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# Regional distribution of blood in the gills of rainbow trout in normoxia and hypoxia: a morphometric study with two fixatives

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Different portions of the gills of rainbow trout were fixed after the fish had been kept under normoxia or hypoxia. Two fixatives were used: a) 2.5 % glutaraldehyde and b) 2.5 % glutaraldehyde + 2.5 % formaldehyde.

Morphometric analyses of light-microscopic sections were carried out for different structural components of the secondary lamellae and comparison made of gills after these different treatments. Overall analyses indicated some differences in the blood components of filaments from dorsal, lateral and ventral regions as well as parts of a filament, but individual analyses showed no significant differences in the small number of samples available.

A number of significant differences were observed, however, between corresponding material fixed in the two ways. These differences were greater during normoxia than hypoxia. The study emphasizes the need for careful control of fixation procedures of the gills before morphometric investigation. Consistent results are obtained with the same fixative but as yet it is difficult to decide which gives the best indication of the normal condition.

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## 1. Introduction

In earlier studies on the morphometry of gills by light and electron microscopy (HUGHES 1972, HUGHES & PERRY 1976) the material for analysis has generally been obtained from one particular region of the gills, e.g. the lateral gill filaments of the third arch (SOIVIO & HUGHES 1978). As shown by these studies as well as earlier work on the distribution of the gill dimensions (HUGHES 1972; MORGAN 1974) and physiological investigations (DAVIS & WATTERS 1970, HOLETON 1972), the gills are by no means homogeneous organs. Consequently morphometric investigations might reflect such a heterogeneity (HUGHES 1975), especially if attention were directed at parameters which indicate the nature of the blood flow through different portions of the gill system.

In the present investigation samples of gill filaments were taken from more widely distributed portions of the gill network. In studies of this kind the most serious methodological problem concerns the ability to fix the material in a condition which closely reflects the normal functioning system. Because of possible variations in the action of fixatives, a comparable series of gill samples was fixed in a second fixative containing formaldehyde in addition to the generally used glutaraldehyde. Although the results gave some indication of regional differences, the variation between specimens made statistical analyses less significant and, consequently, only general trends were recognizable. Of more definite significance were the results of comparing material from similar regions when fixed in the two solutions. This is of great interest in itself, and raises many general problems with respect to future investigations.

## 2. Materials and methods

Altogether 16 specimens of rainbow trout (*Salmo gairdneri*) in their second year were used. Although of the same age they varied in size (body weight 146g — 546g, total body length 23—35 cm). The fish were part of the Kamloops — 74/80 stock of the Laukaa Fish Culture Research Station, Finland. Before experimentation, the fish were transferred from outside pools into holding tanks within the hatchery building, where they were kept for 1 week in tanks of 4 $m^2$. The water temperature was 13.5° C (glutaraldehyde material) and 16.5° C (glutaraldehyde + formaldehyde material). Before an experiment each fish was transferred to a cylindrical experimental chamber (Soivio *et al.* 1975) through which water flowed at a rate of 3 l/min. Eight fish were exposed to hypoxia (3h at 40 % oxygen saturation), the other eight being kept under normoxic conditions (over 90 % oxygen saturation). The water was made hypoxic by pumping it through a partial vacuum with an apparatus slightly modified from that described by Mount (1961).

After this treatment fish were stunned by a blow on the head and the blood circulation to the gills was restricted by clamping the ventral aorta. The gills were fixed in a solution of 2.5 % glutaraldehyde (G) in 0.1 M phosphate buffer (pH 7.2), or of 2.5 % glutaraldehyde + 2.5 % formaldehyde (G/F) in 0.1 M phosphate buffer (pH 7.2). To do this the head of the stunned fish was held immersed in the fixative. The fish was moved up and down in the fixative throughout a 30-min period. After this the third right gill arch was removed from each fish for fixation *in vitro* in the same solutions for 23 h.

