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BODY WEIGHT AND THE HAEMATOLOGY OF THE AMERICAN PLAICE *HIPPOGLOSSOIDES PLATESSOIDES*

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SUMMARY

The relationship between haematological variables and body weight (W) is studied in the American plaice. Haematocrit, haemoglobin concentration, and cell volume and numbers are directly correlated with W, indicating that small fish have low blood oxygen solubility (αB_{O_2}) in spite of a high weight-specific oxygen consumption ($\dot{V}_{O_2}/W$).

Examination of analytical models of the branchial apparatus suggests that, in small fish, αB_{O_2} is lowered to minimize blood viscosity and that a high $\dot{V}_{O_2}/W$ is maintained by increasing the arteriovenous oxygen partial pressure difference.

Mean cell haemoglobin content is positively correlated while mean cell surface area per unit haemoglobin tends to be negatively correlated with W.

Mean erythrocyte residence time in the secondary lamellae is shown analytically to increase with W, possibly accounting for these relationships.

INTRODUCTION

The oxygen consumption rate ($\dot{V}_{O_2}$) of fish and many other animals is related to body weight (W) by a relationship of the form

$$\dot{V}_{O_2} = aW^{\gamma}. \tag{1}$$

The constant a defines the metabolic rate for a fish of unit weight. The exponent γ, which defines the rate of change of metabolism with W, has a value of 0·8 in the American plaice (MacKinnon, 1972). That is, metabolic rate per unit weight of this species, $\dot{V}_{O_2}/W$, decreases with increasing weight to the power $-0{\cdot}2$. This may be expressed as

$$\dot{V}_{O_2}/W = aW^{-0{\cdot}2}. \tag{2}$$

Since oxygen consumption is thus a function of W, it is possible that changes in W may result in altered values for certain haematological parameters. To my knowledge, no adequate examination of this possibility has been attempted for fish. The object of this investigation was to determine what relationships, if any, exist between W and the levels of various blood parameters in a field population of the American plaice, *Hippoglossoides platessoides*, of St Margaret's Bay, Nova Scotia. The findings, which are

illustrative of haematological acclimation to W-dependent variations in oxygen uptake, were then interpreted with reference to analytical models of the respiratory exchange system of fishes devised by Hughes & Shelton (1962) and Muir & Brown (1971).

MATERIALS AND METHODS

List of abbreviations

W body weight (g)
$\dot{V}_{O_2}$ oxygen consumption rate (ml O_2/min)
αB_{O_2} blood oxygen solubility (ml O_2/ml blood mmHg); varies according to O_2 dissociation curve
P_{a,O_2}, P_{v,O_2}, P_{i,O_2} partial pressure of oxygen in arterial blood, venous blood and the inspiratory water current respectively (mmHg)
$P_{a,O_2}-P_{v,O_2}$ the arteriovenous oxygen partial pressure difference (mmHg)
Ht haematocrit (the proportion of whole blood occupied by cells × 100)
PPr plasma protein concentration (mg/ml plasma)
Hb blood haemoglobin concentration (mg/ml blood)
N blood cell numbers (10^{-6} cells/mm^3 whole blood)
MCV mean blood cell volume (μm^3)
Hb/N haemoglobin per cell (pg/cell)
A/Hb blood cell surface area per unit haemoglobin (μm^2/pg)
A_g gill surface area (mm^2)
Δp blood pressure drop across the gill (dynes/cm^2)
l average length of the secondary lamellar blood channels of the gill (mm)
d the average diameter of those channels (mm)
V mean blood flow velocity for the gill (mm/sec)
n the number of secondary lamellar blood channels
Δt average time for a blood cell to pass through the lamellar circulation
η blood viscosity (g/cm sec).

Collection of fish

American plaice were captured (by otter trawl in 35–40 fathoms of water) in St Margaret's Bay, Nova Scotia, on or near the measured mile shown on the Canadian Hydrographic Service chart of the area (chart no. L (D7) 4386).

