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MORPHOMETRY OF THE GILLS OF THE ELASMOBRANCH *SCYLIORHINUS STELLARIS* IN RELATION TO BODY SIZE

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SUMMARY

In order to study the dependence of the dimensions of the respiratory apparatus on body size and to provide a morphometric basis for the analysis of branchial gas exchange function, the gills of 12 specimens of *Scyliorhinus stellaris* L., weighing 0·58–2·62 kg, were examined morphometrically. The average values and the local variations of the structural parameters determining diffusive gas transfer properties of the gills were determined. Particular attention was paid to corrections for shrinkage effects in surface area measurements and to corrections for the Holmes and slant effects in measurements of paraffin sections.

The shape and size of secondary lamellae varied according to the sampling site on the filament, and filament length varied with its location on the gill arch. Also the water–blood distance varied, mainly because of frequent occurrence of thickenings at mid-height of the secondary lamellae.

The total gill surface area increased proportionally to (body mass)$^{0·78}$, mainly because of an increase in surface area of individual lamellae rather than an increase in their number. Since the thickness of the secondary lamellae varied little with body mass, the observed increase in total filament length in proportion to body mass is attributed to an increase in interlamellar distance. The water–blood distance varied little with body mass.

The extent of shrinkage was found to be about 10% of filament length, but because of the compensating increase in secondary lamellar frequency this had no effect on gill area estimates, although it did affect the interlamellar dimensions. Shrinkage of individual secondary lamellae was extremely difficult to estimate, partly because of non-isometric shrinkage within the gill system. Underestimation of secondary lamellar area using paraffin sections could approach 30% mainly because of a reduction in the proportion of the pillar cell system exposed above the level of the gill filaments.

INTRODUCTION

Several studies have been made of the physiology of gas exchange in the elasmobranch *Scyliorhinus stellaris* (Piiper & Schumann, 1967; Piiper & Baumgarten-

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Schumann, 1968*a,b*; Baumgarten-Schumann & Piiper, 1968; Piiper, Baumgarten & Meyer, 1970; Scheid & Piiper, 1976; Piiper, Meyer, Worth & Willmer, 1977). One of the aims of those studies was to arrive at a quantitative estimate of the 'diffusing capacity' (diffusive water-to-blood conductance) as a factor limiting branchial gas exchange of oxygen and carbon dioxide.

The diffusing capacity is expected to be related to the total effective surface area and to the effective water–blood distance. However, theoretical and model analyses have indicated that an important part of the total resistance to oxygen transfer must reside in the interlamellar water (Hughes, 1966; Hills & Hughes, 1970; Scheid & Piiper, 1971, 1976). To quantify the fractional resistances of water and tissue, the length and height of the secondary lamellae and the interlamellar distances must be known (Scheid & Piiper, 1971), in addition to the conventionally measured total surface area of the secondary lamellae (Hughes, 1972, 1984*a*). The present study attempts to obtain measurements of all these parameters in specimens of different body sizes, using morphometric analysis of paraffin wax and Epon sections. Some of the techniques and problems of morphometric studies in fish gills have been discussed previously (Gray, 1954; Hughes, 1966, 1972, 1984*a,b*; Muir & Hughes, 1969; Hughes & Morgan, 1973). The problem of histotechnical and measuring artefacts associated with the paraffin technique receives particular attention. The application of the morphometric data obtained in this study to the analysis of gas exchange by physiological methods is given in a following report (J. Piiper, S. F. Perry, P. Scheid & G. M. Hughes, in preparation).

MATERIALS AND METHODS

Thirteen specimens of *Scyliorhinus stellaris* (larger spotted dogfish, or nurse-hound), body mass 570–2615 g, were caught during March in the Bay of Naples and kept for 1–3 weeks in large holding tanks at the Zoological Station, Naples. Three additional specimens used for estimation of secondary lamellar shrinkage were obtained at the laboratory of the Marine Biological Association, Plymouth. Results obtained from previous studies of gill morphometry on the same species collected at Plymouth are also included.

Fish were anaesthetized with MS222 ($0{\cdot}1\,g\,l^{-1}$ sea water). Except in those specimens used for the study of secondary lamellar shrinkage, the entire branchial region was removed. Samples for electron microscopy were taken from five of the 'Naples' fish. The remaining branchial tissue was allowed to stand for 1–6 days in Bouin's fixative (prepared using sea water) before being transferred to 50% ethanol for storage. Gill filaments from four such fish were subsequently embedded in paraffin for morphometry using standard histological procedures. Samples for ultramicrotomy were fixed in 3% glutaraldehyde in $0{\cdot}1\,mol\,l^{-1}$ phosphate buffer (pH 7·4) containing 4% (w/v) sucrose and 3% (w/v) sodium chloride, postfixed in cold 1·0% osmium tetroxide in the same buffer and embedded in Epon. 1-μm sections were stained with toluidine blue for light microscopy.