Samples from the gills were stored in 0.1 M phosphate buffer (pH 7.2) at + 4° C for 3 weeks, then post-fixed in 1 % $OsO_4$ in 0.1 M phosphate buffer (pH 7.2) for 3 h, dehydrated in ethanol and embedded in Epon 812 (Luft 1961). Half-micron sections were cut parallel to the long axis of the gill filaments and perpendicular to the surface of the secondary lamellae with a Sorvall Mt 2B ultramicrotome. The mounted and stained (1 % toluidine blue) sections were examined under oil immersion (1410 x) with a Wild automatic sampling microscope (Weibel 1970) fitted with a projection head containing a rectilinear grid. Point-counting was carried out for the following structures of the secondary lamellae:

1. E Erythrocytes in secondary lamellae
2. Pl Plasma in secondary lamellae
3. PC Points falling on pillar cells
4. EPT Tissue of the epithelium covering the secondary lamella.
5. EPN Non-tissue (lymphoid) space of the epithelium
6. R Other points falling on the pillar cell system

The total number of points falling on a secondary lamella section (P) is the sum of counts 1—6. Of the points counted Nos. 1—3 fall on the pillar cell system and correspond to those used in a previous study (for definitions see Hughes & Perry 1976). The diagram (Fig. 1) illustrates the positions of the points counted.

Six slides were mounted for the material from each fish; each slide contained sections from the dorsal, lateral and ventral regions of the third gill arch, from

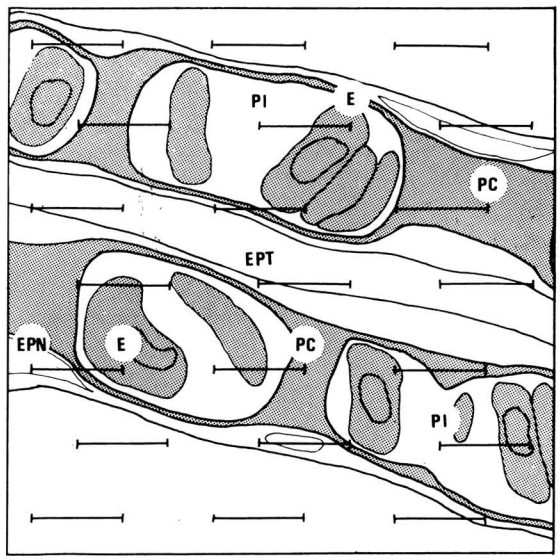


Fig. 1. Diagram of a section through a secondary lamella of a trout gill. The superimposed grid indicates the points counted in the present study.

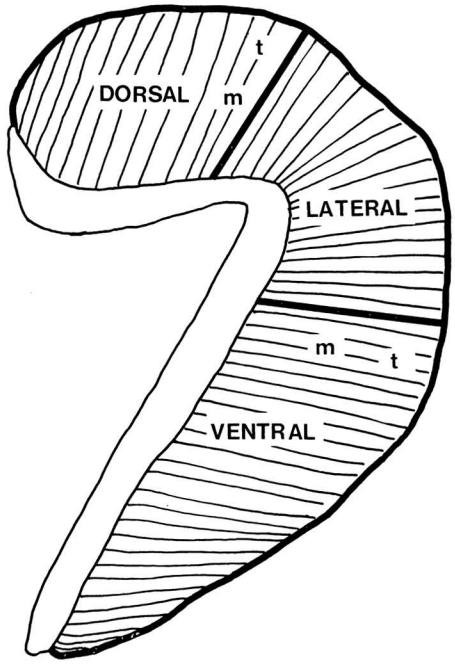


Fig. 2. Diagram of a single gill arch of a rainbow trout showing the sub-division into dorsal, lateral and ventral regions. The position of the tip (t) and middle (m) parts of a gill filament are indicated.

both the middle and the tip of the filament (Fig. 2).

Counts were made from three sections on each of the slides. A preliminary test showed that this method, when used with a coarser grid, gave a result very similar to that found when more detailed counts were made on single sections with the finer grid of the previous study (Soivio & Hughes 1978). It had the advantage, however, of ensuring that a larger sample was taken for each specimen.

The following ratios were calculated from the results of point counting:

$$\frac{E}{P};\ \frac{Pl}{P};\ \frac{Pl+E}{P};\ \frac{PC}{P};\ \frac{E}{E+Pl};\ \frac{R}{P};\ \frac{EPT}{EPN};$$

$$\frac{EPT+EPN}{P};\ \frac{EPT}{P};\ \frac{EPN}{P};\ \frac{EPT}{EPN+EPT};$$

$$\frac{EPN}{EPN+EPT};\ \frac{EPT+PC}{P};\ \frac{Pl}{PCS};\ \frac{E}{PCS};$$

$$\frac{Pl+E}{PCS};\ \frac{PC}{PCS};\ \frac{PC}{EPT}.$$

Marginal channel diameters were measured with an ocular micrometer at a magnification of 1560 x. Diameters were measured perpendicular to the lamellar axis.