Dragging time was kept short (about 8 min) since longer times resulted in large catches and damage to the fish in the net. The catch was immediately placed in tanks of running sea water. A group of females of appropriate weight was then isolated in a separate tank; inactive and damaged specimens being excluded. The required sample of fish was then randomly drawn from this group without regard to state of maturity.

Sorting of the catch proceeded during the return journey (about 15 min). At the dock, weighing, measuring and blood sampling were done without delay. Stevens (1968) found in the trout that a few minutes' exercise resulted in gradually increasing Ht, PPr and Hb values. Phillips (1947) and Starmach (1970) found a haemoconcentration in response to hypoxia. Stevens (1968) also found that violent exercise decreased splenic blood volume. The detailed response of the American plaice to the

rigours of trawl capture is unknown. As there undoubtedly is an effect on blood parameters resulting from the abnormal conditions, and since this effect is likely to be time dependent, it was considered necessary to diminish this influence as much as possible by taking the blood samples as soon as could be arranged, generally between 30 min and 2 hr following capture of the fish. Although disturbances of ionic and water balance due to stress may be maximal during this period (Wood & Randall, 1973), the sampling regimes were identical for all sampling classes (size groups). The technique should thus permit comparison of blood parameter values between different size groups on a relative basis.

To ascertain the relationship between log W and the logarithm of the various blood parameters, the population was divided into a number of size groups according to log W. The range of body weights included was doubled in each succeeding size group, i.e. the smallest size group comprised 25–50 g fish, the next 50–100 g, and so on up to 800–1600 g. As approximately equal numbers of fish were included in each size group, the sample was evenly distributed on the log W scale.

Data were obtained from four sampling series during 1971 and 1972. These series with the dates of capture were: series A (29 May to 5 June 1972); series B (27–30 June 1972); series C (31 July to 2 August 1971), and series D (29 August to 1 September 1972). A total of 534 fish was used.

Blood sampling

In order to exclude damaged individuals from the sample, blood was taken only from fish which exhibited strong activity when handled. The fish were gently blotted with a damp towel, weighed, and measured before blood sampling. Blood samples were taken from unanaesthetized fish out of the water.

Arterial blood samples were obtained by inserting a hypodermic needle (18 G) into the unexposed dorsal aorta of an intact fish near the posterior end of the abdominal cavity. In nearly all cases blood flowed easily into the syringe indicating that the aorta, rather than a vein, had been penetrated. Any difficulty in withdrawing blood, which might indicate penetration of a vein, resulted in excessive foaming and haemolysis, and the sample was discarded. Blood was generally withdrawn until it ceased to come easily, but the amount taken was not recorded. Whether this procedure resulted in proportionately greater volumes being taken from small fish is not known. This could possibly bias the results if, for example, splenic blood reserves were released during sampling and if this blood had different properties than regular blood. The blood was withdrawn into a 5 cc plastic syringe containing a few crystals of Na or K heparin (Nutritional Biochemicals or Sigma) and gently mixed therewith. Great care was necessary to avoid foaming when withdrawing the blood as this readily resulted in haemolysis. The needle was then removed from the syringe and the blood gently expelled into a plastic sample tube containing a few more crystals of heparin and slowly agitated once more. The sample tube containing the blood was immediately placed on ice for the journey to the laboratory where the analysis was carried out. Refrigerated blood samples could be retained in the laboratory up to 5 days without noticeable changes in the variables studied: analysis of the blood samples, however, always took place either on the day of capture or the next day.

Analysis of blood

Blood was placed in heparinized microhaematocrit tubes for Ht determinations: sealing these tubes with clay (Seal-Ease, Clay-Adams) instead of rubber caps resulted in many fewer leaks. The tubes containing the samples were centrifuged at room temperature for 30 min at 12500 rev./min in a Clay-Adams Autocrit centrifuge. Haematocrits were read using the scale in the centrifuge head.

PPr was determined using a Goldberg-type refractometer (American Optical Ts Meter). Plasma was obtained by cutting the microhaematocrit tube and expelling the plasma on to the optical surface of the refractometer.