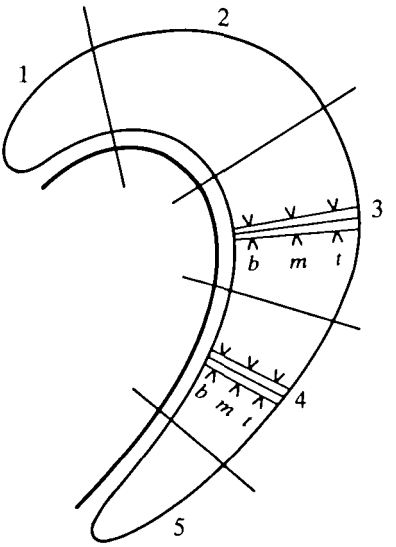

Fig. 1. Diagram of single hemibranch to show five regions used in sampling filaments. In regions 3 and 4, three filaments are drawn to show the positions at base (*b*), middle (*m*) and tip (*t*) where secondary lamellae were sampled. Small cuts in the outer two filaments are used to define the position of the part of the central filament that is measured.

Measurements and morphometric analysis

Frequency and average surface area of the secondary lamellae, and total length of gill filaments were estimated in unembedded alcohol-stored gills of thirteen *Scyliorhinus stellaris*. Every fifth filament from all hemibranchs on one side of a fish was measured for filament length. For the measurement of secondary lamellar frequency and area, filaments were sampled from the second gill on the right side of the fish. A weighting method (Muir & Hughes, 1969; Hughes, 1984*a*,*b*) was employed using secondary lamellae from the tip, middle and base of every fifth filament.

For histological study, the second or third gill arch was removed from the fixed alcohol-stored branchial region and divided into five regions (Fig. 1). Three adjacent filaments were removed from each of the regions 2, 3 and 4, divided into tip, middle and base portions, and embedded in Paraplast. Orientation was such that one filament would be sectioned horizontally, one obliquely, and the third vertically with respect to the filamentary axis. The surfaces of the secondary lamellae, however, were oriented at approximately 90° to the plane of sectioning. Tissue was sectioned at 5 μm and stained with Cason trichrome stain (Humason, 1961). These sections of paraffin-embedded tissue were used for measurement of secondary lamellar thickness, interlamellar distance, water–blood distance, and secondary lamellar blood channel thickness.

Evenly spaced, non-overlapping fields (Weibel, 1969) were viewed through a photomicroscope, and for each sample the first four fields in which secondary lamellae and their associated interlamellar spaces made up at least one-half of the

field were then photographed. Positives of the 35 mm films were projected at a final magnification of 333× onto a square lattice using a microfilm reader.

Five 1-μm sections of Epon-embedded material, each section from a different specimen, were photographed at a magnification of 1040×. No attempt was made in either paraffin or Epon sections to standardize the orientation of the tissue with respect to the frame of the photographic field. These sections were used to measure thickness of the secondary lamellae and water–blood barrier.

The measured and calculated morphometric gill parameters are schematically shown in Fig. 2. Estimates of the total thickness of a secondary lamella were obtained by measuring the perpendicular distance across each lamella at the point of intersection with the test lines of a semicircular test grid (Merz, 1967). Similarly, the thickness of the water–blood barrier was measured from these intersection points using the methods of Hughes & Perry (1976). The blood side of the water–blood barrier was assumed to be the inner limit of the basement membrane as the pillar cell flange cytoplasm was not distinguishable in paraffin sections. A total of 2998 measurements of secondary lamellar thickness and 1094 measurements of water–blood distance were made. Both arithmetic and harmonic means of the

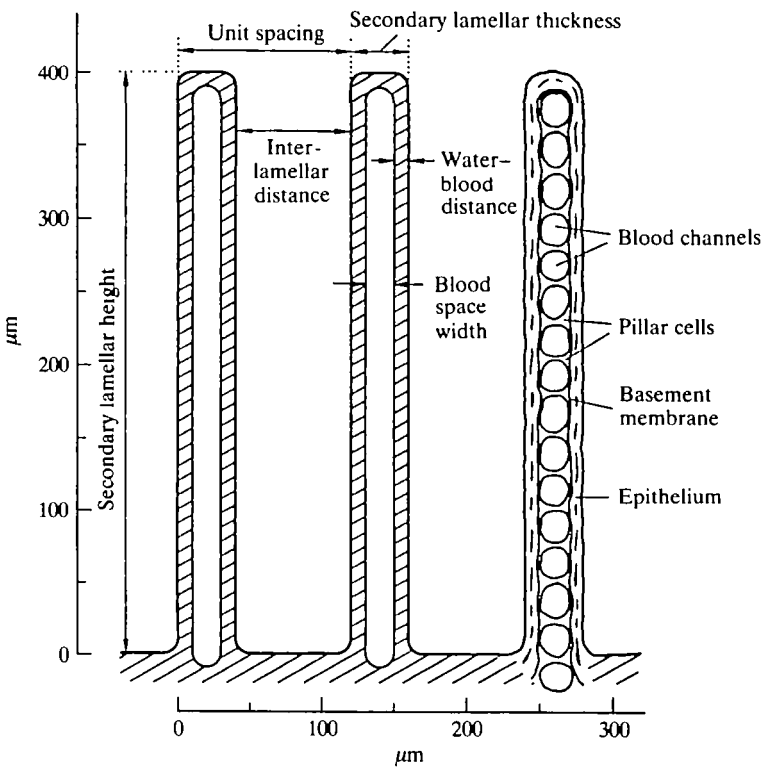

Fig. 2. Section through two adjacent secondary lamellae to show the anatomical structures (right) and simplified geometric model parameters (centre and left). The scales show approximate dimensions.

water–blood distances were calculated. The mean interlamellar distance was obtained by subtracting secondary lamellar thickness from the unit width (i.e. the reciprocal of secondary lamellar frequency). The difference between secondary lamellar thickness and twice the arithmetic mean water–blood distance was taken as an approximation for the blood channel thickness.

