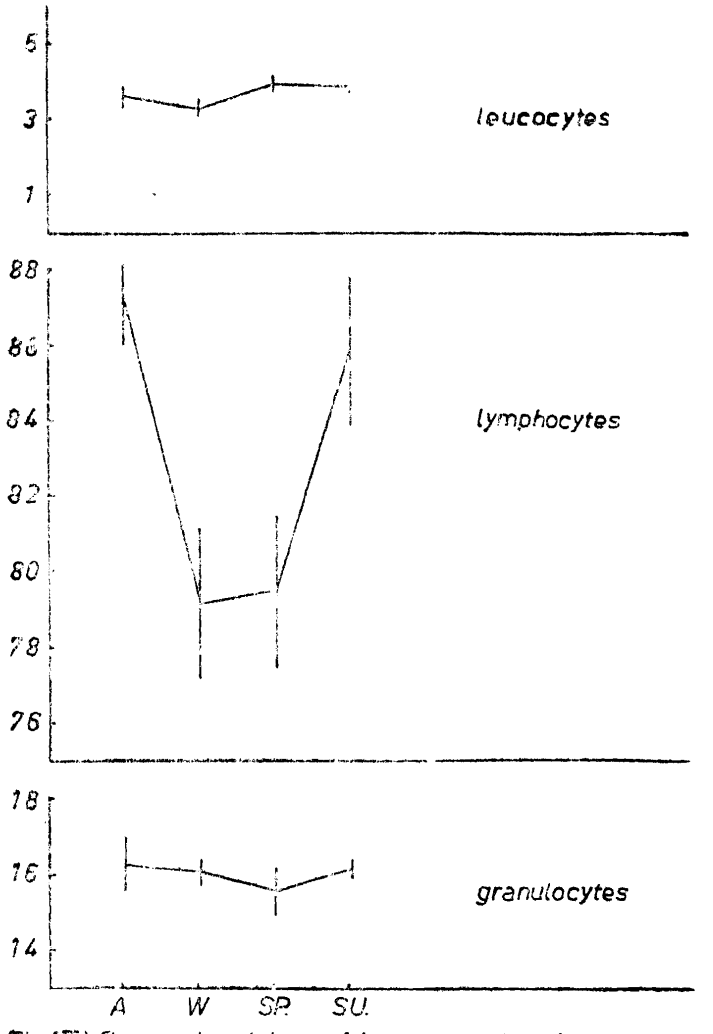

Fig. (I) Seasonal variations of leucocytes, lymphocytes and granulocytes of Anguilla vulgaris