Hb was determined using the cyanmethaemoglobin method. Highly purified horse haemoglobin (Nutritional Biochemicals) was used as a calibration standard. Optical densities of cyanmethaemoglobin solutions were determined at 545 nm using a Bausch and Lomb Spectronic 20 colorimeter. Whole blood (100 μl) was added to 9·9 ml of Drabkin's reagent in a centrifuge tube and mixed vigorously. This mixture was centrifuged at 10000 *g* for 10 min to remove nuclei and cell debris.

There has been some controversy concerning the suitability of the cyanmethaemoglobin method for the determination of Hb in fish blood (e.g. Klawe, Barrett & Klawe, 1963). The pyridine haemochromagen method recommended by them was tested and, although more sensitive than the cyanmethaemoglobin method, was much more laborious. The absorbance spectrum of pyridine haemochromogen was determined using a Bausch and Lomb 505 scanning spectrophotometer calibrated using Hg emission lines at 546·1 and 577 nm. Two absorbance peaks were found: a major one at 555·3 nm and a minor one at 523 nm. It is noteworthy that the wavelength of 545 nm recommended by Klawe *et al.* (1963) for the determination of the optical density of pyridine haemochromogen solutions occurs in a region where absorbance changes very rapidly with wavelength. The cyanmethaemoglobin method is rapid, sufficiently sensitive, and gives a stable product provided the samples are kept cool, covered and out of direct sunlight.

For N and MCV determinations, whole blood was generally diluted 50000-fold in Cortland saline (Wolf, 1963) modified for use with marine fish (D. J. Randall, personal communication). The composition of this saline was: NaCl, 72·5 g; $CaCl_2.2H_2O$, 2·3 g; KCl, 3·8 g; $NaH_2PO_4.H_2O$, 4·1 g; $MgSO_4.7H_2O$, 2·3 g; glucose, 7 g, $NaHCO_3$, 10 g; and distilled water to a total volume of 7·5 *l*. At 5 °C, American plaice blood diluted in this saline remained essentially unchanged for several hours. In subsequent studies, even greater stability was obtained using the saline devised by Baines (1975). Samples were counted immediately after dilution and no measureable degradation of the diluted blood occurred in this period. Diluted samples could not be stored overnight, however. Either a Model B or a Model T Coulter counter (Coulter Electronics, Hialeah, Florida) was used to evaluate both these parameters. For a closely graded distribution of particle sizes, as is the case with blood cells, the Model B Coulter counter is the superior instrument for determining MCV due to its greater resolving ability (R. W. Sheldon, personal communication). Either instrument was satisfactory for the determination of N.

Hb/N and A/Hb were calculated from other parameters. Mean cell surface area

Table 1. *Results of simple linear regressions of the logarithms of the various blood parameters on the logarithm of body weight in the American plaice*

(Series A – captured 29 May to 5 June 1972)

Parameter	Regression coefficient	Log intercept	Intercept	r	P
Ht	0·0506	1·171	14·825	0·216	< 0·05
PPr	0·0512	1·338	21·777	0·205	< 0·05
Hb	0·1605	1·266	18·450	0·539	< 0·01
N	0·0217	0·091	1·233	0·096	N.S.
MCV	−0·0498	2·150	141·254	−0·325	< 0·01
Hb/N	0·1390	1·175	14·962	0·520	< 0·01
A/Hb	−0·1733	0·946	8·831	−0·613	< 0·01

r is the correlation coefficient for the simple linear regressions of the logarithm of the various blood parameters on the logarithm of the body weight. P is the probability that the value of r conforms to the null hypothesis that there is no simple linear relationship between the logarithm of the body weight; N.S. means that a particular probability exceeds 0·05 and is, therefore, not significant. r has 109 degrees of freedom.