Tissue distortion and shrinkage

The most readily accessible parts of the system for measurement are the gill filaments. The position of points on single filaments was obtained using the method indicated in Fig. 1. The frequency of secondary lamellae and the distance between the marked points were determined in fresh material and following fixation and storage in 50% ethanol. Mean shrinkage in filament length obtained in the present study was 10%. The corresponding increase in frequency of the secondary lamellae showed that the overall result of these distortions in terms of total numbers of secondary lamellae effectively cancel out. The more important but much more difficult correction concerns the area shrinkage of the secondary lamella. The accuracy of any such estimate is very limited because of their small size and the inaccessibility of their attached sides for measurement. The following method was adopted at Plymouth.

After the second or third gill arch of an anaesthetized fish had been tied off to prevent excessive blood loss, a filament was carefully removed and fastened to a cork plate (Fig. 3). Secondary lamellae to be measured were separated using a human hair. The preparation was then placed in a dish which contained sea water and held at an inclination of approximately 60°. A stream of sea water was directed through a hypodermic needle at the selected lamella, flattening it against its neighbours and making its total height visible. The shape of this secondary lamella as well as two reference lines was traced three times using a dissecting microscope fitted with a *camera lucida*.

Bouin's fluid was then allowed to enter the inflow tube, replacing the sea water and fixing the traced lamellae *in situ*. After remaining overnight in Bouin's fluid, the tissue was placed in 50% ethanol for at least 14 h. Three more tracings of the same secondary lamella were then made, taking care that the reference lines matched those of the previous tracing.

The tissue and carriers were then dehydrated through graded alcohols and cleared in xylene, and paraffin wax was allowed to infiltrate overnight, as with the experimental tissue. It was then cleared in xylene in order to allow a third set of tracings. The total area of each set of three tracings was determined by point counting (Weibel, 1979) and the percentage shrinkage and ratios of surface areas in the various states were calculated.

It was found that the results of these methods varied in different parts of the gills. Furthermore, non-uniform fixation of secondary lamellae produces curling and consequently an overestimate of their shrinkage based on projected area. Non-uniform tissue distortion is even more exaggerated during further processing with

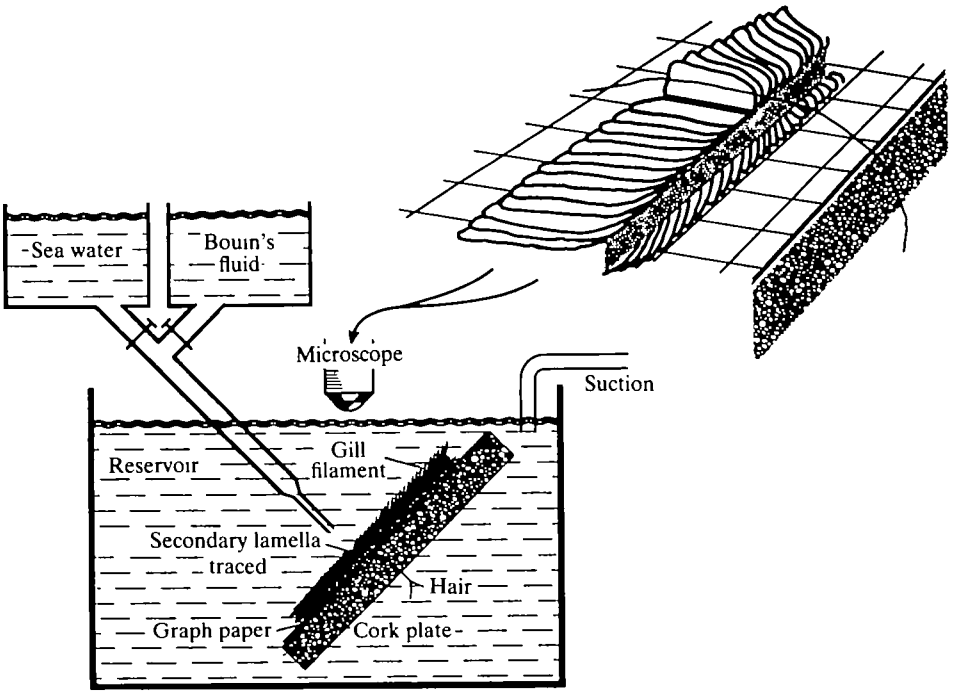

Fig. 3. Arrangement for measurement of single secondary lamellae attached to filament. For description see text.

paraffin embedding. As curling alone would not result in any reduction in lamellar area or diminution in intralamellar distances (i.e. water–blood distance or secondary lamellar thickness), the assumption was made that the values obtained for secondary lamellar shrinkage by these procedures were too large. This conclusion was further supported by comparison of mean values from paraffin-embedded tissue with those from semithin sections (1 μm) after fixation and embedding for electron microscopy. Although these sections produced only a small number of measurements from three animals, water–blood distances obtained were similar to those derived from paraffin sections after correction for Holmes effect and geometric error alone, i.e. without any correction for shrinkage.

