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THE DIMENSIONS OF FISH GILLS IN RELATION TO THEIR FUNCTION

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INTRODUCTION

It is known from the extensive measurements of Gray (1954) that the gills of marine fishes of the Atlantic Coast of North America show a wide variation in the extent of their gill area. This is true whether the area is based on the unit of body surface area or on a unit of body weight. Little data are available, however, for fishes from European waters (Byczkowska-Smyk,* 1957, 1958, 1959). The present investigation was undertaken because earlier work (Hughes & Shelton, 1958) suggested that the nature of the gill resistance is an important variable in the ventilating mechanism. Therefore, as part of a comparative study of the respiratory mechanism of marine fishes (Hughes, 1960) the opportunity was taken to make certain measurements of the gills which might give some indication of the variations in gill resistance of the species studied. From these measurements it was also possible to calculate the gill area. Because of the extremely fine dimensions of the gill sieve some have doubted whether the respiratory current flowed between the secondary lamellae. This is especially relevant as the mean differential pressure across the gills of many fish has been shown to be under 0·5 cm. water (Hughes, 1960). In addition to the British marine fishes studied, data are also presented from two freshwater fishes and from the gills of two specimens of the Antarctic icefish, *Chaenocephalus*, which is of great interest because haemoglobin is absent from its blood. Consequently its oxygen-carrying capacity is only 0·67 vol. %, whereas the blood of other fish in these regions have O_2 capacities of 6–7 vol. % (Ruud, 1954). Some of the data have already been published (Hughes & Shelton, 1962).

MATERIALS AND METHODS

The gills were dissected from the fish and some measurements made immediately on the fresh gills of one side. Later measurements were carried out on material fixed in Bouin's solution and transferred to 70% alcohol after 6 hr. In this way it was possible to make some allowance for any shrinkage which occurred as a result of fixation but it was found to be only slight. All measurements were made under a binocular microscope using a micrometer eyepiece. The total number of filaments on each gill arch was counted and the length of every tenth one was determined. The spacing of the secondary lamellae was measured on several filaments from each of the gill arches but it was found that variations in this parameter were small for a given

* Byczkowska-Smyk (personal communication) has intimated that the figures for gill areas given in these papers are up to ten times too great, in consequence of errors in the estimation of the area of the individual secondary lamella.

specimen, and in fact for a given species. The area of the secondary lamellae is not so constant, being larger for those lamellae nearest the base of the filaments. As a sampling method, the areas of a number of secondary lamellae from different levels of a given filament were measured; the measurement was repeated on filaments from different gill arches. The shape of the secondary lamella was traced on graph paper by means of a camera lucida and its area was determined by counting the number of squares, excluding those which formed the axis of the filament. The average of these areas was doubled to give the total surface of a standard secondary lamella. Multiplying this figure by the total number of secondary lamellae gave a value for the gill area of the whole fish whose weight was measured. No attempt was made to determine the surface area of the fish.

The fixed material was also sectioned and stained for histological examination. Sections were made in the three planes at right angles to one another, namely, (*a*) transverse to the axis of the branchial arch, and (*b*) longitudinally through the arch so as to cut the filament also in longitudinal plane, and (*c*) so as to cut the filaments transversely. These sections gave further material for the confirmation of some of the measurements made on the fresh or fixed material. It was particularly easy to measure the frequency and dimensions of the secondary lamellae from these sections but allowance had to be made for shrinkage. Most of the sections were stained in Azan but in some cases iron haematoxylin or acid haemalum were found useful.

A detailed account of the histological findings is not given here because a subsequent electron microscopy study (Hughes & Grimstone, 1965) has proved to be of greater value.

RESULTS

(1) *Equation for the gill area*

In order to discuss the different ways in which the gill area of fishes may vary it is useful to express this area in terms of the measurements which can easily be made. The dimensions (measured in mm.) are shown in Fig. 1 which indicates diagrammatically a small part of the sieve of two gill filaments. If it is assumed for this purpose that the secondary lamellae of adjacent filaments lie opposite to one another, then the individual pores become rectangular in cross-section and their height is equal to twice the height ($\frac{1}{2}b$) of an individual secondary lamella.* It is clear that the total surface area of each secondary fold equals bl. The total length (L) of all the filaments gives an indication of the size of the sieve as a whole. The relationship between the spacing ($1/d'$) of the secondary lamellae and the distance (d) between individual secondary folds varies because of difference between species in the thickness of the folds; but in general $1/d'$ gives a good indication of the fineness of the sieve. From the spacing of the secondary lamellae (remembering that they are found on both sides of a filament) and the filament length (L), their total number may be obtained and hence

$$\text{the total area of the secondary lamellae} = (2L/d')bl.$$

Clearly the total number of pores (N) and their individual dimensions are important parameters when considering the rate and volume of flow through the sieve.