As point counting of sections of a three-dimensional structure gives an estimate of the volume of the component counted (Underwood 1970), most of these ratios give relative values for the proportion of the reference volume (total volume of secondary lamella P, or volume of pillar cell system, PCS) occupied by a particular component.

Table 1. Material fixed with 2.5 % glutaraldehyde. Means ± SEM for sections from different gill regions.

Dorsal region

| | Middle of filament | | Filament tip | |
|---|---|---|---|---|
| Ratio | Normoxia (n = 4) | Hypoxia (n = 4) | Normoxia (n = 4) | Hypoxia (n = 4) |
| E/P | .216±.037 | .219±.017 | .178±.026 | .207±.042 |
| Pl/P | .087±.023 | .122±.031 | .074±.016 | .111±.020 |
| (Pl+E)/P | .302±.059 | .340±.038 | .251±.037 | .318±.043 |
| PC/P | .182±.011 | .181±.013 | .184±.016 | .172±.026 |
| E/(E+Pl) | .727±.026 | .656±.052 | .708±.034 | .638±.057 |
| EPT/EPN | 3.452±.420 | 2.504±.697 | 5.912±2.594 | 3.618±.623 |
| (EPT+EPN)/P | .492±.052 | .459±.045 | .537±.029 | .482±.054 |

Lateral region

| | Middle of filament | | Filament tip | |
|---|---|---|---|---|
| | Normoxia (n = 2) | Hypoxia (n = 4) | Normoxia (n = 4) | Hypoxia (n = 2) |
| E/P | .126±.039 | .297±.010 | .227±.022 | .233±.009 |
| Pl/P | .044±.006 | .133±.004 | .086±.013 | .103±.028 |
| (Pl+E)/P | .170±.045 | .430±.010 | .312±.028 | .335±.020 |
| PC/P | .160±.009 | .177±.008 | .197±,023 | .173±.026 |
| E/(E+Pl) | .729±.040 | .690±.012 | .726±.036 | .698±.067 |
| EPT/EPN | 4.640±.034 | 2.751±.808 | 4.509±1.060 | 3.858±1.671 |
| (EPT+EPN)/P | .648±.044 | .370±.012 | .467±.037 | .464±.044 |

Ventral region

| | Middle of filament | | Filament tip | |
|---|---|---|---|---|
| | Normoxia (n = 3) | Hypoxia (n = 4) | Normoxia (n = 2) | Hypoxia (n = 3) |
| E/P | 282± 032 | 177± 051 | 230± 009 | .199±.056 |
| Pl/P | .116±.011 | .070±.026 | .089±.006 | .113±.032 |
| (Pl+E)/P | .399±.041 | .247±.074 | .319±.003 | .312±.088 |
| PC/P | .166±.034 | .162±.007 | .181±.027 | .181±.017 |
| E/(E+Pl) | .707±.014 | .734±.036 | .721±.020 | .638±.001 |
| EPT/EPN | 3.653±.646 | 9.135±3.477 | 2.852±.276 | 14.108±10.217 |
| (EPT+EPN)/P | .413±.013 | .564±.069 | .463±.024 | .488±.101 |

*Analysis of data.* Analysis of variance was carried out with a Genstat programme and a model similar to that used by HUGHES & FLOS (1978), which also gave the means and SEM values for all groups. Student's *t* test was applied to the mean values for ratios obtained from material fixed in two different ways.

It is largely on the basis of these tests that the degrees of significance are given in Tables 1, 2 and 3.

## 3. Results

As mentioned in the introduction, the primary aim was an analysis of differences between regions of the gills and of changes in morphometry possibly produced by hypoxia. The results have given clear indications that the type of fixative used may significantly affect the results of morphometric investigations.

### A. Regional differences in morphometry of the gill system

Analyses were directed to comparisons between different regions of the third gill arch and differences between the tip and middle parts of individual filaments. The results are summarized in Tables 1 and 2, together with the number of specimens (n) for each part of the system.

