J. exp. Biol. **123**, 27–41 (1986)
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EFFECTIVE AND MORPHOMETRIC OXYGEN-DIFFUSING CAPACITY OF THE GILLS OF THE ELASMOBRANCH *SCYLIORHINUS STELLARIS*

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Accepted 23 January 1986

SUMMARY

Calculations of the effective O_2 conductance (diffusing capacity or transfer factor, D_{eff}) of fish gills, obtained from experimental data on gill O_2 exchange, were compared with the predicted O_2-exchange properties of gill models based on morphometric measurements of the elasmobranch, *Scyliorhinus stellaris*. D_{eff} was calculated from O_2 uptake and P_{O_2} in gill water and blood, using a modified Bohr integration technique. In the morphometric gill model, O_2 conductance was considered for both the water–blood tissue barrier (D_m) and the interlamellar water (D_w). D_m was calculated from the total secondary lamellar surface area, the harmonic mean water–blood barrier thickness, and an assumed Krogh O_2-diffusion constant for gill tissue. D_w was estimated from the dimensions of the interlamellar spaces, the mean respiratory water flow velocity, and the diffusion coefficient of O_2 in water.

The ratio D_m/D_w was 1·84 in quiescently resting, 1·68 in resting alert, and 1·47 in swimming fish, showing that diffusion across interlamellar water was somewhat more important than that across the water–blood barrier in limiting the diffusive O_2 transfer between water and blood. The total morphometric diffusing capacity, D_{morph}, estimated by the combined membrane-and-water diffusing capacity, D_{m+w}, which is defined as $1/D_{m+w} = 1/D_m + 1/D_w$, was similar to D_{eff}, the ratio D_{m+w}/D_{eff} being 1·64 for quiescently resting, 1·02 for resting alert, and 0·92 for swimming fish. The good agreement between the effective and morphometric D estimates validates the approach, and leaves, at least for the alert and swimming fish, little space for functional inhomogeneities, which are expected to reduce D_{eff} as compared to D_{m+w}.

INTRODUCTION

There is a distinct discrepancy in fish between the effective conductance (diffusing capacity or transfer factor) for gill O_2 exchange as determined by physiological methods and morphometric measurements of gill secondary lamellae (cf. Hughes,

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Key words: fishes, elasmobranchs, diffusing capacity for O_2, gills, diffusion, morphometry.

1972). One reason for the physiological estimates being lower than the morphometric has been claimed to be the diffusion resistance offered by the water passing through the interlamellar space (Scheid & Piiper, 1971; Hills & Hughes, 1970). The first attempt at a comparison of the effective, physiological conductance for O_2 (D_{eff}) with morphometric measurements that accounted for resistance in water showed a reasonable agreement between D_{eff} and preliminary morphometric data for the gills in the elasmobranch *Scyliorhinus stellaris* (Scheid & Piiper, 1976).

Recently, the morphometric gill data of the same species have been reanalysed and completed, with particular attention paid to corrections for shrinkage and for optical artefacts (Hughes, Perry & Piiper, 1986). The aim of this study is to compare diffusing capacity for O_2 derived from this newer set of morphometric data with D_{eff} calculated from physiological measurements in the same species both at rest and during swimming activity (Baumgarten-Schumann & Piiper, 1968; Piiper & Baumgarten-Schumann, 1968*b*; Piiper, Meyer, Worth & Willmer, 1977). This comparison is based on the approach used in the previous study (Scheid & Piiper, 1976).

MATERIALS AND METHODS

A hypothetical P_{O_2} profile across a secondary lamella and the adjacent interlamellar water space is schematically shown in Fig. 1, which is based on the morphometry of Hughes *et al.* (1986). The P_{O_2} profile results from the resistances to O_2 diffusion, in interlamellar water and in the tissue barrier, and to the O_2 uptake resistance offered by the blood. Since the diffusivity of O_2 in water is about twice that in tissue (in terms of both Krogh's diffusion constant, K, and diffusion coefficient, $d = K/\alpha$; where α is the solubility of O_2) but the maximum diffusion pathway in water, equal to one-half the interlamellar distance (b), is about five times the thickness of the water–blood tissue barrier (s), an appreciable part of the total O_2 pressure drop is expected to reside within the interlamellar water.

An attempt will be made to estimate the relative magnitudes of the resistances to O_2 diffusion in interlamellar water and across the water–blood barrier and to compare their sum with *in vivo* measurements of branchial O_2 transfer. In accordance with customary usage, the reciprocal of O_2 diffusion resistance, i.e. the O_2 conductance or O_2-diffusing capacity, will be used as the characteristic parameter. In particular, we intend to compare the 'membrane' O_2-diffusing capacity (D_m) with that of interlamellar water (D_w) and both of these with the effective diffusing capacity (D_{eff}), which includes both components, tissue barrier ('membrane') and water.