From these studies it was concluded that there was negligible shrinkage of Bouin-fixed/alcohol-stored material and that the linear shrinkage for the paraffin-embedded material was 11%. The latter is in good agreement with the 15% linear shrinkage observed for paraffin-embedded lizard lungs (Perry, 1983). Paraffin sections rarely regain their original dimensions after sectioning but tend to remain contracted in the direction of the section by approximately 25%. Since this error only applies in the x or y direction but not both, the mean apparent shrinkage in area is $25/2 = 12{\cdot}5$ %.

It was therefore concluded that shrinkage and sectioning artefacts together could amount to a total systematic underestimate of linear dimensions in paraffin-sectioned material by as much as 20–30% (i.e. $11+12{\cdot}5 = 23{\cdot}5$%), and a 25% value has been applied in the present study (Table 3). For the Bouin-fixed/50% ethanol-stored

material used to determine secondary lamellar area, uncorrected data are presented but corrections have been carried out for other calculations such as those concerning dimensions of the interlamellar spaces. One difficulty in determining secondary lamellar area is the removal of whole individual lamellae since they are longest at their attachment to the filament. The magnitude of potential underestimates by incomplete removal could be substantial, although it is reduced if complete sections are made across individual filaments as in many investigations (e.g. Hughes, 1966, 1984*a*; Muir & Hughes, 1969). Furthermore, it has been accepted for some time that there are differences in the proportion of the pillar cell system which may be exposed to the water under different conditions, and in rainbow trout this has been confirmed (Part, Tuurala, Nikinmaa & Kiessling, 1984; Tuurala, 1983). For these reasons the uncorrected values given in the present study should be interpreted as a minimum estimate of secondary lamellar area. To correct the data to give maximum values 30% should be added to the lamellar area measurements to compensate not only for shrinkage in Bouin and 50% ethanol but also retraction of the pillar cell system from the lamellae into the filament.

Corrections for non-perpendicular sectioning

Although an attempt was made to cut all secondary lamellae perpendicular to their flat surface a deviation of up to 10° must be expected. When plate-like structures are not cut perpendicular to their plane the thickness is overestimated. The effects are as follows.

(1) *Slant effect*. The apparent length (l_{app}) is increased from the true length (l_o) depending on the angle of deviation from the perpendicular (φ):

$$l_{app} = l_o/\cos\varphi. \quad (1)$$

(2) *Holmes effect*. Thickness of opaque structures is overestimated when they are not cut perpendicularly because of the finite thickness of the section. This overestimation (l) is independent of thickness of the structure but proportional to section thickness (t) and to the tangent of the deviation angle (φ):

$$l = t\tan\varphi. \quad (2)$$

The thickness of transparent structures (e.g. interlamellar spaces) is reduced by the same amount.

(3) The *combination* of these two effects yields the following correction formula for opaque structures:

$$l_o = l_{app}\cos\varphi - t\tan\varphi. \quad (3)$$

In the present study it was assumed that the histological sections included deviations as large as 10° from normal and the mean deviation angle was 5°. Thus, for 5-μm sections the corrected l_o is:

$$l_o = 0{\cdot}996\, l_{app} - 0{\cdot}44, \quad (4)$$

where l_o and l_{app} are in μm.

RESULTS

Filament length

As in most elasmobranchs only a single hemibranch (posterior) is present on the first arch, while arches 2–5 have both anterior and posterior hemibranchs. For the second and third arches, filaments of the posterior hemibranchs are longer than those of the anterior hemibranch (Fig. 4). However, this relationship is reversed for the last arch, which is also characterized by a pronounced change in filament length along the hemibranch. Differences in filament length are related to the orientation of the gill arches in the branchial system and serve to ensure the correct juxtaposition of the tips of the filaments of the two hemibranchs in a given gill slit. In this way, water passing from the orobranchial to parabranchial cavity tends to flow between the filaments rather than be shunted past the filament tips (Hughes, 1972; de Vries & de Jager, 1984).