* The leading edge of each secondary lamella is usually higher than the rest of the lamella (Fig. 1), but in all cases a mean figure has been used.

(2) *The gill areas of some marine and freshwater teleosts*

From the first it became clear that the values obtained were in substantial agreement with the areas obtained by Gray (1954) for fishes of similar modes of life. It was therefore decided not to study such a large number of individuals or of species as he had done but rather to pay more attention to variations in histological structure and to investigate the ways in which fishes of different species can have similar gill areas because of variations in different parameters and to consider this in relation to the

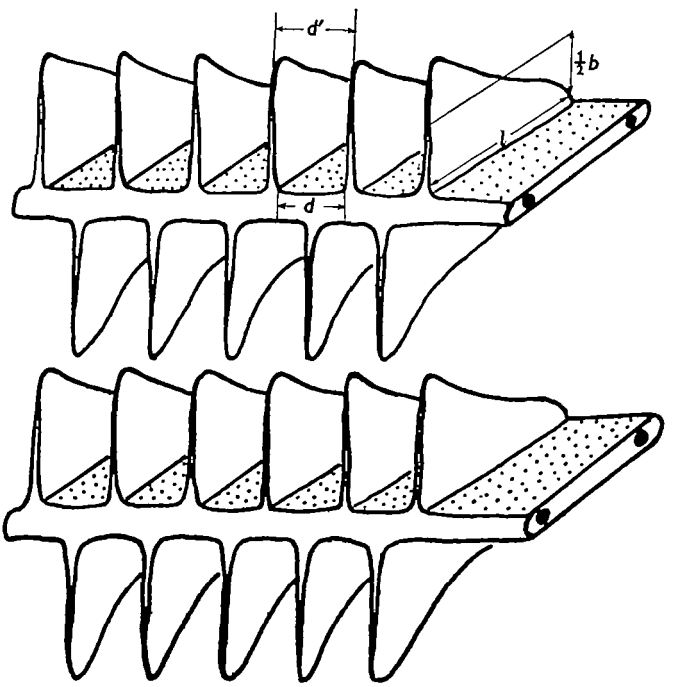

Fig. 1. Diagram of a part of two adjacent gill filaments to show the dimensions measured. The secondary lamellae shown above and below the plane of each filament. Notice the higher leading edge of each secondary lamella.

mechanism of gill ventilation. In Table 1 are collected some of the data obtained on fourteen species and it will be seen that the gill areas range from about 150 to 1200 mm.^{2}/g. This range is less than that obtained by Gray, but the present study does not include the mackerel and other comparable fishes in which he found the largest areas. As in the case of the North American species a clear relationship exists between gill area and the presumed activity of the fish.

The measurements on the specimens of *Chaenocephalus* have shown that its gill area/g. is about average, but it is distributed in such a way that the resistance to water flow is less than in any other fish studied. This is mainly because of the wide spacing of the secondary lamellae (12/mm.), but also because of the large cross-sectional area of the whole sieve, even allowing for the larger size of these specimens. When compared with a specimen of *Lophius* which was twice the size but only of two-thirds the

Table 1. *Gill areas and the dimensions that were measured (the fish are mainly arranged in descending order of gill areas)*