RESULTS

Measurements

Physiology

Calculations are based on measurements of ventilation and gas exchange in *Scyliorhinus stellaris* at rest and during exercise. In the experiments of Baumgarten-

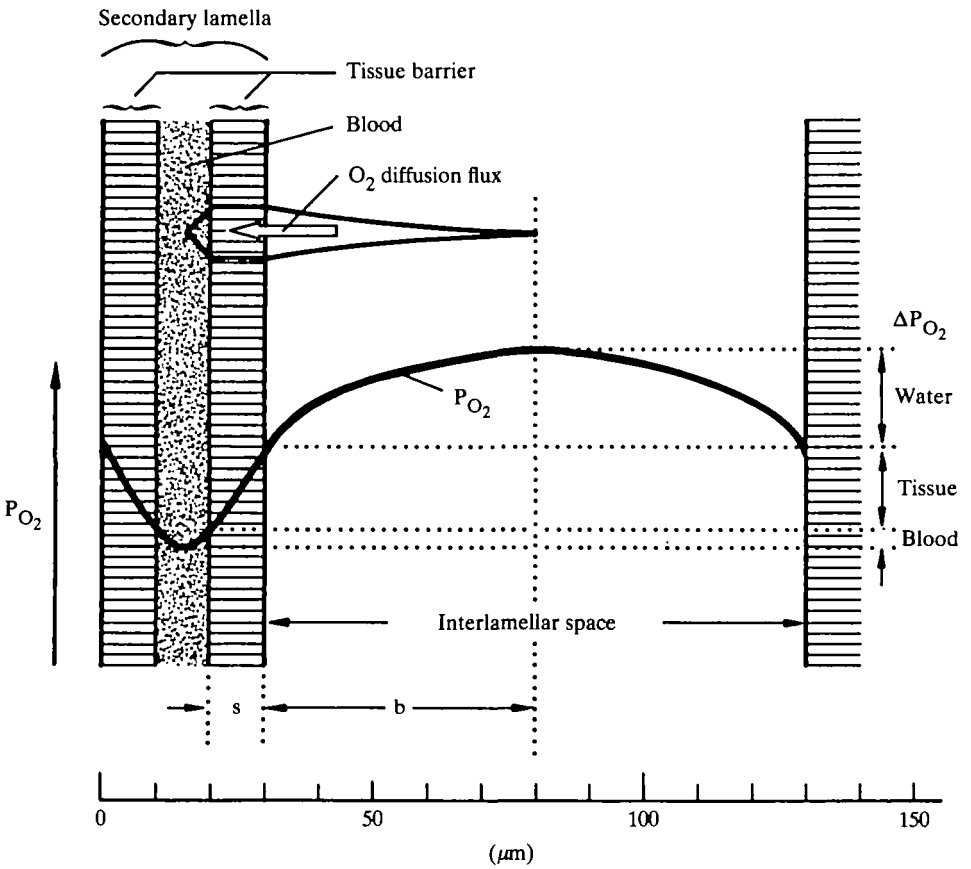

Fig. 1. Schematic cross-section through a secondary lamella and an interlamellar space. The P_{O_2} profile and the diffusion flux of O_2 are indicated. The scale refers to dimensions in 2·5-kg *Scyliorhinus stellaris*.

Schumann & Piiper (1968), the animals were quiescently resting; i.e. although awake and unanaesthetized, their metabolic rate was probably close to basal. In the more recent experiments of Piiper *et al.* (1977) the same species was investigated in conditions of spontaneous periodic swimming and resting periods between swimming bouts. These resting periods can be regarded as a state of alertness, the metabolic rate being above basal. Table 1 shows ventilation and O_2 uptake for these series.

Morphometry

The measurements of Hughes *et al.* (1986) were used, which were obtained on 12 specimens of *Scyliorhinus stellaris* with body mass ranging from 0·58 to 2·62 kg. From linear regressions of the logarithms of the morphometric variables against the logarithm of body mass the values for fish of 2·18 and of 2·53 kg, corresponding to the mean body mass of the fish used in physiological measurements (Table 1), were obtained.

The morphometric values required for this study, corrected for shrinkage as well as for the slant and Holmes effects (both due to non-perpendicular sectioning) are presented in Table 2, which also lists the magnitudes of the corrections.

Calculations

Effective diffusing capacity (D_{eff})

The effective O_2-diffusion conductance of any gas exchange system can be obtained as the ratio of O_2 uptake and mean P_{O_2} difference between medium, e.g. water, and blood (cf. Piiper & Scheid, 1975). In Table 1 the effective diffusing capacity (= transfer factor) for O_2 (D_{eff}) was calculated from experimental data of O_2 uptake ($\dot{M}_{O_2}$) and of P_{O_2} in inspired water (PI), expired water (PE), mixed venous blood (Pv) and arterial blood (Pa) using three different methods.

(1) $\dot{M}_{O_2}$ divided by the arithmetic mean water − blood P_{O_2} difference [i.e. (PI+PE−Pa−Pv)/2] (Randall, Holeton & Stevens, 1967).

(2) According to the theory of the counter-current model, assuming all resistance to O_2 diffusion to reside in a membrane separating blood and water, and the blood O_2 dissociation curve to be linear (Scheid & Piiper, 1976).

(3) The same as method 2, but using the blood O_2-dissociation curve and a graphical Bohr integration technique adjusted to the counter-current model (Piiper & Baumgarten-Schumann, 1968*b*; cf. Piiper & Scheid, 1984).