Table 2. *Results of simple linear regressions of the logarithms of the various blood parameters on the logarithm of body weight in the American plaice*

(Series B – captured 27–30 June 1972)

Parameter	Regression coefficient	Log intercept	Intercept	r	P
Ht	0·1096	0·998	9·954	0·410	< 0·01
PPr	0·0684	1·330	21·380	0·276	< 0·01
Hb	0·1773	1·275	18·836	0·555	< 0·01
N	0·0398	0·044	1·107	0·170	N.S.
MCV	0·0432	1·895	78·524	0·172	< 0·05
Hb/N	0·1346	1·237	17·258	0·479	< 0·01
A/Hb	−0·1231	0·744	5·546	0·438	< 0·01

r is the correlation coefficient for the simple linear regressions of the logarithm of the various blood paramaters on the logarithm of the body weight. P is the probability that the value of r conforms to the null hypothesis that there is no simple linear relationship between the logarithms of the blood parameters and the logarithm of the body weight; N.S. means that a particular probability exceeds 0·05 and is, therefore, not significant. r has 129 degrees of freedom.

was calculated from MCV assuming a spherical cell, although the cells are somewhat elliptical in the American plaice.

RESULTS

The results of simple regressions of the logarithms of the seven blood parameters versus log W for individuals in each of the four sampling series (A–D) are given in Tables 1–4 respectively. The logarithms of Ht, PPr, Hb and Hb/N are all negatively correlated with log W, while log N shows no significant correlation in series A fish (Table 1). The results for series B (Table 2) are similar, but log MCV is now positively correlated with log W. Log N is positively correlated with log W by the one-tailed t test ($0{\cdot}01 < P < 0{\cdot}05$) but r is not significant. For series C (Table 3) the logarithms of Ht, Hb, N and Hb/N are all positively correlated with log W. Log A/Hb is negatively correlated with log W by the one-tailed t test ($0{\cdot}01 < P < 0{\cdot}05$) while r is

Table 3. *Results of simple linear regressions of the logarithms of the various blood parameters on the logarithm of body weight in the American plaice*

(Series C – captured 2 August 1971)

Parameter	Regression coefficient	Log intercept	Intercept	r	P
Ht	0·1327	1·052	11·272	0·603	< 0·01
PPr	0·0020	1·566	36·813	0·008	N.S.
Hb	0·1530	1·330	21·380	0·630	< 0·01
N	0·1093	−0·125	0·750	0·507	< 0·01
MCV	0·0055	2·032	107·647	0·028	N.S.
HB/N	0·0437	1·455	28·510	0·348	< 0·01
A/Hb	−0·0176	0·525	3·350	−0·153	< 0·01

r is the correlation coefficient for the simple linear regressions of the logarithm of the various blood parameters on the logarithm of the body weight. P is the probability that the value of r conforms to the null hypothesis that there is no simple linear relationship between the logarithms of the blood parameters and the logarithm of the body weight; N.S. means that a particular probability exceeds 0·05 and is, therefore, not significant. r has 142 degrees of freedom.

Results of simple linear regressions of the logarithms of the various blood parameters on the logarithm of body weight in the American plaice

(Series D – captured 29 Aug. to 1 Sept. 1972)

Parameter	Regression coefficient	Log intercept	Intercept	r	P
Ht	0·1155	1·082	12·078	0·401	< 0·01
PPr	0·0068	1·601	39·902	0·022	N.S.
Hb	0·1409	1·339	21·827	0·500	< 0·01
N	0·0979	−0·030	0·933	0·353	< 0·01
MCV	0·0309	1·972	93·756	0·234	< 0·01
HB/N	0·0409	1·374	23·659	0·170	< 0·05
A/Hb	−0·0209	0·625	4·217	−0·094	N.S.

r is the correlation coefficient for the simple linear regressions of the logarithm of the various blood parameters on the logarithm of the body weight. P is the probability that the value of r conforms to the null hypothesis that there is no simple linear relationship between the logarithms of the blood parameters and the logarithm of the body weight; N.S. means that a particular probability exceeds 0·05 and is, therefore, not significant. r has 146 degrees of freedom.

non-significant. In series D fish (Table 4) the logarithms of Ht, Hb, N, MCV and Hb/N are all positively correlated while log PPr and log A/Hb are not correlated with log W.