	Weight (g.)	Filaments on both sides (×4)	Average filament length (mm.)	Total length (mm.) of all filaments (L)	Secondary lamellae permm. 1/$\bar{d}'$	Distance between secondary lamellae (d)	Surface area of a sec. lamella (mm.²)	Max. length of sec. lamella (l) mm.	Ave. height of sec. lamella ($b/2$)	Total surface area (mm.²/g.)
Trachurus trachurus	12	412	2·37	3,920	38	0·017	0·03	0·18	0·07	745
	40	420	4·18	7,035	39	0·017	0·06	0·25	0·10	822
	125	478	6·16	11,777	38	0·017	0·15	0·70	0·20	1,074
	135	463	6·14	11,305	39	0·02	0·17	0·75	0·15	1,110
Clupea harengus	85	308	4·46	5,490	33	0·018	0·15	0·60	0·17	845
Gadus merlangus	51	271	3·18	3,450	21	0·03	0·15	0·60	0·20	426
Onos mustela	20	149	2·39	1,428	26	0·023	0·082	0·45	0·15	302·5
	80	135	4·66	2,513	20	0·03	0·2	0·55	0·27	251
Crenilabrus melops	65	198	2·96	2,360	21	0·04	0·22	0·85	0·30	336
Salmo trutta sp.	175	324	5·44	7,048	21	0·023	0·2	0·70	0·20	339
Tinca tinca	140	335	8·00	10,720	25	0·025	0·1	0·86	0·10	383
Chaenocephalus sp.	750	363	11·96	17,378	12	0·053	0·8	1·60	0·50	445
	790	391	9·84	15,386	12	0·057	0·84	1·50	0·55	393
Lophius piscatorius	1550	385	13·31	20,500	11	0·07	0·50	1·10	0·40	143
Pleuronectes platessa	86	218	5·08	4,426	20	0·04	0·21	0·75	0·20	433
Zeus faber	300	356	4·97	7,075	15	0·04	0·25	1·10	0·11	177
Trigla gurnardus	17·8	308	1·84	2,270	22	0·02	0·04	0·35	0·06	224
Cottus bubalis	40	150	2·86	1,716	16	0·04	0·33	0·80	0·30	453
	52	153	3·00	1,835	16	0·04	0·22	0·80	0·25	250
Callionymus lyra	64♂	124	2·86	1,418	15	0·05	0·43	0·80	0·35	292
	46♂	114	2·56	1,169	16	0·03	0·22	0·70	0·25	198
	24♀	120	1·77	848	17	0·047	0·14	0·60	0·15	168

gill area, it is apparent that the gill area of *Chaenocephalus* is relatively large. In fact it is comparable in weight and gill area with the sea trout (Gray, 1954). The latter fish, however, has 27 secondary lamellae/mm. and hence the same area can be accommodated in a smaller space but the resistance is higher. Apart from the lowered gill resistance, examination of the gills of these fish lacking haemoglobin has not suggested any striking adaptation of their respiratory pumps, but this needs to be established experimentally. Because of the low oxygen capacity of their blood it is possible that there are adaptations whereby the volume of blood flowing through the gills is relatively high as this will equalize the capacity rates of the two exchanging fluids.

The larger gill area of more active fish is probably related to their need for greater oxygen consumption. This extra oxygen could be obtained by an increase in the volume of water passing through the gills (Vw), or by increasing the effectiveness of the transfer of oxygen from the water to the blood which would be achieved by increasing the number of transfer units (gill area $\times$ a coefficient which depends upon the diffusion conditions; Hughes, 1964). As the percentage utilization of oxygen seems to be high even in less active fish it seems very probable that the greater oxygen consumption must be associated with an increase in the rate of water flow. The disadvantage of a system depending solely on an increase in flow is that it results in a fall in utilization, probably due to the rising proportion of water which does not pass between the secondary lamellae but is shunted past the tips of the gill filaments (Fig. 4*b*); but even so this may produce a net increase in oxygen absorption. A further factor is that its velocity of flow through the secondary lamellar spaces will be high and there may be insufficient time for diffusion to occur and allow equilibrium to be established between water and blood (Lloyd, 1961). An increased gill area therefore usually means that the total resistance of the sieve will be reduced and ensures that an effective utilization of oxygen can take place at greater ventilation volumes. There are, however, relatively few measurements of ventilation volume in fishes.

In a system as complex as that of fish gills many different ways are possible by which the gill surface might be increased. From the figures obtained in the present work and from those of Gray it appears that the spacing between the secondary lamellae remains relatively constant for a given species, although in developmental studies (Price, 1931) it has been shown that even this parameter changes rapidly during the early stages (Table 2). In general it seems that more active fish have a larger number of filaments which are of greater length, thus giving rise to a greater total filament length and hence a greater total number of secondary lamellae. The secondary lamellae are more closely packed but are smaller in area than those of more sluggish fish. Thus for a fish such as *Callionymus*, average weight about 50 g., the surface of a secondary lamella is about 0·3 mm.^{2}. For a more active fish of twice the size, e.g. the horse mackerel, the area of each secondary lamella is half the size. The very large area of the secondary lamellae in the icefish is particularly striking in this feature, but allowance must be made for the much greater weight of these specimens.