Since method 3 is the most accurate in theory, the D_{eff} values based on this method are used in the present study. The method is shown diagramatically in Fig. 2. D_{eff} is calculated as

$$D_{eff} = \frac{\dot{M}}{Ca - Cv} \times \sum_{n=1}^{N} \frac{\Delta C}{(Pw - Pb)_n}, \quad (1)$$

where $\dot{M}$ is O_2 uptake, Ca and Cv are O_2 concentrations in arterial and mixed venous blood, N is the number of (not necessarily constant) blood O_2 concentration

Table 1. *Physiological measurements in* Scyliorhinus stellaris

	Resting		
	Quiescent*	Alert†	Swimming†
Water temperature (°C)	17	18·3	18·3
Body mass (kg)	2·18	2·53	2·53
Ventilation, $\dot{V}$ (ml min^{-1})	425	810	2320
O_2 uptake, $\dot{M}_{O_2}$ (μmol min^{-1})	62	124	218
Effective O_2 diffusing capacity, D_{eff} (μmol min^{-1} Torr^{-1})	0·83	1·62	1·95

* Baumgarten-Schumann & Piiper (1968). D_{eff} calculated from data of these authors by Piiper & Baumgarten-Schumann (1968*b*).

† Piiper, Meyer, Worth & Willmer (1977). $\dot{V}$ and $\dot{M}_{O_2}$ from their table 1, D_{eff} calculated from data of their table 4, using a mass of 2·53 kg which is the average body mass of their entire series (their table 1).

Table 2. *Morphometric measurements of gill structures in* Scyliorhinus stellaris *of 2·18 and 2·53 kg body mass, used for calculations of morphometric* O_2 *diffusing capacity*

	Body mass (kg)		Ratio corrected to uncorrected
	2·18	2·53	
Total secondary lamellar surface area, A (cm^2)	3218	3614	1·30
Harmonic mean thickness of water–blood barrier, s (μm)	9·4	9·4	1·13
Interlamellar distance, 2b (mm)	0·102	0·112	1·12
Base length of a secondary lamella, *l* (mm)*	1·8	2·0	1·09
Height of secondary lamella, h (mm)	0·45	0·45	1·10
Total number of secondary lamellae, N	226 000	234 000	1·00
Total cross-sectional area of interlamellar spaces, F (cm^2)	103·7	117·9	1·23
Base-to-top taper index, λ	0·75	0·75	—
O_2 diffusing capacity of membrane, D_m (μmol min^{-1} $Torr^{-1}$)	3·87	4·42	—

Correction factors for shrinkage and distortion of tissue after Hughes, Perry & Piiper (1986).
* Value adjusted to fit the A, N and h values using a base-to-top taper index, $\lambda = 0{\cdot}75$.

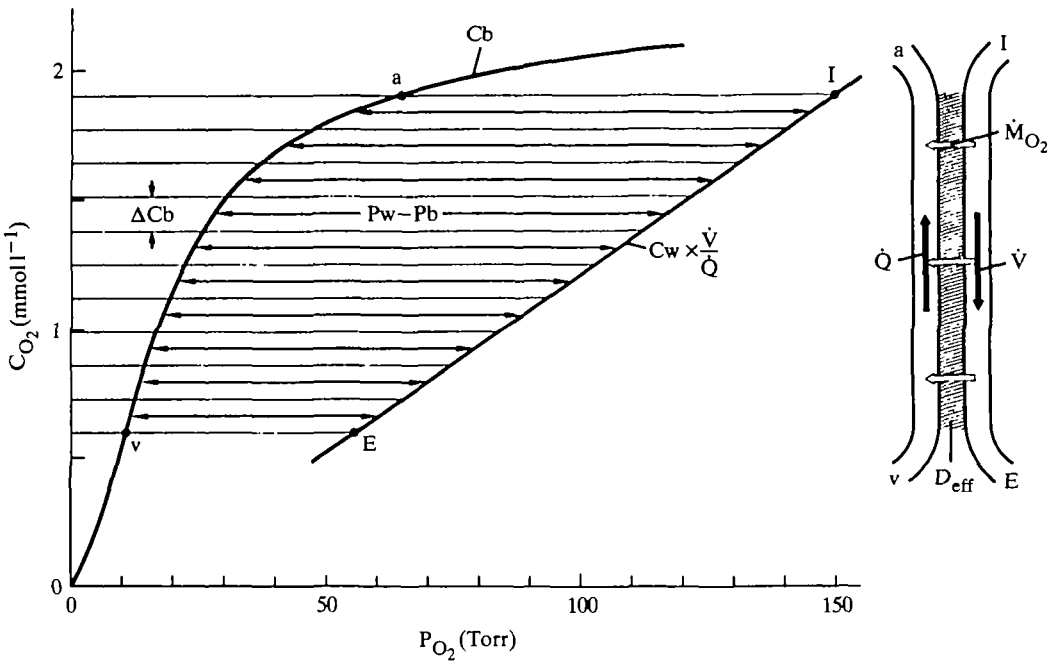

Fig. 2. Right-hand side: counter-current model for O_2 exchange in fish gills. $\dot{V}$, water flow; $\dot{Q}$, blood flow; $\dot{M}_{O_2}$, O_2 uptake. Left-hand side: Bohr integration technique for determination of effective O_2-diffusing capacity (D_{eff}). Cb is the effective blood O_2 dissociation curve (Piiper & Baumgarten-Schumann, 1968*a*). The straight line is its water counterpart (Cw) standardized to the same total O_2 concentration change (by multiplication by $\dot{V}/\dot{Q}$). The subdivision of the O_2 content change in blood (ΔCb) and water into 10 elements is shown by the thin lines. The double-headed arrows indicate the O_2 pressure difference effective for O_2 uptake (Pw−Pb). Note that equal ΔC values do not correspond to equal D_{eff} elements (due to variation of Pw−Pb). I, inspired; E, expired; a, arterial; v, venous.

increments (ΔC) in the interval Ca−Cv, Pw and Pb are the P_{O_2} values of water and blood, respectively; for the integration, the limiting values of Pw−Pb are PE−Pv and PI−Pa.