Thus, for all series, Ht and especially Hb are consistently related to W by a relationship of the form

$$y = a_0 W^b ,$$

or

$$\log y = \log a_0 + b \log W,$$

where y is the level of a particular blood parameter, a_0 is a constant defining the level of a given parameter for a fish of unit W and the exponent b describes the rate of change of y with W. Holeton & Randall (1967) have shown that blood oxygen capacity increases directly with Ht in the rainbow trout and it is reasonable to assume a rather similar relationship between blood oxygen solubility (αB_{O_2}) and Hb. Where blood parameters are measured in terms of concentration per unit volume of blood

and the exponent b takes a positive value, therefore, small fish have a lower αB_{O_2} than larger fish. This is true for Ht and Hb throughout the series of measurements.

The value of Hb/N also increases with size in the American plaice. The relationships of PPr, MCV, N and A/Hb to W vary among the different series of experiments, however, and may be affected by seasonal or other phenomena.

DISCUSSION

1. *Ht, Hb and W*

Since small American plaice clearly have lower αB_{O_2}'s (as indicated by Ht and Hb levels) than larger individuals, the question arises of how smaller fish maintain their relatively high $\dot{V}_{O_2}/W$.

Hughes & Shelton (1962) give the relationship of $\dot{V}_{O_2}$ to αB_{O_2}, the cardiac output ($\dot{Q}$), and the oxygen partial pressure difference between venous and arterial blood ($P_{a,O_2}-P_{v,O_2}$) as

$$\dot{V}_{O_2} = \dot{Q}.\alpha B_{O_2}.(P_{a,O_2}-P_{v,O_2}). \tag{3}$$

In order to answer the question posed above it is necessary to examine the relationships to W of the variables on the right of equation (3).

Hart (1943) has apparently been the only investigator to attempt direct measurements of $\dot{Q}$ as a function of W for fish. Unfortunately, his methods involved serious perturbations of normal cardiorespiratory function, heart rates were given as the mean value for several fish, and individual stroke volumes were presented graphically. However, I calculated from fig. 2 of Hart (1943) that stroke volume in the sucker is a function of W to the 0·67 power. Since the sucker mean heart rate had a very low variance (presumably this variance would be high if heart rate varied systematically with W), one may tentatively conclude that $\dot{Q}$ is a function of W to the 0·7 power, provided that the stroke volume/W relationship was not affected by the experimental technique. Hart (1943) also obtained similar data for the carp, bowfin and catfish.

Comparable conclusions about the $\dot{Q}$–W relationship may be deduced from the data of Garey (1970) for the carp and Hanson & Johansen (1970) for the dogfish. In these studies, $\dot{Q}$ increased linearly with $\dot{V}_{O_2}$. This may be expressed as

$$\dot{Q} = c+m\dot{V}_{O_2}. \tag{4}$$

The intercept c represents the amount of oxygen a fish can obtain without utilizing its circulatory system: c must therefore be essentially zero. If equation (4) applies to the American plaice, then substituting from equation (1)

$$\dot{Q} = eW^{0·8}, \tag{5}$$

where $ma = e$.

Clearly, then, the W-dependency of $\dot{V}_{O_2}$ observed in American plaice (MacKinnon, 1972) (equation 2) may be accounted for by a similar W-dependency of $\dot{Q}$ (equation 5), provided the sum of the exponents relating αB_{O_2} and $P_{a,O_2}-P_{v,O_2}$ to W equals zero (equation 3).