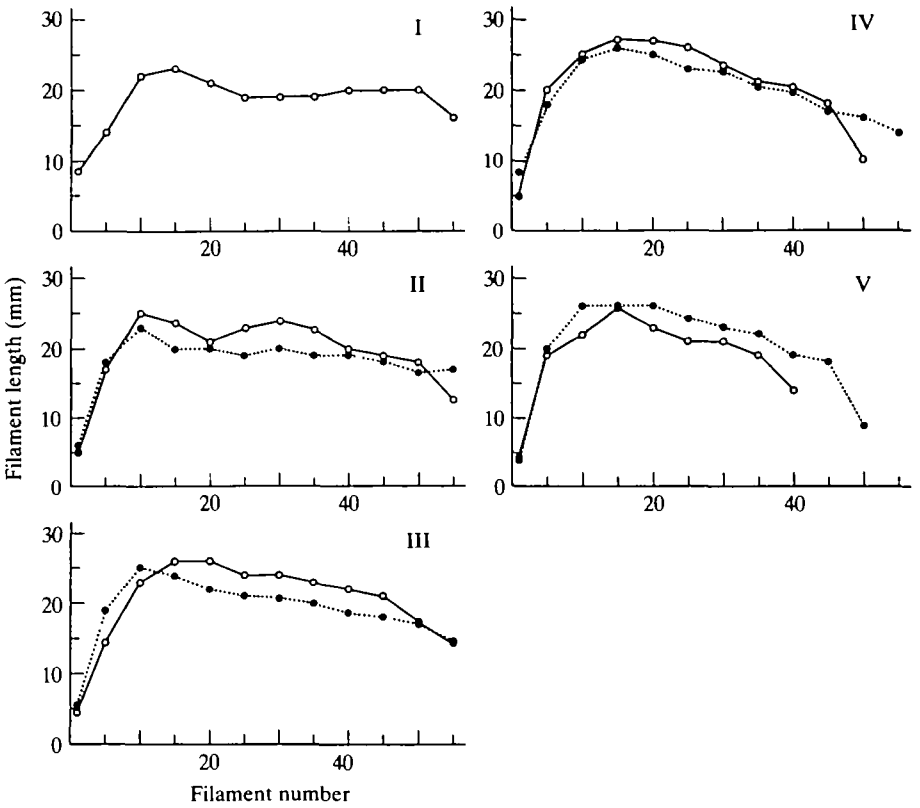

Fig. 4. Diagrams to show distribution of filament length along the gill arches (Roman numerals) of a specimen of *Scyliorhinus stellaris*. Open symbols (and continuous lines) indicate filaments of posterior hemibranchs, closed symbols (and dotted lines) are for anterior hemibranchs.

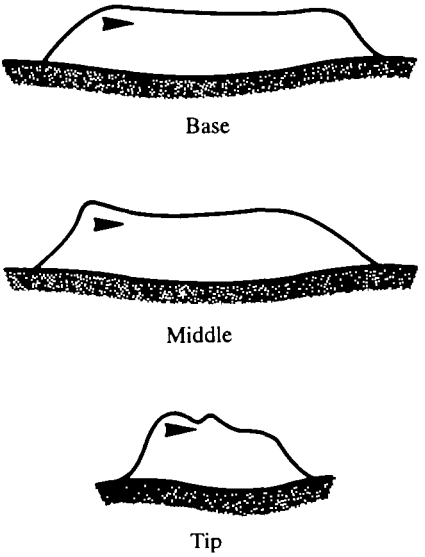

Fig. 5. Typical profiles of the secondary lamellae from three regions of a single filament. The arrowheads indicate water flow direction.

Shape and distribution of secondary lamellae

The shape and dimensions of the secondary lamellae vary with their position along each filament (Fig. 5; Table 1). Lamellae at the tip of the filaments are shorter and higher than at the base. The surface area of individual secondary lamellae appears to be maximal in the middle portion of each filament.

Gill surface area

The measurements of total gill surface area and its component parameters were carried out on the Bouin-fixed, alcohol-stored specimens (Table 2). Values refer to the total gills of the whole fish, although in most cases only gills from the right side were measured. As in studies on other species, control measurements showed very good agreement between values obtained for gills of the right and left side of the same

Table 1. *Measurement of maximum length and average height of secondary lamellae from 11 fish, mean body mass 1·37 kg and, in parentheses, for a single specimen of 2·47 kg*

Position on filament	Maximum length [Range] (mm)		Average height [Range] (mm)	
Base	1·59 [1·2–2·0]	(1·65)	0·35 [0·2–0·5]	(0·40)
Middle	1·61 [1·2–2·4]	(2·4)	0·41 [0·2–0·6]	(0·45)
Tip	1·30 [0·7–1·5]	(1·55)	0·48 [0·3–0·9]	(0·60)
Mean	1·50	(1·87)	0·41	(0·48)

specimen. Results for variables summarized in Table 2 are plotted against body mass on double-logarithmic coordinates in Fig. 6. This analysis is based on the allometric relationship:

$$Y = a\,W^b \tag{5}$$

$$\log Y = \log a + b \log W, \tag{6}$$

where Y is the variable considered and plotted on the ordinate (Fig. 6), W is the wet body mass of the whole fish, a is the value for Y when W = 1, and b is the allometric exponent representing the slope of the regression lines in Fig. 6. The lines have been fitted by the method of least squares and values for standard deviation of the slope (b) are given in Table 2B. Examination of this Table and Fig. 6 reveals the following.