Evidently equation 1 defines a mean Pw−Pb ($= \dot{M}/D_{eff}$) as a harmonic mean. The same applies to method 2, whereas method 1 uses an arithmetic mean.

The mean D_{eff} values thus obtained are presented in Table 1.

Diffusing capacity of the water–blood barrier (D_m)

According to Fick's diffusion equation, the diffusive conductance or diffusing capacity of a (tissue) sheet depends on the following physical and geometrical properties: d, diffusion coefficient; α, solubility; K, Krogh's diffusion constant; A, surface area; s, thickness:

$$D_m = d \times \alpha \times A/s = K \times A/s. \quad (2)$$

The values for secondary lamellar surface area (A) and harmonic mean thickness of water–blood (tissue) barrier, s, can be taken from Table 2.

Unfortunately, no experimental data exist for d, α or K of secondary lamellar tissue for O_2. We adopted the K_{O_2} value for human lung tissue (Grote, 1967) extrapolated to 17 and 18·3°C, the average water temperature in the experiments (Table 1). These values are listed in Table 3.

The values for D_m thus calculated from equation 2 are listed in Table 2.

Diffusing capacity of interlamellar water (D_w)

Scheid & Piiper (1971) have analysed the resistance to O_2 diffusion offered by the interlamellar water, using simple geometric models of secondary lamellae. In these models they calculated the P_{O_2} profiles in the interlamellar water which entered the interlamellar space at a partial pressure, PI, the P_{O_2} at the secondary lamellar membrane being kept constant at Po. Using the P_{O_2} in mixed water leaving the gill

Table 3. *Diffusivity and solubility values for O_2 in tissue and water at 17 and 18·3°C*

	Temperature (°C) 17	18·3
Krogh diffusion constant of O_2 in tissue, K (μmol min^{-1} Torr^{-1} cm^{-1})	$1{\cdot}13\times10^{-6}$	$1{\cdot}15\times10^{-6}$
Diffusion coefficient of O_2 in water, d (cm^2 s^{-1})	$2{\cdot}16\times10^{-5}$	$2{\cdot}22\times10^{-5}$
Solubility of O_2 in sea water, α (μmol ml^{-1} Torr^{-1})	$1{\cdot}59\times10^{-3}$	$1{\cdot}62\times10^{-3}$

K and d after Grote (1967); α after Piiper & Schumann (1967), using the temperature dependence of α in water (Grote, 1967) to extrapolate to 18·3°C.

model, PE, they defined the equilibration inefficiency, ε, to quantify the equilibration deficit due to the diffusion resistance in interlamellar water:

$$\varepsilon = \frac{P_E - P_O}{P_I - P_O}. \tag{3}$$

They showed that the magnitude of ε for given secondary lamellar geometry and given velocity profile in the secondary lamellar water can be described as a function of the dimensionless equilibration resistance index, φ:

$$\varphi = \frac{b^2 \times \bar{v}}{l \times d}, \tag{4}$$

where b is one-half the interlamellar distance; $\bar{v}$ is the mean water velocity; l is the length of secondary lamella at the base of the lamella and d is the diffusion coefficient of O_2 in water. A large value of φ indicates poor conditions for O_2 equilibration.

Fig. 3 illustrates the relationship between ε and φ according to model B of Scheid & Piiper (1971) in which water flow is laminar in the interlamellar space (parabolic velocity distribution across the secondary lamellar space). These two curves represent limiting cases of lamellar shape as expressed by the base-to-top taper index, λ, i.e. the ratio of lamellar length at the top to that at the base. For $\lambda = 1{\cdot}0$ (rectangular secondary lamella) the water velocity is independent of the height, whereas there is a hyperbolic flow distribution for $\lambda = 0{\cdot}5$, accounting for the smaller resistance to water flow at the shorter top compared with the bottom.

The value of φ can be calculated from the data presented in Tables 1–3. The mean velocity, $\bar{v}$, is calculated from the measured ventilation, $\dot{V}$, and the total cross-sectional area of the interlamellar spaces, F:

$$\bar{v} = \dot{V}/F. \tag{5}$$

F is given by the individual cross-sectional area of pores (width, 2b, multiplied by height, h) multiplied by their total number (N):

$$F = 2b \times h \times N. \tag{6}$$

Values for the mean water flow velocity ($\bar{v}$) obtained from $\dot{V}$ (Table 1) and F (Table 2) are presented in Table 4 which also contains the resulting values for the equilibration resistance index φ for rest and swimming activity.

Using these values for φ, and a mean taper index of $\lambda = 0{\cdot}75$ (Table 4), the corresponding values for the equilibration inefficiency, ε, can be obtained from Fig. 3. They are listed in Table 4.