Under routine conditions where the arterial blood in the efferent branchial arteries is nearly saturated with oxygen, there must be an essentially direct relationship between

αB_{O_2} and Ht and especially Hb. In the case of series C (Table 3), for example, the empirical relationship between Hb and W is adequately described by the equation

$$\text{Hb} = qW^{0.153}, \tag{6}$$

where $q = 21{\cdot}38$. Taking this example for simplicity, Hb may be replaced by αB_{O_2} in equation (6) as follows:

$$\alpha B_{O_2} = k_0 W^{0.153}. \tag{7}$$

If, as already suggested, the sum of the exponents relating αB_{O_2} and $P_{a,O_2} - P_{v,O_2}$ to W must equal zero, then

$$P_{a,O_2} - P_{v,O_2} = k_1 W^{-0.153}. \tag{8}$$

Equation (8) predicts that $P_{a,O_2} - P_{v,O_2}$ is inversely related to W in the American plaice. If true, this provides a tentative answer to the question posed at the beginning of this section: that is, the pressure difference driving oxygen diffusion across the branchial epithelium is greater in smaller fish, which would account, in part at least, for their relatively greater $\dot{V}_{O_2}/W$ with blood of low αB_{O_2}.

$P_{a,O_2} - P_{v,O_2}$ can be increased either by decreasing P_{v,O_2} or by augmenting P_{a,O_2}. Lowering P_{v,O_2} not only increases the gradient down which oxygen diffuses into the blood, but also indicates a greater tissue oxygen utilization. Also, P_{a,O_2} has an upper limit (i.e. P_{i,O_2}). Effectiveness of oxygen transfer to the blood was defined (Hughes & Shelton, 1962; Hughes, 1964) as the ratio between the amount of oxygen transferred ($= \dot{V}_{O_2}$) to the maximum which could possibly be taken up by the blood. Randall *et al.* (1967), using the expression

$$E_b = \dot{V}_{O_2} \cdot 100/\dot{Q}\alpha B_{O_2}(P_{i,O_2} - P_{v,O_2})$$

obtained values near 100% in the resting rainbow trout, decreasing only slightly during moderate exercise. If a similar conclusion applies to the American plaice, then, when effectiveness is nearly 100%, $P_{a,O_2} \simeq P_{i,O_2}$. As P_{i,O_2} cannot vary with W, it is probable that P_{a,O_2} does not change with W in fish under routine conditions. This also follows as the alternative to near saturation of the arterial blood is a wastage of energy through excess cardiac and osmotic work. If P_{a,O_2} is not related to W, then it may be replaced by a constant in equation (8) and hence

$$dP_{v,O_2}/dW = 0{\cdot}153k_1. \tag{9}$$

The hypotheses that $P_{a,O_2} - P_{v,O_2}$ is larger in smaller fish, that this is achieved by diminishing P_{v,O_2}, and that P_{v,O_2} is lowered by decreasing haemoglobin oxygen affinity are currently being tested in this laboratory.

It is also necessary to inquire why small American plaice have a diminished αB_{O_2}. This requires an examination of the W-dependency of the physical properties of the branchial apparatus and the various parameters affecting gill blood flow. This leads to the hypothesis that small fish must minimize blood viscosity (η).

If A_g is a function of $W^{0.8}$ (Price, 1931; Muir, 1969; Muir & Hughes, 1969) and since $\dot{Q}$ may also be a function of $W^{0.8}$ (equation 5), it is possible that the ratio $\dot{Q}/A_g$ remains rather constant for different sizes of the same species (Muir & Brown, 1971). That is $\dot{Q}/A_g = f(W^0)$. On the other hand, Hughes (1972) suggested that it is

more common to find that A_g increases with W more rapidly than $\dot{V}_{O_2}$. Thus if, for the sake of argument, we take $A_g = f(W^{1.0})$, then, from equation (5).

$$\dot{Q}/A_g = f(W^{-0.2}). \tag{10}$$

The consequences of both conditions will be examined.