Table 2. Material fixed with 2.5 % glutaraldehyde + 2.5 % formaldehyde. Means ± SEM for sections from different gill regions.

| Dorsal region | Middle of filament | | Filament tip | |
|---|---|---|---|---|
| | Normoxia (n = 4) | Hypoxia (n = 4) | Normoxia (n = 3) | Hypoxia (n = 3) |
| E/P | .256±.024 | .221±.046 | .234±.013 | .222±.042 |
| Pl/P | .116±.018 | .139±.025 | .160±.040 | .120±.033 |
| (Pl+E)/P | .327±.017 | .360±.054 | .393±.029 | .342±.029 |
| PC/P | .175±.006 | .182±.014 | .192±.015 | .244±.007 |
| E/(E+Pl) | .686±.048 | .606±.056 | .606±.080 | .657±.045 |
| EPT/EPN | 7.639±2.398 | 4.380±2.205 | 4.910±.970 | 13.517±5.289 |
| (EPT+EPN)/P | .426±.017 | .427±.062 | .386±.031 | .388±.067 |

| Lateral region | Middle of filament | | Filament tip | |
|---|---|---|---|---|
| | Normoxia (n = 3) | Hypoxia (n = 2) | Normoxia (n = 3) | Hypoxia (n = 4) |
| E/P | .259±.021 | .229±.016 | .269±.016 | .260±.022 |
| Pl/P | .153±.025 | .117±.010 | .139±.023 | .111±.016 |
| (Pl+E)/P | .412±.028 | .346±.026 | .408±.038 | .372±.011 |
| PC/P | .217±.022 | .250±.047 | .190±.006 | .212±.012 |
| E/(E+Pl) | .631±.047 | .661±.004 | .665±.027 | .699±.044 |
| EPT/EPN | 6.851±3.217 | 5.896±1.422 | 5.949±2.342 | 5.145±2.183 |
| (EPT+EPN)/P | .345±.007 | .381±.018 | .380±.033 | .393±.016 |

| Ventral region | Middle of filament | | Filament tip | |
|---|---|---|---|---|
| | Normoxia (n = 4) | Hypoxia (n = 3) | Normoxia (n=2) | Hypoxia (n=3) |
| E/P | .196±.045 | .204±.041 | .233±.014 | .234±.021 |
| Pl/P | .149±.017 | .055±.015 | .157±.018 | .061±.007 |
| (Pl+E)/P | .345±.084 | .259±.046 | .391±.004 | .295±.020 |
| PC/P | .199±.018 | .228±.018 | .182±.006 | .170±.021 |
| E/(E+Pl) | .581±.033 | .789±.064 | .598±.042 | .792±.028 |
| EPT/EPN | 8.232±2.067 | 5.204±1.098 | 6.859±3.444 | 3.656±1.939 |
| (EPT+EPN)/P | .423±.087 | .488±.037 | .395±.004 | .516±.041 |

The overall computer analysis of the data for normoxic and hypoxic material suggested that there were significant differences between different regions, i.e. between the dorsal, lateral and ventral regions of the gill arch. These data were mainly gathered from material fixed in 2.5 % glutaraldehyde but such differences were also indicated for the G/F material.

Analyses using more specific tests tended to confirm that the lateral region of the gills differed in certain respects from the dorsal and ventral parts. For example, in the lateral region the blood values relative to the volume of a secondary lamella (see ratios $\frac{E}{P}$, $\frac{Pl + E}{P}$) were greater during hypoxia (H) than in normoxia (N) ($\frac{E}{P}$ = 0.297; 0.126, in normoxia: $\frac{Pl + E}{P}$ = 0.430 (H); 0.170 (N)). These differences were noticeable in the middle parts of the filaments but not in the tips. This parameter showed little difference between normoxia and hypoxia for the dorsal gill region. The relation in the ventral region was in the opposite direction, but not significantly. In the material fixed in glutaraldehyde (G) the relative volume of blood ($\frac{Pl + E}{P}$) was greater in the middle region than in the tips of the filaments from the dorsal and lateral regions during hypoxia, but the opposite was true in normoxia, at least for the lateral region.

For the material fixed in glutaraldehyde and formaldehyde (G/F) no significant differences were found for any of the blood ratios between the middle and tip portions of gill filaments from the dorsal and lateral regions in either normoxia or hypoxia; some suggestion of a greater relative volume of erythrocytes was found for the tip portions of the ventral region. For material fixed in this solution in normoxia, no regional differences were found between filament tips, but compared with the other regions the middle portions of the ventral region had a lower haematocrit value and this showed a marked increase in hypoxia.