(1) Total length of the gill filaments increases much less than directly proportionately to body mass (b = 0·365). This increase is due to the increase in mean length rather than in the number of filaments.

Table 2. A. *Measurements of gill parameters for 13 specimens of* Scyliorhinus stellaris, *Bouin-fixed and stored in 50% ethanol. Not corrected for shrinkage (see text).* B. *Results of regression analyses on the basis of the allometric equation, log Y = log a + b log W [Y, the variable; W, body mass in kg; b, allometric exponent (slope); a, Y value for W = 1 kg]; r, correlation coefficient; S_b, standard deviation of slope (b)*

	No.	Body mass (kg)	Filaments: Total number	Filaments: Total length (m)	Secondary lamellae: Frequency (mm^{-1})	Secondary lamellae: Average area (mm^2)	Total area (cm^2)	Remarks
A	1	0·585	914	8·35	10·39	0·635	1102	
	2	0·630	964	9·11	9·55	0·633	1102	
	3	0·730	942	8·77	9·77	0·553	947	
	4	0·750	966	9·17	10·03	0·577	1057	
	5	0·850	956	9·78	9·70	0·586	1111	
	6	1·033	922	9·11	10·21	0·755	1405	
	7	1·033	962	10·87	9·50	0·612	1264	*
	8	1·090	912	9·35	10·70	0·618	1235	*
	9	1·500	978	11·99	9·44	0·686	1553	
	10	1·760	986	13·79	8·13	0·953	2136	RS
			970	12·39	8·74	0·995	2155	LS*
	11	2·150	932	15·07	7·59	2·087	4778	†
	12	2·470	988	13·91	7·44	1·264	2617	RS+LS*
	13	2·615	958	14·53	8·33	1·523	3700	
B		*r*	0·50	0·95	0·80	0·88	0·94	
		a	951	10·13	9·55	0·698	1350	
		b	0·028	0·365	−0·167	0·580	0·779	
		S_b	0·014	0·036	0·039	0·096	0·088	

RS, right side; LS, left side; RS+LS, both sides.
* Fish used for analysis of sectioned material.
† Fish omitted from regression analysis given in B.

(2) The increase in filament length is associated with a decrease in secondary lamellar frequency (b = −0·167). Thus the total number of secondary lamellae increases only slightly with increase in body mass (b = 0·198).

(3) The total gill surface area increases with body mass to the 0·779 power. This is mainly due to an increase in the mean area of individual secondary lamellae (b = 0·580), the increase in the number of secondary lamellae contributing to a lesser extent (b = 0·198).

Dimensions of gas exchange unit

The mean results of various measurements on paraffin-embedded material are presented in Table 3. All these values have been corrected for the Holmes effect, section slant and shrinkage.

Since the secondary lamellar thickness shows no correlation with body mass, but the unit spacing (d′ = thickness of a secondary lamella plus an interlamellar space) obtained as the reciprocal of secondary lamellar frequency, tends to increase, the interlamellar distance also appears to increase with body mass.

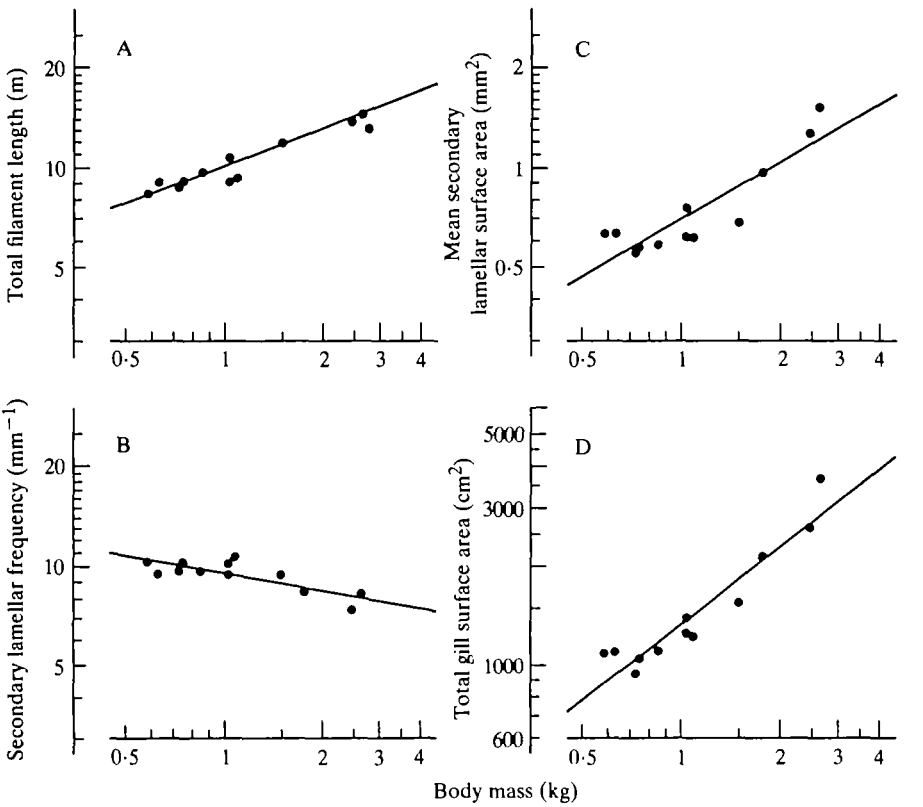

Fig. 6. Double-logarithmic plots of the total gill surface area (D) and its component parameters (A–C) against body mass. Each point represents one fish using values (uncorrected) given in Table 2. The slopes of the lines equal to 'b' of equations 5 and 6 are: A, 0·365; B, −0·167; C, 0·580; D, 0·779.