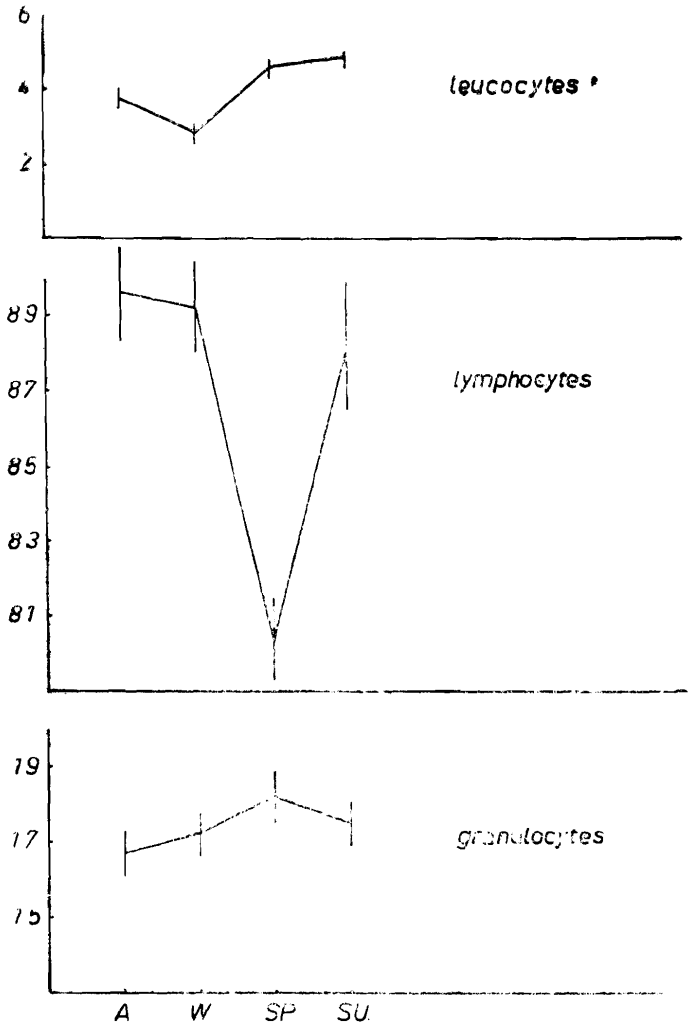

Fig. (III) Seasonal variations of leucocytes, lymphocytes and granulocytes of *Mugil cephalus*

Recently Mcknight (1966) reported the following averages : small lymphocytes 37.5%, large lymphocytes 2.4% and neutrophilic granulocytes 66.1% for the mountain white-fish, *Prosopius williafsoni*. He also reported that basophils were seen in few fishes, neither esinophil nor monoceytes were found in the blood of these species. Badawi (1968) reported that lymphocytes ranged from 75-94.5% and from 80.5-94.2% for males and females

rainbow trout taken from Oriental Yest Co. and a range of 79.2-92.5 and from 75.7-92.2% for males and females taken from Nikko. While granulocyte showed a range of 13.4-18.8% and a range of 14.2-19.1% for males and females taken from Oriental Yest Co. and a range of 14.3-18.8% males and a range of 14.5-18.9% for females taken from Nikko.

It is evident from tables and the graphs, that the total leucoyte counts of both species undergo seasonal changes, its behaviour is similar to the erythrocyte counts.

Seasonal cycle is also demonstrated in the leucocyte differential count.

Blood sedimentation rate :

In these experiments blood sedimentation rate has been determined seasonaly from autumn 1972 until summer 1973. The results of these determinations are given in table (4) and are shown graphically in figs. (7,8). *Anguilla vulgaris* had a range of 2.2-4.0 mm/h, while *Mugil cephaleus* had a range of 2.4-4.30 mm/h. No clear seasonal variations could be observed for erythrocyte sedimentation rate. We could only found Sano (1957) who determined the erythrocyte sedimentation rate for the Japanese eel *Anguilla japonica* and reported a range of 2.0-2.9 mm/h.

Haematocrit :

Experimental results of haematocrit determinations are reported in table (1) and are shown graphically in Figs. (1, 2). In these studies *Anguilla vulgaris* ranged from 27.5-31.5%, while *Mugil cephalus* ranged from 27.2-31.5%. From the tables and the graphs one can easily recognize that there is a good correlation between the red cell count and haematocrit value.

McCay (1930) reported a range of 26-40% for Congo eel. Wintrobe (1934) gave a range of 36.0-39.8% for the American eel *Anguilla rostrata*. Kisch (1949) reported a range of 35-36.2% for *Anguilla bostoniensis*. Sano (1957) gave a range of 24.0-45.2% for the Japanese eel, *Anguilla japanica*. Poluhowich & Parks (1972) reported, a range of 11.0-43.8% (mean 31.8) for the fresh-water American eel *Anguilla rostrata*, and a range of 3.00-44.0% (mean 37.95%) for the marine American eel *Anguilla rostrata*. Cameron (1970) gave a range of 25.9-31.1% for *Mugil cephalus*. Our results are in harmony with the higher values Cameron obtained for *Mugil cephalus*.

Specific gravity

Results of specific gravity determination are given in table (4) and are shown graphically in figs. (VII, VIII). *Anguilla vulgaris* showed a range of 1.035-1.043, while *Mugil cephalus* showed a range of 1.038-1.044. No significant seasonal variation was observed regarding this parameter.

TABLE 4. SPECIFIC GRAVITY AND ERYTHROCYTE SEDIMENTATION RATE OF HEALTHY *Anguilla vulgaris* AND *Mugil cephalus* MEASURED SEASONALY FROM AUTUMN 1972 TO SUMMER 1973.