Inspection of the data for the epithelium of G-fixed material suggested a greater relative volume in the middle parts of filaments from the lateral regions, which became significantly reduced during hypoxia ($\frac{EPT + EPN}{P}$ = 0.646 (N); 0.370 (H). In other parts of the dorsal and lateral regions there was also a reduction in this volume during hypoxia, but the opposite tendency was apparent in the results for the ventral areas, i.e. the total volume of the epithelium was slightly increased, although these differences were not statistically significant. The G/F material showed relatively few changes during hypoxia with the exception of the tip portions from the ventral regions, where there was a marked increase in $\frac{EPT + EPN}{P}$ during hypoxia. This material showed a general increase in this ratio for tip and middle filament portions during hypoxia, but again almost none of the differences were statistically significant.

### B. Comparison of material obtained from normoxic and hypoxic fish

The overall nature of differences between normoxic and hypoxic gills is summarized in Table 3, where there are a number of statistically significant results. Most of them relate to the G/F material. For example, there was a decrease in the relative volume of the blood plasma and of the total blood volume in hypoxia. The haematocrit value shows a statistically significant increase in the G/F material, whereas it shows a significant decrease in the G material. Similar differences are apparent when the ratios are calculated with respect to the volume of the pillar cell system (PCS) rather than to the total volume of the secondary lamella. There was, however, a significant increase in the volume of the pillar cells from the G/F material during hypoxia, although no significant changes were apparent in the G material. The most significant changes observed in the G material during hypoxia were a decrease in haematocrit value and an increase in the volume of the plasma relative to the volume of the pillar cell system. In the epithelium there was a decrease in the tissue component during hypoxia with both fixatives. The G/F material suggests a slightly ($P < 0.1$) significant rise in the volume of the secondary lamella occupied by the epithelium during hypoxia.

### C. Comparison of material fixed in two different ways

Both fixatives were made up in 0.1 M phosphate

Table 3. Means ± SEM of all ratios calculated for the whole material. A = normoxia with 2.5 % glutaraldehyde; B = normoxia with 2.5 % glutaraldehyde + 2.5 % formaldehyde; C = hypoxia with 2.5 % glutaraldehyde; D = hypoxia with 2.5 % glutaraldehyde + 2.5 % formaldehyde. Δ = discrepancy (see text). For other symbols, see text.

| | Normoxia | | | Hypoxia | | | | |
|---|---|---|---|---|---|---|---|---|
| Ratio | A (n=19) | *P* | B (n=19) | C (n=21) | *P* | D (n=19) | *P* A/C | *P* B/D |
| E/P | .213±.015 | o | .240±.012 | .222±.016 | NS | .230±.013 | NS | NS |
| Pl/P | .084±.007 | *** | .144±.012 Δ | .109±.010 | » | .102±.011 | o | xx |
| (Pl+E)/P | .292±.021 | ** | .384±.019 | .331±.023 | » | .332±.018 | NS | x |
| PC/P | .181±.008 | NS | .192±.006 | .174±.006 | ** | .211±.009 | » | o |
| E/(E+Pl) | .719±.011 | *** | .630±.019 Δ | .675±.017 | NS | .697±.024 | x | x |
| EPT/EPN | 4.286±.588 | * | 6.860±.886 | 5.814±1.658 | » | 6.159±1.231 | NS | NS |
| (EPT+EPN)/P | .497±.021 | *** | .396±.019 Δ | .471±.025 | » | .432±.020 | » | o |
| EPT/P | .390±.020 | * | .329±.021 Δ | .366±.029 | » | .335±.018 | » | NS |
| EPN/P | .108±.010 | ** | .067±.010 | .105±.012 | » | .101±.019 | » | » |
| EPT/(EPT+EPN) | .782±.018 | NS | .828±.025 | .766±.025 | » | .786±.033 | » | » |
| EPN/(EPT+EPN) | .219±.018 | NS | .172±.025 | .234±.025 | » | .214±.033 | » | » |
| (EPT+PC)/P | .570±.023 | NS | .521±.020 Δ | .540±.026 | » | .545±.021 | » | » |
| Pl/PCS | .171±.010 | *** | .245±.015 Δ | .209±.013 | » | .185±.016 | x | xx |
| E/PCS | .436±.020 | NS | .414±.015 | .430±.016 | » | .422±.018 | NS | NS |
| (Pl+E)/PCS | .607±.025 | o | .658±.016 Δ | .639±.020 | » | .606±.017 | » | x |
| PC/PCS | .393±.025 | o | .342±.016 | .361±.020 | » | .393±.017 | » | x |
| PC/EPT | .481±.029 | ** | .623±.039 Δ | .534±.042 | o | .656±.044 | » | NS |
| Marginal channel diameter μm | 12.0±.5 | x | 10.2±.4 | 11.4±.3 | xx | 9.7±.4 | NS | NS |

o $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

buffers (pH 7.2) but presumably the osmolarity of the G/F mixture was greater. There was also a slight difference in the temperature during fixation.