The frequency distribution of secondary lamellar thickness and of the water–blood distance is represented in Fig. 7. The asymmetry and tailing towards higher values, showing a small second peak for the water–blood distance but not for the secondary lamellar thickness, is due to the presence of thickened areas. On the average, only about 11% of the epithelium was found to be thickened. This asymmetry of distribution brings about a considerable difference between the arithmetic and harmonic mean values.

DISCUSSION

Critique of methods

Use of Bouin-fixed material and paraffin sections

Gill surface areas have often been determined using Bouin-fixed material, sometimes after extensive storage in ethanol. Of the standard histological fixatives, Bouin's is believed to cause least tissue distortion without undue hardening. Extensive alcohol storage increases shrinkage (Hughes, 1984*a*). The only difference introduced in the present study was storage in 50% ethanol rather than 70% ethanol. The use of light microscopy of sectioned material for measurement of secondary lamellar thickness and water–blood distances has been found suitable in other studies (Hughes & Perry, 1976). Light microscopic examination has the advantage over electron-microscopic investigations in that it allows a sampling of a greater portion of the material; in the present work complete sectioning of the portions of gill filaments from the tip, middle and base. Unfortunately, in this study insufficient suitably embedded material was available to allow a complete dependence on semithin sections. Nevertheless those measurements made have fully confirmed the results obtained using paraffin wax sections, provided suitable correction is made for the extensive contraction artefacts which occur during such treatments. As a result of these investigations it is recommended that paraffin-sectioned material should not be relied upon if at all possible.

Table 3. *Dimensions of gill elements in* Scyliorhinus stellaris *after measurements in paraffin sections*

Fish no.	Body mass (kg)	Secondary lamellar thickness	Water–blood distance Arithmetic mean	Water–blood distance Harmonic mean	Unit spacing	Inter-lamellar distance	Blood space width
7	1·033	37·3	12·5	8·2	113·7	76·4	12·3
8	1·090	44·6	15·8	11·9	101·0	56·4	13·0
10	1·760	38·5	13·3	9·3	128·0	90·5	11·9
12	2·470	36·1	11·2	8·3	145·3	109·2	13·7
Mean	1·590	39·1	13·2	9·4	122·0	83·1	12·7

Values corrected for Holmes effect, slant and shrinkage.
All values in μm.

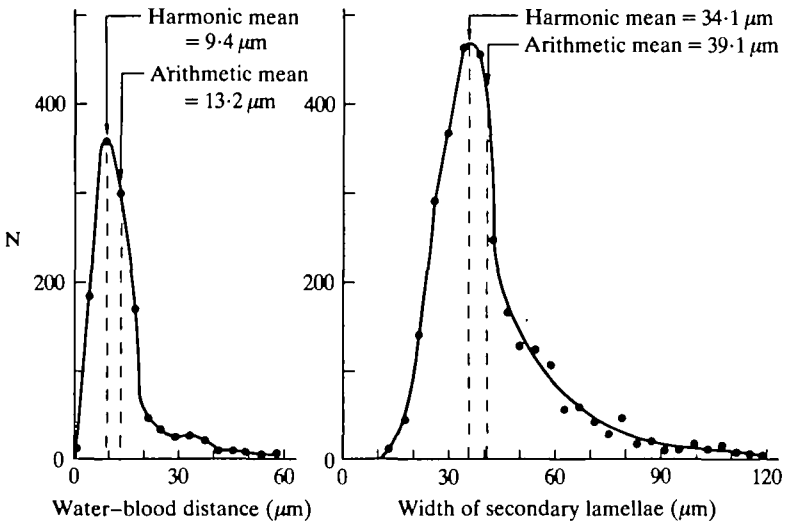

Fig. 7. Frequency distribution of corrected values for water–blood distances and secondary lamellar thickness. N is the number of occurrences in a class. Summated measurements of specimens listed in Table 2.

Determination of secondary lamellar area

Total area of the secondary lamellae on one side of the fish was estimated as in previous studies and multiplied by 2 in order to give the figure for the whole fish. Control measurements on both sides, however, showed that, as in most free-swimming species, there is no significant difference between the two sides. Values given in Table 2 and plotted in Fig. 6 are uncorrected and therefore comparable with those in most other studies. They represent minimum values, whereas corrected areas (= uncorrected+30%) are considered to be maximum values for this species. It is important to stress that all the corrections given in this study are only applicable to the present material. Appropriate correction factors could be calculated for other gills using comparable methods.