The inefficiency parameter, ε, has the meaning of a fractional effective water shunt: it defines what fraction of the respiratory water may be considered as shunted (because the P_{O_2} value is unchanged) when the remainder is assumed to equilibrate completely with the secondary lamellae. Scheid & Piiper (1971) have used ε to calculate the effective diffusing capacity of interlamellar water, D_w. The equivalent model used for this analysis is shown in Fig. 4B, in which the continuously

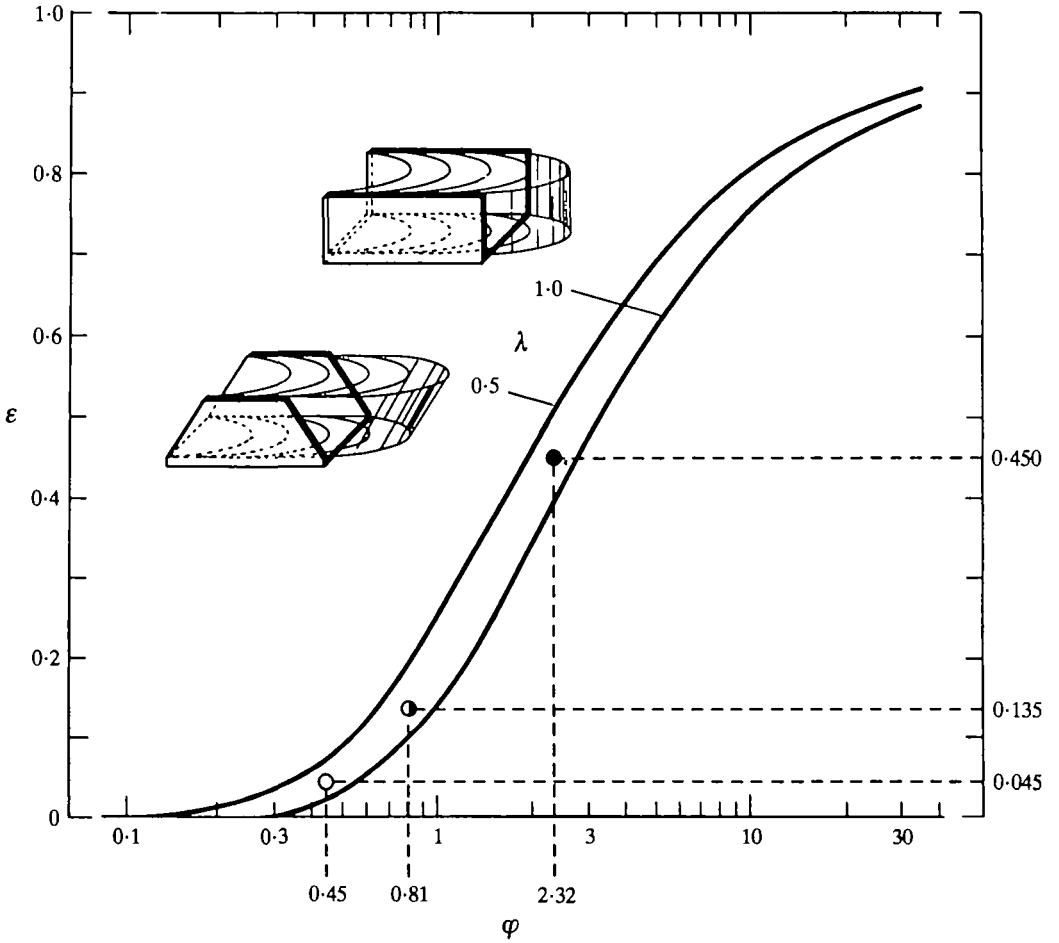

Fig. 3. Plot of 'equilibration inefficiency', ε (equation 3), against 'equilibration resistance index', φ (equation 4). Abscissa (φ), logarithmic; ordinate (ε), linear. The two curves are for a rectangular lamella ($\lambda = 1{\cdot}0$) and for a trapezoidal lamella, of same base length, but tapering to one-half length at the top edge ($\lambda = 0{\cdot}5$). The experimental points (open circle, quiescently resting; half-closed circle, resting; filled circle, swimming) are in the middle, corresponding to $\lambda = 0{\cdot}75$.

distributed water velocity of the laminar flow model is replaced by a model with a stagnant water layer lining the secondary lamellar surface and a central core of mixed flow. In this model the central core equilibrates with the wall according to the equation:

$$\varepsilon = \exp\left[-D_w/(\dot{V} \times \alpha)\right], \tag{7}$$

where $\dot{V}$ is ventilation (water flow) and α the solubility of O_2 in water. Transformation yields:

$$D_w = \dot{V} \times \alpha \times \ln(1/\varepsilon). \tag{8}$$

With values for $\dot{V}$ (Table 1), α (Table 3) and ε (Table 4), one obtains the D_w values listed in Table 4.

The thickness of the equivalent stagnant layer, s_{st}, can be calculated from the Fick diffusion equation:

$$s_{st} = d \times \alpha \times A/D_w . \quad (9)$$

The values for s_{st} and for the ratio s_{st}/b are presented in Table 4.

Combination and comparison

The values of D_{eff} (Table 1), D_m (Table 2) and D_w (Table 4) are compiled and compared in Table 5.