When the results for data from all regions of the gills are collated (Table 3), there are more results showing significant differences between normoxic gills fixed by the two different methods than in any other part of the table. In hypoxia only the relative volume of the pillar cells is significantly greater in material fixed in the G/F mixture. A similar trend is present in the normoxic material. The response to hypoxia indicated by material fixed in these two different ways sometimes shows opposite tendencies, marked with Δ in Table 3. For example, in the G-fixed material the ratio $\frac{E}{E + Pl}$ decreases in hypoxia, whereas in gills fixed with the G/F mixture it increases. These differences are mainly due to changes in plasma volume; the red cell volume changes to a lesser degree but shows the same tendency.

Marginal channel diameters show a significant difference in material fixed in the two different solutions. There appears to be no correlation between body size and the diameter of these blood channels.

With both types of fixation the non-tissue volume of the epithelium relative to the total epithelial volume increases during hypoxia and the tissue component decreases. Both types also show an increase in volume of the pillar cells relative to the volume of the epithelial tissue during hypoxia.

## 4. Discussion

As the analysis shows, far more material needs to be studied before truly significant differences can be assessed. Nevertheless certain trends do suggest themselves. These include the possibility of regional differences between parts of the gill system, but their precise nature is uncertain. Of great interest are inconsistencies which seem to be associated more with the nature of the fixative than with the other variables investigated. It is well known that the circulation through the fish gill is a labile system which can be influenced by many nervous and hormonal factors (Rankin & Maetz 1971, Wood 1974, Payan & Girard 1977). Clearly the precise way in which any fixative affects this