$\dot{Q}$ and A_g are related to other factors affecting the branchial circulation by the equation of Muir & Brown (1971), i.e.

$$\dot{Q}/A_g = \Delta p \pi d^3/256\,\eta l^2, \tag{11}$$

where l is the length and d the diameter of the individual secondary lamellar blood channels and Δp is the blood pressure drop across the gills. If $\dot{Q}/A_g = f(W^0)$ then

$$(\dot{Q}/A_g)_L = (\dot{Q}/A_g)_S \tag{12}$$

(where L and S denote large and small fish respectively) and

$$[(\Delta p/\eta)(\pi d^3/256 l^2)]_L = [(\Delta p/\eta)(\pi d^3/256 l^2)]_S. \tag{13}$$

Muir & Brown (1971) give values of $l = 2{\cdot}3$ mm and $d = 6{\cdot}2\ \mu$m for a 19 kg cod (*Gadus morhua*) and $l = 1{\cdot}5$ mm and $d = 4{\cdot}5\ \mu$m for a 1–2 kg cod. (The value $l = 1{\cdot}5$ mm for the latter fish was read off fig. 4 of Muir & Brown (1971).) Thus $(\pi d^3)/256\ l^2$) equals $0{\cdot}508\ \mu m^3/cm^2$ for the 19 kg cod and $0{\cdot}497\ \mu m^3/cm^2$ for the smaller cod. These numbers cannot be considered significantly different and hence

$$(\Delta p/\eta)_L = (\Delta p/\eta)_S. \tag{14}$$

I am not aware of any observations on the relationship of Δp to W. However, ventral aortic pressure increases with W in the salmon (Green, 1905) and similar increases in the trout are associated with increased Δp (Stevens & Randall, 1967). Thus, if Δp is less in smaller American plaice then, according to equation (13), η must be similarly diminished. If, however, we take $\dot{Q}/A_g = f(W^{-0.2})$, then it follows that

$$\Delta p/\eta = f(W^{-0.2}). \tag{15}$$

In this case, the same increase in η in a small individual would result in a greater increase in Δp than in a larger fish. Clearly, it would be advantageous to minimize η in these circumstances.

Poiseuille's law, from which equation (11) was derived, is known to adequately predict blood flow in vessels of large diameter. As the vessel diameter decreases to about the size of arterioles, however, Δp takes much smaller values than predicted by Poiseuille's law using η's found adequate for larger tubes. This is due to the phenomenon of axial concentration of red cells (Fahreus–Lindquist effect). Lighthill (1975) has pointed out, however, that the Fahreus–Lindquist effect is first abolished and then strongly reversed as tube diameters decrease to the size where the red cells must undergo distortion to pass through the vessel. Dintenfass (1968) has observed this result for blood flow in a parallel plate viscometer. Red cells are deformed within the vessels of fixed and stained secondary lamellar preparations (unpublished observations, this laboratory). Therefore it may be necessary to raise η to a power greater than 1 (equation 11) for the secondary lamellar circulation. If this is so, then the requirement to regulate blood η becomes more pressing.

Since both η (Ruch & Fulton, 1960) and αB_{O_2} (Holeton & Randall, 1967) are directly related to Ht, one may postulate that the observed relationship between Ht, Hb and W in the American plaice is a result of a requirement to minimize η in smaller individuals. That is, the physical nature of the branchial apparatus, the haemorheology of the microcirculation, and the arrangement of the gills in series with the heart are such that if smaller fish were to maintain an αB_{O_2} equal to that of larger fish, as might have been expected from equations (3) and (5), then blood η would be disproportionately increased in smaller individuals. This would result in an increase in Δp or a decrease in $\dot{Q}$, necessitating augmented cardiac work if $\dot{V}_{O_2}/W$ and dorsal aortic blood pressure were to be maintained.

One may ask why do not larger fish also save energy by lowering blood η since this avenue seems to be open to smaller fish? Data given by Hughes (1972) are suggestive of an increase in the thickness of the branchial water/blood barrier with W in the tench. This would result in a diminished oxygen diffusing capacity of the gill of larger fish. The increase in αB_{O_2} with W in the American plaice may indicate an attempt to overcome this problem.

2. *Hb/N and W*

Hb/N has been shown to increase with W in the American plaice (Tables 1–4). This result can be anticipated analytically.