The ratio D_m/D_w is higher than unity, implying that the limitation to O_2 diffusion is greater in interlamellar water than in the water–blood tissue barrier.

In order to compare the results of model calculations with D_{eff}, a 'total membrane-and-water' diffusing capacity, D_{m+w}, is approximated by the addition of the reciprocal 'component' D:

$$1/D_{m+w} = 1/D_m + 1/D_w . \quad (10)$$

The D_{m+w}/D_{eff} ratio for quiescent fish, 1·64, is significantly above unity, but the ratios for resting alert and swimming fish (1·02 and 0·92, respectively) are close to unity, signifying a remarkably good agreement between gas exchange measurements, physical properties of tissue and water, and morphometric values.

DISCUSSION

Physiological conditions

In two previous studies on resting fish (Baumgarten-Schumann & Piiper, 1968; Piiper *et al.* 1977), there were important differences in the conditions under which relevant measurements (e.g. of ventilation) were made. In the former study, the fish were in a prolonged state of inactivity, whereas the animals in the latter study were alert, the relatively short resting periods (averaging about 30 min) being interrupted by spontaneous swimming periods. This is evident from the marked differences in both ventilation and O_2 uptake. Such an increase in D_{eff} may in part be due to increased water velocity in the interlamellar space, with an associated increase in water diffusing capacity, D_w (see below). On the other hand, it is conceivable that in the resting quiescent condition the full capacity of the gill apparatus is not used,

Table 4. *Values used in calculating the interlamellar water O_2 diffusing capacity (D_w), derived from data of Tables 1–3 according to the text*

	Resting		
	Quiescent	Alert	Swimming
Mean water flow velocity, $\bar{v}$ ($mm\,s^{-1}$)	0·68	1·15	3·28
Equilibration resistance index, φ	0·45	0·81	2·32
Equilibration inefficiency, ε	0·045	0·135	0·450
O_2 diffusing capacity of interlamellar water, D_w ($\mu mol\,min^{-1}\,Torr^{-1}$)	2·10	2·63	3·00
Thickness of stagnant layer, s_{st} (μm)	31·6	29·7	26·0
Ratio of stagnant layer thickness to half interlamellar distance, s_{st}/b	0·62	0·53	0·46

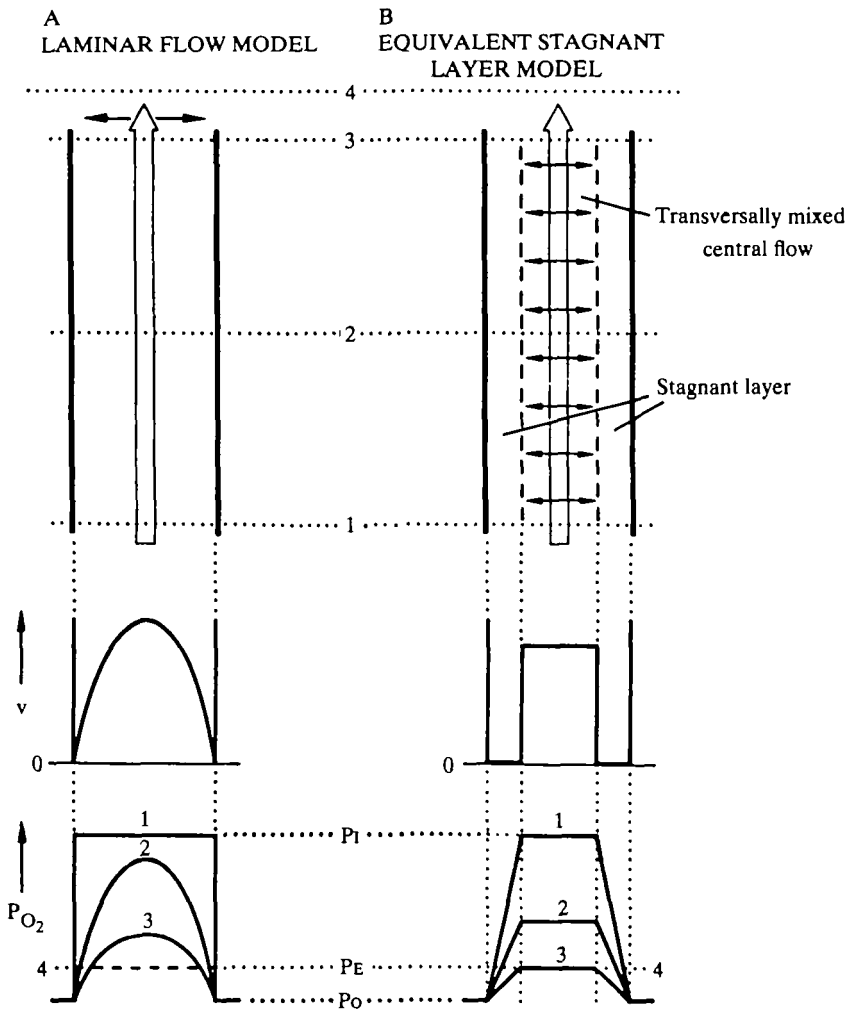

Fig. 4. The laminar flow model (A) and the equivalent stagnant layer model (B). Top: section perpendicular to secondary lamellae, parallel to the filament. Water flow direction is indicated by open arrows. Numbers refer to positions: 1, inflow end; 2, middle; 3, outflow end; 4, respired water after leaving the interlamellar space. In B, equivalent cross-sectional mixing within the flowing water is indicated by transverse arrows; the equivalent stagnant water layer is separated from flowing water by a broken line. Middle: water velocity (v) profiles: parabolic in A, step-like in B, with $v = 0$ in the stagnant layers; v is constant in the central flow. Bottom: cross-sectional O_2 pressure (P_{O_2}) profiles at positions 1–4 of the model. Continuous profiles in A. In B, linear P_{O_2} drop in the stagnant layer, no P_{O_2} gradient within the flowing water.