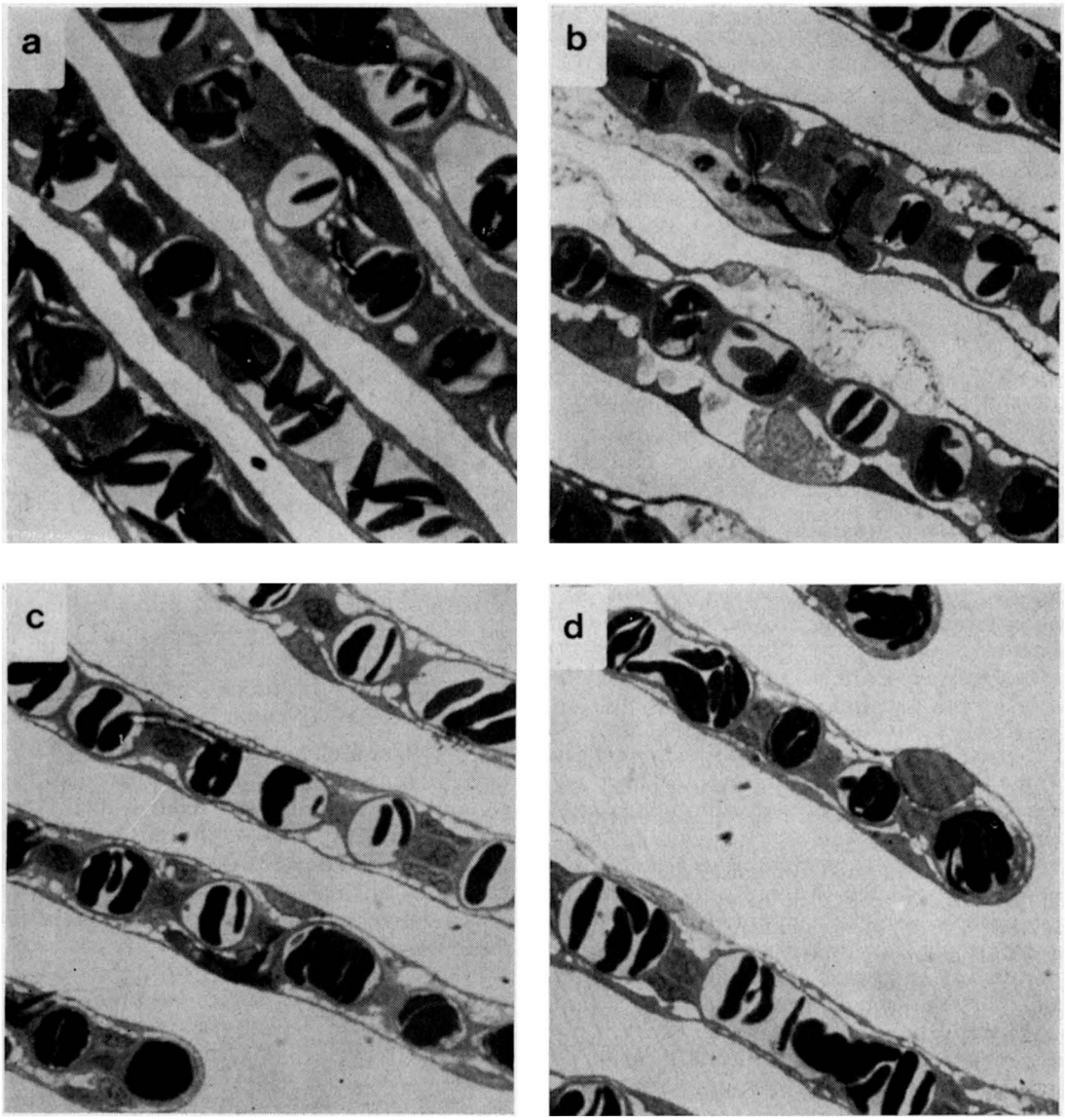


Fig. 3. Light micrographs of sections of secondary lamellae. a: hypoxia, gill fixed with 2.5 % glutaraldehyde, b: normoxia, gill fixed with 2.5 % glutaraldehyde, c: hypoxia, gill fixed with 2.5 % glutaraldehyde and 2.5 % formaldehyde, d: normoxia, gill fixed with 2.5 % glutaraldehyde and 2.5 % formaldehyde. Magnification × 613.

system will have a considerable influence upon the final result. Another problem facing anyone attempting a more detailed morphometric analysis is associated with the greater degrees of freedom during normoxia at both the morphological and physiological levels. Most fish function with a large safety factor which becomes progressively reduced in hypoxia. Consequently the system being investigated is probably far less variable during hypoxia than in normoxia. This gives one possible explanation for the finding that there are more significant differences correlated with the type of fixative during normoxia. During hypoxia it is possible that some of the effects tend to reduce the differences due to the fixation procedure in normoxia and con-

sequently these differences would no longer be statistically significant. The one ratio which seems to show a similar direction of change during hypoxia in material fixed by the two different methods is that concerning the structure of the epithelium. As indicated above, many other ratios show opposite tendencies. Another factor in this complex situation is the possibility that the physiological condition of the fish may affect the efficacy of a given fixative and thus with one fixative material may be fixed better during normoxia than in hypoxia and with another vice versa.

This study has certainly emphasized some of the problems concerned with the study of gill morphometry and immediately suggests that attention should be directed to the best way of fixing gill material. It seems that results are consistent, however, provided the same fixative is used. Thus a comparison of the changes in the ratios for blood during normoxia and hypoxia using glutaraldehyde fixation has given essentially the same results in both the present and the initial study (SOIVIO & HUGHES 1978) on portions from the lateral part of the third gill arch of fish accumated at 10°. Contradictory results have been obtained using two different fixatives and this raises the question of which is preferable. Unfortunately, we do not know which most closely reflects the situation in living gill tissue. Visual inspection is equivocal, the appearance being best in secondary lamellae fixed during hypoxia in either fixative (Fig. 3).

Most of the morphometric comparison has been made on the basis of ratios of points counted; absolute values are given only for measurements of the marginal channel diameter. It is therefore of especial interest that these values showed a significant difference between the fixatives. One possible explanation for such differences might be the different osmolalities of the fixatives.

Although the evidence is insufficient for detailed statements regarding differences in regional distribution of blood during normoxia and hypoxia, nevertheless it is clear that the gills are not homogeneous in this respect. This adds further to knowledge derived from gross anatomical studies. For example, the size of individual secondary lamellae varies not only along the length of a given filament, but also in filaments from different parts of a gill arch. Likewise, the frequency of the secondary lamellae is variable (HUGHES 1972) and consequently the gas exchange area contained in a unit portion of the gill system is not constant.