Muir & Brown (1971) give the mean blood flow velocity (V) for the entire gill as

$$V = 4\dot{Q}/n\pi d^2, \tag{16}$$

where n is the number of secondary lamellar blood channels. The relationship between n and A_g is

$$n = A_g/2dl \tag{17}$$

(Muir & Brown, 1971). Substituting for n in equation (16),

$$V = (\dot{Q}/A_g)\,(8l/\pi d). \tag{18}$$

If, as previously suggested, the ratio $\dot{Q}/A_g = f(W^0)$, then

$$8\dot{Q}/\pi A_g = k_2, \tag{19}$$

a constant, for different sizes of the same species.

Letting

$$v = V/k_2, \tag{20}$$

$$v_L = l_L/d_L$$

and

$$v_S = l_S/d_S,$$

where L and S denote large and small fish respectively. Using the data of Muir & Brown (1971) for the 19 and 1–2 kg cod once again, $v_L = 0{\cdot}371$ and $v_S = 0{\cdot}333$. The residence time (Δt) for the average erythrocyte in the lamellar vessels is

$$\Delta t = lv^{-1}. \tag{21}$$

Thus, $\Delta t_L = 6{\cdot}2$, $\Delta t_S = 4{\cdot}5$ and $\Delta t_L/\Delta t_S = 1{\cdot}38$. This ratio nearly doubles if the relation between $\dot{Q}/A_g$ and W given in equation (10) is assumed.

Since $\Delta t_S < \Delta t_L$, it is probably an adaptive advantage to correlate Hb/N and W in

the same manner. In the absence of this adaptation either small fish would tend to produce unsaturated arterial blood or large fish would tend to expose already fully saturated blood at the respiratory surface.

3. *MCV and W*

The relationship of MCV to W is complex and seasonally variable, passing from a high negative correlation in May to high positive correlation in late August. A comparison of the seasonal cycle of MCV in 400–800 g female American plaice (Smith, 1977) with that of 100–200 g fish (unpublished data, this laboratory) indicates that smaller fish have the lower MCV's throughout most of the year, indicating that MCV and W are usually positively correlated. This relationship may indicate W-related space limitations in the secondary lamellar vessels. However, at those times of the year when the rate of erythropoesis is greatest, MCV declines sharply since young red cells appear to be smaller than old red cells (Blaxhall & Daisley, 1973). If the spring increases in the rate of erythropoesis were to occur in larger fish before doing so in smaller fish then MCV and W might be negatively correlated for a time: this would appear to be the case for series A (Table 1).

4. *A/Hb and W*

Both MCV and Hb increase with W. The rate of increase is somewhat less for MCV than for Hb, resulting in a tendency for A/Hb to be inversely related to W. This finding is expected since it has been deduced above that the erythrocyte Δt in the secondary lamellar blood vessels, and hence the time available to saturate the cellular haemoglobin, is less in smaller fish. Staub, Bishop & Forster (1962) have shown that the rate of exchange of oxygen with intact red cells may limit, in part at least, the rate of exchange of oxygen in the lungs. Under these circumstances, the inverse relationship of A/Hb to W in the American plaice is to be expected.

5. *N and W*

N also shows a tendency to increase with W although this relationship was not always very strong. The product of N and MCV is directly proportional to Ht and, hence, a function of η which must be minimized in small fish. It is to be expected, therefore, that N would tend to be lower in smaller fish.

6. *PPr and W*

The functions of plasma proteins are so varied that it is difficult to see why PPr should be related to $\dot{V}_{O_2}$, and hence W, in a simple way. If PPr contributes significantly to η, it is likely that PPr would be directly related to W. This is observed in series A (Table 1) and series B (Table 2). No such relationship was observed for series C (Table 3) and series D (Table 4). Furthermore, comparison of the seasonal cycles of PPr in 400–800 g American plaice (Smith, 1977) with similar unpublished data for 100–200 g specimens indicates a significant negative correlation of PPr with W at certain seasons, but no clear general trend.

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