On the basis of the present measurements, estimates of the diffusing capacity have been made and give values of 3·87–4·42 μmol min^{-1} Torr^{-1}. Such values are somewhat higher than those obtained by physiological methods (Piiper *et al.* 1977; Scheid & Piiper, 1976). However, these determinations are only for the structural barrier between the water and blood and take no account of the resistance to gas transfer which resides in the water. The latter has been shown to form a significant portion of the overall resistance (Hills & Hughes, 1970; Scheid & Piiper, 1971, 1976) and the new morphometric data obtained in this study have stimulated a renewed analysis using methods similar to that used by Scheid & Piiper (1971). A major feature of this new analysis (J. Piiper, P. Scheid, S. F. Perry & G. M. Hughes, in preparation) is the much closer agreement between the morphometrically determined and physiological values for overall diffusing capacity, especially during exercise.

Comparison with data on other fishes

Present data are in essential agreement with those for a 795-g dogfish (*Scyliorhinus canicula*; G. M. Hughes, unpublished data, cited by Piiper, 1971). The gill area of the spiny dogfish (*Squalus acanthias*), however, is double that predicted by the present study. This discrepancy lies in the large size of the individual secondary lamella in *Squalus* reported by Boylan (1967). Previously reported values for water–blood distance, 9·6 μm in *Scyliorhinus stellaris*, 11·3 μm in *Scyliorhinus canicula* and 10·1 μm in *Squalus acanthias* (Hughes & Wright, 1970) are similar to those in the present study (9·4 μm in Table 3). Such thick diffusion barriers are seen among teleosts only in species which are inactive bottom dwellers (e.g. *Ameiurus* = *Ictalurus nebulosus,* Steen & Berg, 1966) or which possess accessory respiratory organs (e.g. *Anabas testudineus*, Hughes & Munshi, 1968).

It appears that in *S. stellaris* the water–blood barrier is relatively thick and total gill area fairly small, and consequently the diffusing capacity less than in most fish species. This accords with the normal life habits of these fish which spend much of their time ventilating rhythmically at rest on the sea bottom. When swimming, many elasmobranchs obtain 'extra' ventilation because of the ramjet component (Hughes, 1960), and in most active sharks this forms the main mechanism of gill ventilation. It is under such conditions that physiological diffusing capacity can be expected to be highest and close to the morphometrically-determined values. At rest, however, there is incomplete ventilation of the gill pouches, at least in *Scyliorhinus canicula*, and this heterogeneity is a further factor which would reduce the resting physiological diffusing capacity of the whole gill system (Hughes, 1973).

The gill area of a 1-kg specimen of *S. stellaris* is very clse to the ray (*Raia clavata*) but is almost twice that (76 $mm^2 g^{-1}$) for *Torpedo marmorata* and the latter has a very low resting $\dot{V}_{O_2}$. As a consequence, the ratio $\dot{V}_{O_2}$/area is very similar (Hughes, 1978) for these three species and also for *S. canicula* in which the gill area (200 $mm^2 g^{-1}$) is greater than that of the other three elasmobranchs.

On this basis we can suppose that *S. stellaris* is a fish of activity intermediate between that of *S. canicula* and *T. marmorata* and is similar to that of *R. clavata*. Of these four species, *S. stellaris* is the only one in which the slope of the regression line for gill area *vs* body mass is less than 0·9. Perhaps this suggests that large *S. stellaris* are less active relative to the other three species.

With respect to the allometric analysis the general nature of the results is similar to that obtained for other fishes. As would be expected from the foregoing discussion, the elevation ('a' value) of the gill area line is lower than that of most fishes (Hughes, 1984*b*). The relationship for total gill surface area with a slope of about 0·78 is well within the range (0·5–1·0) found for fish gill areas (Hughes, 1984*a,b*) and its component parameters have similar slopes to those of many other fish species. However, the slope of the gill area line of three other elasmobranchs seems to be close to 1·0, the difference being mainly due to the area of individual lamellae (Table 4).

The present study probably represents a more complete determination of the dimensions of the gill sieve in relation to the gas exchange function of the secondary lamellar surface than has been achieved for any other fish species. In the course of the

Table 4. *Slope (b) of regression analyses for gill area and component parameters of four species of elasmobranch*

	*Raia clavata**	*Torpedo marmorata*†	*Scyliorhinus canicula*‡	*Scyliorhinus stellaris*§
Total area of secondary lamellae	0·97	0·937	0·961	0·779
Bilateral area of average secondary lamellae	0·713	0·706	0·684	0·580
Secondary lamellae mm^{-1}	−0·154	−0·167	−0·071	−0·167
Total filament length	0·40	0·397	0·351	0·365

* Hughes, 1977.
† Hughes, 1978.
‡ Hughes, 1972.
§ Present study.

work many problems have been shown to exist which make it difficult to obtain reliable data concerning these dimensions in the living, unfixed condition. It is only by repetition of studies of this kind using other species that a more complete understanding can be obtained of the functioning of fish gills in which not only the structural parameters but also the flow conditions on either side of the water–blood interface must be taken into account.

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