Similar features emerge from an analysis of Gray's figures (Table 3). For example, a toadfish of about 300 g. has a secondary lamellar area of 0·9 mm.^{2}, whereas a mackerel of slightly smaller size has an area of 0·41 mm.^{2}, although its total gill area is nearly eight times that of the toadfish. An exception to this generalization, however, is to be found in the menhaden which, of the fish investigated by Gray, has the largest

Table 2. Micropterus dolomieu (*Lecepede*). *Dimensions of gill sieve for different sizes of fish (after Price, 1931)*

The rate of water flow is calculated from the equation

$$q = \frac{p_1 - p_2}{\eta} \cdot \frac{5d^3b}{24l}.$$

Weight (g.)	Total filament length (L) (mm.)	d' (mm.)	Total no. of pores (N) × 10^4	Pore dimensions (mm.)			Water flow (q) (c.c./pore/ cm. H_2O/ sec.) (× 10^{-4})	Water flow through gills (Nq) (c.c./sec./ cm. H_2O)	Gill area	
				d	$\frac{1}{2}b$	l_{max}			mm.^{2} × 10^4	mm.^{2} /g.
0·332	252	0·0356	0·706	0·025	0·0588	0·133	0·288	0·204	0·024	724
2·71	1,285	0·0346	3·72	0·024	0·0686	0·269	1·472	5·48	0·21	775
25·98	3,409	0·0356	9·56	0·025	0·0808	0·52	0·984	9·40	1·13	436
41·1	4,121	0·0368	11·21	0·026	0·0882	0·63	1·032	11·58	1·69	411
115·72	6,540	0·042	15·56	0·031	0·0882	0·80	1·388	21·6	3·60	311
189·25	8,290	0·0453	18·28	0·032	0·1250	0·83	2·058	37·6	5·08	268·5
288·6	10,010	0·049	20·42	0·034	0·1323	0·92	2·410	49·2	7·10	246
452·0	11,890	0·049	24·22	0·034	0·1617	1·06	2·50	60·6	10·08	239
618·2	14,870	0·056	26·57	0·038	0·1764	1·24	3·262	86·8	14·29	220·5
837·5	16,210	0·0535	30·27	0·036	0·1764	1·46	2·342	71·0	18·89	225

Table 3. *Gill areas and some of the measurements used in their determination for individuals of approximately the same weight*

(Selected from data kindly supplied by Dr I. E. Gray. The species are roughly classified into active, intermediate, and sluggish forms.)

Fish	Activity	Weight (g.)	Total no. of filaments	Total filament length (L) (mm.)	Secondary lamellae per mm.	Total no. of pores (N) ($\times 10^4$)	Average filament length (mm.)	Gill area (mm.^{2})	
								Total per g.	Single lamella
Toadfish	Sl.	305	656	4,752	11	5·2	7·24	151	0·88
		326	720	5,107	11	5·62	7·10	189	0·92
Fluke	Sl.	404	1,706	9,747	19·3	18·8	5·71	247	0·53
Sea Trout	A.	470	2,856	22,650	26	60·6	7·94	?	?
		705	2,908	21,416	28	60·1	7·38	275	0·32
Puffer	I.→ Sl.	326	704	7,587	18	13·65	10·78	423	1·01
Sea Robin	I.	365	1,996	14,022	20	28·04	7·04	432	0·56
Tautog	I.	297	1,548	11,132	17	18·94	7·21	?	?
		466	1,580	12,537	19	23·87	7·94	450	0·88
Butterfish		261	1,548	9,855	32	31·58	6·37	461	0·38
Sheepshead	I.→ A.	544	2,300	17,331	22	38·13	7·54	467	0·67
Scup	I.→ A.	253	2,016	12,277	25·3	31·09	6·12	498	0·40
Mullet	A.	250	2,190	16,399	26·5	43·43	7·49	1,010	0·58
Mackerel	A.	226	2,814	19,271	29·4	56·6	6·84	1,040	0·41
Menhaden	A.	525	2,298	25,138	27	68·9	10·94	1,241	1·02

gill area per gramme. In this very active fish the secondary lamellae are quite large—1·02 mm.^{2} in a 500 g. fish. But even for the menhaden the increase in total filament length and number of secondary lamellae is a very important feature of its gill system.

It is of interest to consider the functional significance of some of these dimensions of the gill sieve. Before this can be done, however, it is necessary to discuss quantitatively the resistance offered by the gills to the flow of water and the conditions for the exchange of the respiratory gases between the water and blood.