	Species	Amplitude	Autumn	Winter	Spring	Summer
Specific gravity	EEL	Range	1.040 1.045	1.033. 1.037	1.040 1.043	1.042 1.045
		Aver.	1.042	1.035	1.042	1.043
		S.D.	0.002	0.001	0.001	0.001
	MULLET	Range	1.039 1.046	1.036 1.040	1.040 1.043	1.037 1.042
		Aver.	1.044	1.038	1.042	1.040
		S.D.	0.003	0.001	0.002	0.002
ESR (mm/h)	EEL	Range	1.95 2.38	3.7 4.3	3.8 4.1	3.3 3.7
		Aver.	2.2	3.9	4.0	3.5
		S.D.	0.22	0.31	0.2	0.20
	MULLET	Range	2.22 2.55	3.35 3.70	4.15 4.44	3.75 4.20
		Aver.	2.4	3.50	4.30	4.0
		S.D.	0.35	0.40	0.25	0.25

Haemoglobin

Haemoglobin has been determined seasonaly from autumn 1972 to summer 1973. *Anguilla vulgaris* showed a range of 6.8-8.7 g/100 ml. and the *Mugil cephalus* showed a range of 7.0-9.15 g/100 ml. The results of these determinations are given in table (1) and are shown graphically in figs. (I, II). Wintrobe (1934) determined the range of 8.0-10.0 g/100 ml. for American eel, *Anguilla rostrata*. Kisch (1949) reported a range of 8.6-10.2 g/100 ml. for *Anguilla bostoniensis*. Poluhowich & Parks (1972) showed a range of 6.63-10.74 g/100 ml. for *Anguilla rostrata*, while Eisler (1965) gave a mean haemoglobin concentration of American eel 6.12 g/100 ml. Cameron (1970) determined the haemoglobin for *Mugil cephalus* and reported a range of 6.73-7.58 g/100 ml.

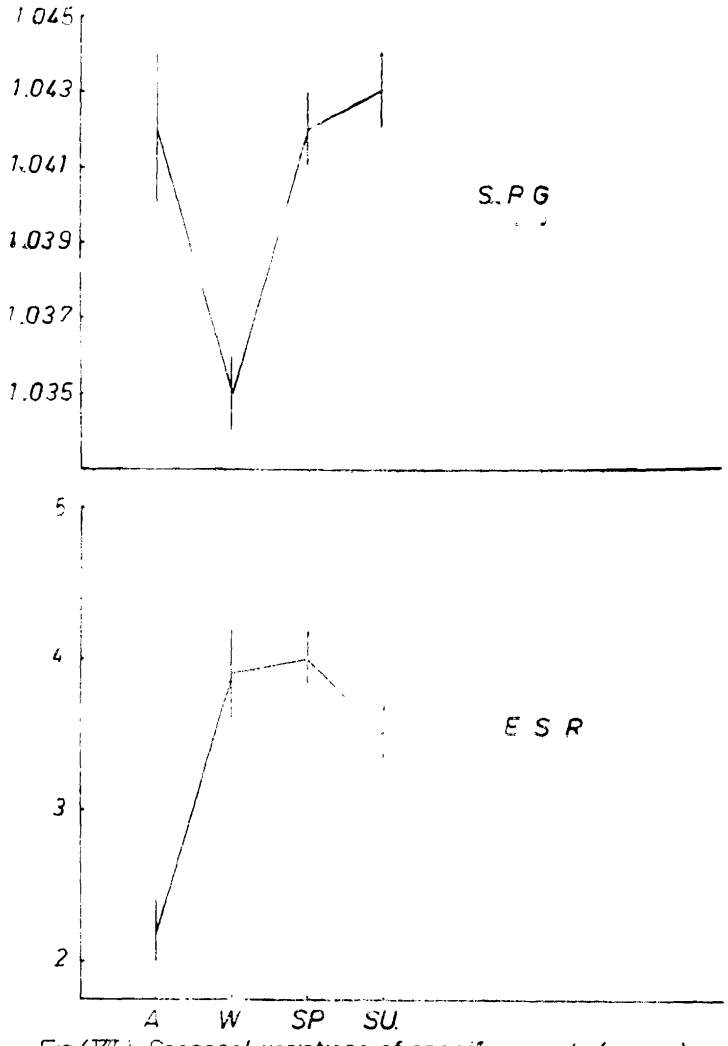

Fig (VII) Seasonal variations of specific gravity (upper) and erythrocyte sedimentation rate (lower) of Anguilla vulgaris