Some general relation can be discerned between surface area and water volume ventilating different parts of the gill. For example, PALING (1968), using glochidia larvae of *Anodon* as markers, found that they became attached preferentially in certain regions of the filaments and concluded that this was related to the larger volume of water flowing through these. Different gill arches also take up different quantities of heavy metal contained in water breathed by trout (HUGHES & FLOS 1978). These quantities are related to the distribution of metal in surfaces and perhaps indicate a relationship between area distribution and volume flow. On the blood side of the exchange surfaces, evidence for recruitment of blood flow in secondary lamellae has also been obtained (CAMERON *et al.* 1971, DAVIS 1972, CAMERON 1974, BOOTH 1978). The combined effect of these differences in ventilation and perfusion result in local variations in the amount of oxygen removed from the water. Evidence of this kind is available from both bony and cartilaginous fishes (HUGHES 1973).

Evidently there are many ways in which local differences in the pattern of blood flow can be altered and these are greater during normoxia than in hypoxia. Clearly these more physiological observations are quite compatible with the results so far obtained with morphometric methods.

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## References

BOOTH, J. H. 1978: The distribution of blood flow in the gills of fish; application of a new technique to rainbow trout (Salmo gairdneri). — J. Exp. Biol. 73: 119—129.

CAMERON, J. N. 1974: Evidence for the lack of by-pass shunting in teleost gills. — J. Fisheries Res.

Board Canada 31:211—213.

CAMERON, J. N., RANDALL, J. C. & DAVIS, J. C. 1971: Regulation of the ventilation-perfusion ratio in the gills of Dasyatis sabina and Squalus suckleyi. — Comp. Biochem. Physiol. 39A:505—519.

DAVIS, J. C. 1972: An infrared photographic technique useful for studying vascularization of fish gills. — J. Fisheries Res. Board Canada 29:109—111.

DAVIS, J. C. & WATTERS, K. 1970: Evaluation of opercular catheterisation as a method for sampling water expired by fish. — J. Fisheries Res. Board Canada 27:1627—35.

HOLETON, G. F. 1972: Gas exchange in fish with and without hemoglobin. — Respiration Physiol. 14: 142—150.

HUGHES, G. M. 1972: Morphometrics of fish gills. — Respiration Physiol. 14: 1—25.

—»— 1973: Comparative vertebrate ventilation and heterogeneity. — In: L. BOLIS, L., SCHMIDT-NIELSEN, K. & MADDRELL, S. H. P., Comparative Physiology: 187—220.

HUGHES, G. M. & FLOS, R. 1978: Zinc content of the gills of rainbow trout (S. gairdneri Richardson) following treatment with zinc solutions under normoxic and hypoxic conditions. — J. Fish Biol. (in the press).

HUGHES, G. M. & PERRY S. F. 1976: Morphometric study of trout gills: A light-microscopic method suitable for the evaluation of pollutant action. — J. Exp. Biol. 64:447—460.

LUFT, J. H. 1961: Improvements in epoxy resin embedding methods. — J. Biophys. Biochem. Cytol. 9:409.

MORGAN, M. 1974: Development of secondary lamellae of the gills of the trout, Salmo gairdneri (Richardson). — Cell. Tissue Res. 151:509—523.

MOUNT, D. I. 1961: Development of a system for controlling dissolved-oxygen content of water. — Trans. Am. Fisheries Soc. 90:323—327.

PALING, J. E. 1968: A method of estimating the relative volumes of water flowing over the different gills of a freshwater fish. — J. Exp. Biol. 48:533—44.

PAYAN, P. & GIRARD, J. P. 1977: Adrenergic receptors regulating patterns of blood flow through the gills of trout. Am. J. Physiol. 232:H18—H23.

RANKIN, J. C. & MAETZ, J. 1971: A perfused teleostean gill preparation: hemodynamic actions of neurohypophysial hormones and catecholamines. — J. Endocrinol. 51:621—635.

SOIVIO, A. & HUGHES, G. M. 1978: Circulatory changes in secondary lamellae of Salmo gairdneri gills in hypoxia and anaesthesia. — Ann. Zool. Fennici 15: 221—225.

SOIVIO, A., NYHOLM, K., & WESTMAN, K. 1975: A technique for repeated sampling of the blood of individual resting fish. — J. Exp. Biol. 62: 207—217.

UNDERWOOD, E. E. 1970: Quantitative Stereology. — 247 pp. Addison Wesley, London.

WEIBEL, E. R. 1970: Automatic sampling stage microscope for stereology. — J. Microscopy 91:1—18.

WOOD, C. M. 1974: A critical examination of the physical and adrenergic factors affecting blood flow through the gills of the rainbow trout. — J. Exp. Biol. 60:241—265.

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