J. mar. biol. Ass. U.K. (1960) 39, 681–687
Printed in Great Britain

RED BLOOD VALUES IN THE PLAICE (*PLEURONECTES PLATESSA* L.)

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(Text-fig. 1)

The work reported in this paper forms part of a programme of investigation into the normal parameters of radiosensitive tissues in the plaice. The work was conducted prior to investigations into the effects of ionizing radiations on these tissues, in order to form a background to examinations of fish from areas of radioactive pollution resulting from the controlled disposal of radioactive effluent. Tissues were selected on the basis of known radiosensitive tissues in mammals and the work on red blood values reported here arose from these preliminary investigations.

In the first instance it was realized from the work of Schaefer (1925), Kawamoto (1929), Yokoyama*, Dombrowski (1953), Antipova (1954) and Kaplan & Crouse (1956), that the value of such indices of the red blood picture as packed cell volume (PCV), red cell count (RBC) and haemoglobin (Hb) might vary both with the size of the animal and with season. A preliminary investigation was therefore carried out in April 1956, using PCV as the index, to determine at what size asymptotic values for red blood were reached. This was followed in 1957 by measurement throughout the year of PCV, RBC, Hb and sedimentation rate (SR) in order to determine the existence and extent of seasonal variation, among fish that were large enough to have reached asymptotic values for red blood.

MATERIALS AND METHODS

The relationship between PCV and size was determined in April 1956 from a sample of 108 freshly caught live plaice. Seasonal variation was determined from samples of twenty to thirty freshly caught live plaice, obtained off Lowestoft at approximately 2-month intervals from the end of February to the beginning of September, together with a final sample examined at the beginning of December. A sample of 0·5 ml. of blood was removed from each fish, by cardiac puncture (Yoffey 1929), using a heparinized 1 c.c. glass syringe with No. 19 needle, and sufficient quantities of blood were transferred

* Yokoyama, H. O. Studies on the origin, development and seasonal variation in the blood cells of the perch (*Perca flavescens*). Ph.D. Thesis, Univ. Wisconsin, 150 pp., 1947.

to a clean paraffin waxed watch glass for each measurement. Using standard techniques (Kolmer, Spaulding & Robinson, 1938), packed cell volumes were measured with Wintrobe microhaematocrit tubes. Red-cell counts were carried out using standard red-cell pipettes and a Hawksley improved Neubauer crystallite counting chamber. Fifty per cent filtered sea water is isotonic with plaice erythrocytes and was used as diluting fluid. It was just coloured with gentian violet to provide sufficient coloration of the cells to facilitate counting. The fluid was freshly filtered prior to each dilution, to remove precipitated stain.

Haemoglobin estimations were made by the alkaline haematin method (Gibson & Harrison, 1945), using the B.D.H. artificial standard and a photoelectric absorptiometer. Sedimentation rates were measured by standard techniques, using microsedimentation tubes. During the early determinations of sedimentation rate measurements were made every fifteen minutes for 1 h, but in later determinations only the hourly rate was recorded.

PACKED CELL VOLUME AND WEIGHT

The data obtained on the relationship between PCV and weight are set out in Table 1. There is an increase in mean PCV with increase in weight of the fish up to a weight of approximately 120 g, when the PCV value stabilizes.

TABLE 1. RELATION BETWEEN PACKED CELL VOLUME AND WEIGHT. DATA FROM A SAMPLE OF FRESHLY CAUGHT LIVE PLAICE, APRIL 1956

No. of fish in sample	Weight range (g)	Mean weight of sample (g)	Mean PCV (%)	Range PCV % in sample	Standard deviation PCV
11	1– 20	14·1	17·2	14·1–20·6	2·2
16	21– 40	32·9	19·5	15·3–23·8	2·8
15	41– 60	52·0	21·9	19·2–24·6	1·7
12	61– 80	72·3	22·9	19·2–26·7	2·4
14	81–120	97·5	25·8	20·1–30·2	3·0
9	121–150	136·0	25·7	15·3–31·5	6·0
8	151–200	174·6	25·0	20·5–32·2	4·3
13	201–300	247·9	25·6	18·0–32·1	4·5
10	301–500	374·2	24·7	20·4–27·7	2·4

Inspection of the data in Table 1 shows that there is a considerable variation about the mean value for PCV, percentage variation ranging from 24 to 62%, which is not uncommon in the determination of PCV in fish blood. Field, Elvehjem & Juday (1943) give percentage variations of 61% for carp blood and 50% for trout blood, and Young (1949), measuring the PCV of individual fish at time intervals of from 10 to 14 days, remarks that the variation in PCV of individual fish at different times is comparable to that between different fishes of the same species at the same time. He gives percentage variation figures of from 15 to 67% of the mean PCV.

The increase of RBC values with increase in size of the fish has been noted before by Dombrowski during a study of the blood of the carp. He found that the erythrocyte counts increased from $1{\cdot}1 \times 10^6$ per mm^3 at hatching to $1{\cdot}5$–$1{\cdot}8 \times 10^6$ per mm^3 at the end of the fourth season, a mean increase of 50%, and that this was accompanied by an increase in haemoglobin from values of 7·3 g/100 ml. to 10·8 g/100 ml. over the same period, an increase of 48%. The increase in mean PCV in the plaice over the weight range 14–120 g (a 3–4 year period) is comparable, i.e. 17·2–25·6, an increase of 49%.