because there is ample functional shunting. This hypothesis is supported by the observations of rapidly changing arterial P_{O_2} in some fish, apparently reflecting changing functional inhomogeneity (Piiper & Schumann, 1967).

Diffusion limitation in interlamellar water

The present analysis shows, in agreement with the previous study (Scheid & Piiper, 1976), that the resistance to O_2 diffusion in interlamellar water plays an

important role in limiting branchial O_2 transfer, both at rest and during swimming activity.

The relative roles of diffusion in interlamellar water and across the water–blood barrier depend on the diffusion distances (Fig. 1) and the diffusion properties of the media. For a first approximation, the average path length for lateral diffusion of O_2 molecules in the interlamellar space may be taken as b/2, and that across the water–blood barrier as s. For *Scyliorhinus stellaris* the b/2:s ratio is between 2·7 and 3 (Table 2). The ratio of the assumed Krogh diffusion constant for tissue–water (Table 3) is between 0·55 and 0·53. Thus the estimated water/tissue diffusion resistance ratio, corresponding to the D_m/D_w ratio, is expected to be between 1·5 and 1·6, which is in reasonable agreement with the calculated results of Table 5.

The mean diffusion path length decreases with increasing water velocity, because gas exchange becomes restricted to layers close to the secondary lamellar surface. This is why D_w increases and the equivalent stagnant water layer decreases with increasing water flow (Table 5). The exact quantitative relationships are influenced by the flow velocity profile (Scheid & Piiper, 1971).

Comparison of morphometric and physiological diffusing capacities

For the quiescently resting fish, the total morphometric diffusing capacity (D_{morph}), estimated by the combined membrane-and-water diffusing capacity (D_{m+w}), is considerably above the effective, physiological diffusing capacity, D_{eff}. This result, which is in qualitative agreement with the earlier analysis of Scheid & Piiper (1976), is not unexpected since in most reported cases D_{morph} has been found to be considerably higher, even by an order of magnitude, than the D_{eff}. This has been repeatedly documented for mammalian lungs (reviewed by Weibel, 1973), but also for reptilian lungs (Perry, 1978) and for avian lungs (Abdalla *et al.* 1982). Only for the skin of a lungless plethodontid salamander (Piiper, Gatz & Crawford, 1976) and for the pleural membrane of dog lungs (Magnussen, Perry, Willmer & Piiper, 1974) has a reasonable agreement been found. But also in these cases, D_{morph} was slightly higher than D_{eff}.

The conventional explanation for $D_{morph}/D_{eff} > 1$ is that the numerous parallel units in the gas exchange organ are inhomogeneous with respect to ventilation,

Table 5. *Comparison of diffusing capacities for O_2 (D)*

	Resting		
	Quiescent	Alert	Swimming
Physiological, D_{eff}	0·83	1·62	1·95
Membrane, D_m	3·87	4·42	4·42
Water, D_w	2·10	2·63	3·00
Membrane-and-water, D_{m+w}	1·36	1·65	1·79
D_m/D_w	1·84	1·68	1·47
D_{m+w}/D_{eff}	1·64	1·02	0·92

D is in μmol min^{-1} Torr^{-1}.

diffusion and perfusion, which, unless properly accounted for, leads to an underestimation of D_{eff}. For example, in mammalian lungs, D for O_2 is determined from alveolar–arterial P_{O_2} differences; since these are also generated by shunt and unequal distribution of ventilation to perfusion, D for O_2 is underestimated if no appropriate corrections are applied (see Piiper & Scheid, 1980).

For fish gills, there is ample possibility of ventilation–perfusion inhomogeneity due to both morphological and functional factors. Extreme cases are blood shunting (e.g. due to perfusion of intrafilamentary afferent–efferent arterial connections or to perfusion of unventilated lamellae) and water shunting (due to passage of water between rows of secondary lamellae or between tips of filaments). Moreover, part of the O_2 uptake resistance may reside in the blood (diffusion limitation; reaction limitation due to slow oxygenation of haemoglobin).

Whereas the finding that $D_{m+w}/D_{eff} > 1$ for the quiescently resting fish thus appears to be readily explained, it was unexpected to find a close agreement between D_{m+w} and D_{eff} for resting and swimming fish. This would call for a critical examination of all the methods, including morphometric techniques, physical properties, models and physiological measurements, for directional errors potentially leading to an overestimation of D_{eff} or to an underestimation of D_{morph}.

Shrinkage

One of the basic problems in morphometry is deformation, due in great part to shrinkage and to the finite sectioning thickness. Hughes *et al.* (1986) have presented a detailed account of the procedures for morphometry and the determination of factors for corrections for deformation (see Table 2).