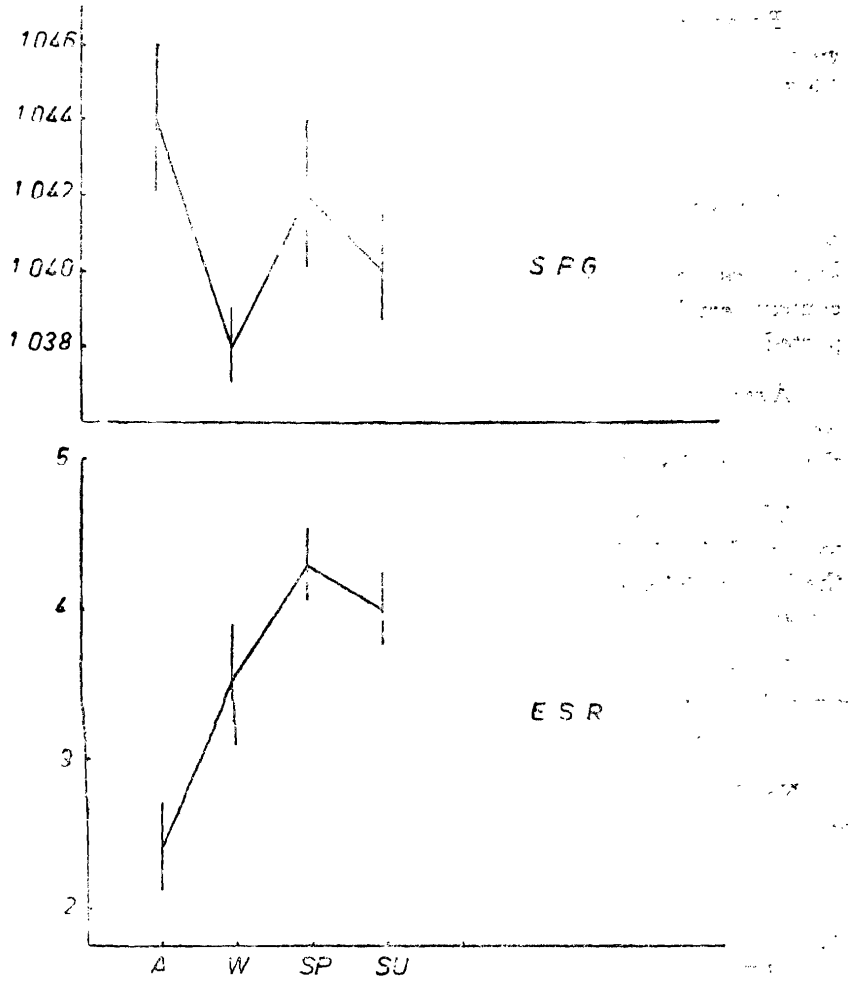

Fig (VIII) Seasonal variations of specific gravity (upper) and erythrocyte sedimentation rate (lower) of Mugil cephalus

In conclusion there is a close correlation between the number of erythrocytes, haematocrit value and haemoglobin content.

Wintrobe (1934) correlation between the three haematological parameters, red cell counts, haematocrit andhaemoglobin content is clearly demonstrated in the blood of both species. The same correlation was also reported by Wintrobe (1934) for all vertebrates he examined. Snieszko (1961) reproted that the correlation between haematocrit, red cellcount and haemoglobin is as good in the trout as in human and other vertebrates.

Recently this correlation was widely demonstrated by various investigators. (Eisler, 1965; Summerfelt et al., 1967b; De Wilde and Houston, 1967; Badawi, 1968, Badawi and Said. 1971 and El-Domiaty 1972.

SUMMARY

The seasonal variations of the cellular blood constituents, erythrocyte counts, size of the erythrocytes andthe erythrocyte nuclei, the number of leucocytes and their differential counts, haematocrit values, haemoglobin content, erythrocyte sedimentation rate and specific gravity have been reported.

Average erythrocyte counts, haematocrit values and haemoglobin content for *Mugil cephalus* were always higher. than those of *Anguilla vulgaris* throughout the whole experimental period.

The three parameters in both species vary considerably with respect to time, being higher in summer than in winter which may be attributed to the high activity of fish due to elevation of water temperature, low oxygen content and to the greater amount of food.

It was concluded that the correlation between the number of erythrocytes, haematocrit values and haemoglobin content is as close in the two species as in humans and other vertebrates.

No clear seasonal variations could be observed for both species in the erythrocyte sedimentation rate and specific gravity.

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