From the preceding plaice data it was decided to confine the study on seasonal variation to fish of not less than 140 g, so as to obviate the fluctuations due to differences in size of the fish. The data that follow are accordingly only applicable to plaice of weights in excess of 140 g.

SEASONAL VARIATION IN RED BLOOD VALUES

The data on seasonal variation of PCV, RBC, Hb and SR given in Table 2 and Fig. 1 show clearly that a process of haemoconcentration occurs throughout the late spring and summer, and that this is followed in the late summer and autumn by haemodilution.

TABLE 2. SEASONAL VARIATION IN RED BLOOD VALUES: PACKED CELL VOLUME (PCV), RED BLOOD CELL COUNT (RBC), HAEMOGLOBIN (Hb) AND SEDIMENTATION RATE (SR), DURING 1957

	Date of sample				
Parameter	28 February	23 April	21 June	29 August	1 December
No. observations	28	26	22	26	24
PCV Range (%)	16·7–27·5	16·2–27·2	22·2–32·2	20·6–32·4	17·4–27·0
Mean (%)	21·4	21·8	27·0	25·6	21·3
Standard deviation	2·9	3·0	3·1	3·3	2·7
No. observations	29	25	22	26	20
RBC Range (10^6/mm^3)	1·52–2·81	1·53–2·54	1·86–2·62	1·79–3·01	1·72–2·42
Mean (10^6/mm^3)	2·05	2·02	2·28	2·38	2·03
Standard deviation	0·35	0·28	0·22	0·29	0·20
No. observations	29	26	22	26	21
Hb Range (g/100 ml.)	5·52–11·06	4·55–7·87	6·70–10·42	4·38–8·51	4·22–6·85
Mean (g/100 ml.)	8·12	5·66	8·75	6·37	5·93
Standard deviation	1·38	0·88	1·03	1·03	0·66
No. observations	26	25	20	20	20
SR Range (mm/hr)	1·0–2·75	0·7–2·05	0·5–1·8	0·3–1·65	0·7–1·75
Mean (mm/hr)	1·65	1·37	1·01	0·96	1·14
Standard deviation	0·49	0·35	0·37	0·34	0·28

The values for PCV and RBC reach their lowest points, in the period examined, at the end of the winter and then increase fairly rapidly during the early summer. The increase in PCV from April to June is rather more rapid than the increase in RBC, and the plasma volume is thus decreasing slightly over the period, i.e. the blood is becoming more concentrated. SR—which is some measure of the concentration of the blood, the rate slowing as the

RBC increases—is consistent with these changes by its significant decline over the same period. It can be calculated from Table 2 that there is no significant difference between PCV values in June and August, nor in RBC over the same period. These values are therefore probably steady during the