D_m is proportional to A/s, thus the correction factor is $1{\cdot}30/1{\cdot}13 = 1{\cdot}15$. This means that without correction, D_m is underestimated by $(1-1/1{\cdot}15)\times 100 = 13\,\%$.

The effects of shrinkage on D_w are more complex. According to equation 4 the anatomical dimensions determining φ are the interlamellar distance (2b) and the length of the secondary lamellae (l), φ being proportional to b^2/l. With the correction factors from Table 2 this yields a combined correction factor for φ of 1·15. Thus shrinkage appears to lead to underestimation of φ and ε, and thus to overestimation of D_w.

This analysis assumes constancy of the interlamellar water velocity, $\bar{v}$. Changes (errors) in the anatomical dimensions, however, influence $\bar{v}$ if a given (constant) $\dot{V}$ is considered. Combination of equations 4, 5 and 6 yields:

$$\varphi = \frac{\dot{V}}{2N \times d} \times \frac{b}{h \times l}. \qquad (11)$$

The combined correction factor of $b/(h\times l)$ is 0·934 (Table 2). In this case shrinkage, if not accounted for, leads to an overestimate of φ and ε, and to an underestimation of D_w. But the error does not exceed 10 % during either rest or exercise.

It is evident from equation 11 that not only the extent, but even more the anisotropy of the shrinkage, i.e. different functional shrinkage of b, h and *l*, play an important role in influencing the conditions for O_2 diffusion in terms of D_w.

Physical diffusion properties

There are only few reports in the literature on measurements of the O_2 diffusion coefficient, d, or of the Krogh diffusion constant for O_2, K ($= d \times \alpha$), in tissues (see Bartels, 1971). Most authors have used the values of Krogh (1918/19) and of Thews & Grote (see Grote, 1967).

There are no measurements on fish gill tissue. We used the values obtained by Grote (1967) on rat lung tissue mainly because they appear to be derived from the most reliable determinations. Not only may the true value for fish gills be different, but also in calculations of D_m for lungs it must be considered that the measurements were performed on slices of degassed whole lung tissue, only a small fraction of which constitutes the gas–blood barrier. A promising approach is determination of D_m from oxygenation and deoxygenation kinetics of red cells in isolated secondary lamellae of fish gills (Hills, Hughes & Koyama, 1982). At present, nothing can be predicted concerning the direction or extent of errors due to the uncertainty about O_2 diffusivity.

For this reason, i.e. lack of reliable data on physical diffusion properties, it appears to be generally preferable to express the results of morphometric studies on medium–blood barrier for gas exchange in terms of the surface area/mean harmonic thickness ratio (A/s; dimension:length) – the 'anatomical diffusion factor' of Perry (1978). The value can then be used for functional estimates in conjunction with the appropriate K value, of which more accurate determinations will be available in the future. In addition, the previously reported values for O_2 diffusivity in water are rather unreliable (see Bartels, 1971).

Models

The flat sheet model for calculation of D_m is rather straightforward. But problems arise from making appropriate allowance for the pillar cells, which support the secondary lamellae and reduce the surface area available for gas exchange.

More critical are the assumptions for calculation of D_w. Unfortunately, the required morphometric measurements in large *Scyliorhinus stellaris* are very limited (Hughes *et al.* 1986). Moreover, the parabolic flow velocity profile, assumed in the model analysis, may not be fully developed in the interlamellar space. A square-front flow would be more efficient for gas exchange and would therefore yield higher D_w values (Scheid & Piiper, 1971).

Transversal mixing of interlamellar water would greatly increase gas exchange efficiency by reducing the P_{O_2} gradient in water (see Fig. 1). However, the flow in the interlamellar space is expected to be fully laminar on the basis of low Reynolds numbers (estimated range 0·1–0·4). But the splitting of flow upon entering the interlamellar spaces may give rise to some transverse mixing which could elevate the O_2 transport efficiency.

The simplified model for isolated quantification of diffusion resistance in interlamellar water (Scheid & Piiper, 1971) does not take into account the change of P_{O_2} in secondary lamellar blood, which in reality increases from the mixed venous to the arterial value. Instead, the lamellar P_{O_2} is assumed to be constant throughout (Po in equation 3 and Fig. 4). Therefore, the simple additive combination of D_w with D_m, yielding D_{m+w} (equation 10), and comparison with D derived from a model that accounts for changing P_{O_2} in intralamellar blood is clearly incorrect because the models are not consistent. However, a recent theoretical study shows that the error produced by this apparent incompatibility is relatively minor (Scheid, Hook & Piiper, 1986).

We conclude that resistance to O_2 diffusion in the interlamellar water ($1/D_w$) exceeds that of the secondary lamellar tissue membrane ($1/D_m$) and that this is particularly pronounced at rest. When comparing morphometric with physiological estimates of the diffusing capacity, the good agreement between the D_{eff} and D_{m+w} values may be interpreted to show that the methods and models used are appropriate. On the other hand, local variations of physiological quantities like ventilation, diffusing capacity and blood flow, in part resulting from morphometric inhomogeneity, are expected to reduce gas exchange efficiency, i.e. to decrease D_{eff}. Possibly some inhomogeneity effects were compensated by physiological control mechanisms. In any case, there seemed to be little space for mechanisms reducing O_2 transfer efficiency, such as blood or water shunts.

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