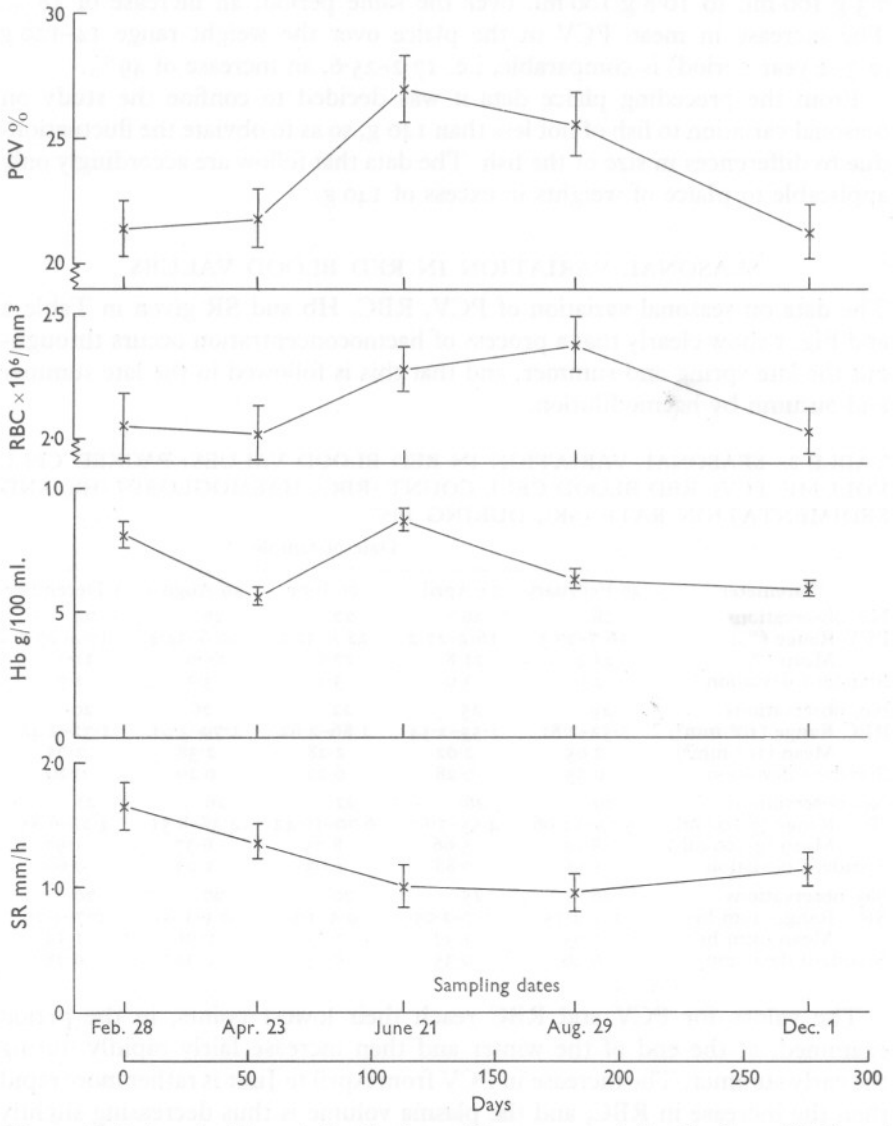

Fig. 1. Variation of plaice red blood values with season; packed cell volume (PCV), red blood cell count (RBC), haemoglobin (Hb), and sedimentation rate (SR), during 1957. The vertical lines show ± twice the standard error of the mean.

summer months, as is the SR, which halts its rapid fall and levels out over this period. From August to December both PCV and RBC fall steadily and the SR increases as the blood becomes more dilute.

It is interesting to note that the concentration of the blood revealed by the relative changes in PCV and RBC from April to June, is foreshadowed by a significant decrease in mean SR over the period February to April.

The values for Hb afford a less clear picture of events, in that they are already high at the end of the winter, falling to their lowest value of the whole period during the early spring. From then on they follow the same general trend as PCV and RBC, though their decline, which commences in June, is somewhat earlier than the decline in RBC.

A similar picture of haemoconcentration is described for another poikilotherm, the frog, by Kaplan & Crouse, though the time and magnitude of events are somewhat different, and the spring spawning obviously interrupts the rhythm.

It seems unlikely that the haemodilution which occurs in the plaice throughout the autumn and winter is a direct result of the decreased food intake occurring at this time. Schaefer, using pumpkin seed fish (*Eupomotis gibbosus* L. Cuvier and Valenciennes), found that these fish exhibited haemodilution during the late autumn and winter, but that the blood was able to concentrate again during the spring in spite of starvation throughout the whole period under investigation.

From the work of Musacchia & Sievers (1956), on cold torpor in the turtle (*Chrysemys picta*), it seems that the mechanism behind these changes is basically one of temperature. They found that turtles subjected to a low environmental temperature, $2 \pm 4^{\circ}$ C, exhibited haemodilution and that this was particularly well shown by the rapid fall in PCV and whole blood specific gravity. Thus the concentration of the blood of the plaice in late spring and summer may be a response to an increase in water temperature, and the subsequent haemodilution later in the year, the result of a falling water temperature.

ABNORMAL SEDIMENTATION RATES

It is well known that in mammals some disease conditions are accompanied by an accelerated SR; this has also been reported by Schumacher, Hamilton & Longtin (1956) for brook trout with furunculosis. These authors commented on the future of the method as a diagnostic tool in fish hatcheries and stressed the necessity for further information with respect to additional species and disease conditions.

During the early months, February and April, of the 1957 sampling programme some plaice were obtained exhibiting necrosis of the caudal fins and the posterior margins of the dorsal and ventral fins. When sedimentation rates were determined for these fish they proved to be abnormally fast (see Table 3).

TABLE 3. ABNORMAL SEDIMENTATION RATES, OF FISH WITH FIN NECROSIS, COMPARED WITH NORMAL FISH, FROM FEBRUARY AND APRIL 1957 SAMPLES

Time of observations (min)	Sedimentation rate: Healthy fish Range (mm)	Healthy fish Average (mm)	Fish with fin necrosis Range (mm)	Fish with fin necrosis Average (mm)
15	0·1–0·8	0·3	1·0–1·2	1·1
30	0·4–1·6	0·8	2·0–2·9	2·5
45	0·8–2·5	1·4	2·9–5·8	4·6
60	0·8–3·5	1·7	4·0–7·8	6·2

It is a pleasure to record my thanks to Mr F. Morgan who initiated and guided this work and to Mr C. Barker for his help throughout.

SUMMARY

The present study was undertaken as part of a programme designed to measure various parameters of normal fish tissues, prior to an investigation into the effects of ionizing radiation upon these tissues. The tissues were selected on the basis of known radiosensitive tissues in mammals. It was found that red blood values for the plaice, as indicated by packed cell volume, increased with the weight of the fish, up to a weight of approximately 120 g. Seasonal variations in packed cell volume, red blood cell count, haemoglobin and sedimentation rate among fish of not less than 140 g in weight were examined. A process of haemoconcentration during the spring and summer and one of haemodilution during the autumn and winter occurred. Data on accelerated sedimentation rates among diseased plaice are recorded.

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