(3) *Equation for the flow of water through the sieve*

Poiseuilles's equation for the laminar* flow of water through a tube is usually given in terms of a circular cross-section of radius, r. [$q = (p_1 - p_2 . r^4)/8l\eta$]. The equation for a capillary of roughly rectangular cross-section may be expressed as follows:

$$q = \frac{p_1 - p_2}{\eta} \frac{5d^3b}{24l},$$

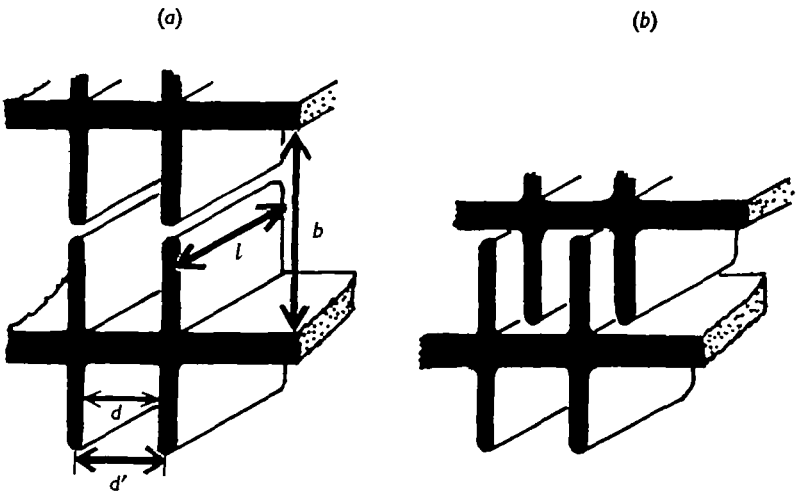

Fig. 2. Diagram of the secondary lamellae attached to two neighbouring filaments of the same gill arch to show the dimensions used in the calculations of gill resistance. In (*a*) the filaments are shown wide apart and with the secondary lamellae opposite one another; in (*b*) the minimum possible size of pores is shown to result from the close interdigitation of the filaments. It is improbable that such close interdigitation is ever found in life.

where p_1 and p_2 are the pressures (dynes/cm.^{2}) on either side of the sieve (i.e. in the buccal and opercular cavities), η is the viscosity (poises) of the fluid, d, b and l are the dimensions (cm.) of the sieve shown in Fig. 2*a*. This gives the flow q, through a single pore in c.c. per second. The total flow through the gills is $Q = Nq$, where N is the total number of pores in the sieve and is equal to the total filament length, L, times the spacing, $1/d'$.

This equation has been applied to the gills of a tench which had been used in experiments to determine the flow of water pumped by the fish per minute. In this

* This is highly probable because the Reynolds number must be less than 10 at such low velocities (Huhes & Shelton, 1962).

equation it is assumed (1) that all the pores of the sieve are equally accessible to the water throughout the whole of the respiratory cycle, and (2) that the pressure gradient across them remains constant and equal to the mean of the differential pressure measured simultaneously. (3) In order to simplify the calculation the shape of each pore has been assumed to be as shown in Fig. 2*a*. In fact the secondary lamellae of adjacent filaments alternate with one another (Fig. 1) and are not opposite as in this diagram.

An example of the use of this equation is given below:

Volume of fluid flowing through a pore of rectangular cross-section, $q = \frac{p_1 - p_2}{\eta} \frac{5d^3b}{24l}$.

$p_1 - p_2$ = 0·5 cm. H_2O = $\frac{981}{2}$, say 500 dynes/cm.^{2}
η = 0·01 poises.
d = 0·025 mm. = $2{\cdot}5 \times 10^{-3}$ cm.
b = 2×10^{-2} cm.
l = $8{\cdot}6 \times 10^{-2}$ cm.

$$q = \frac{500}{10^{-2}} \frac{5(2{\cdot}5 \times 10^{-3})^3 \times 2 \times 10^{-2}}{24 \times 8{\cdot}6 \times 10^{-2}}$$
$$= \frac{78{\cdot}125 \times 10^{-4}}{206{\cdot}4}$$
$$= 0{\cdot}379 \times 10^{-4} \text{ c.c./pore/sec.}$$

Number of pores in gill sieve, $N = 26{\cdot}8 \times 10^4$.

Therefore total flow of water through gills, Nq = 10·13 c.c./sec. Normal volume of water pumped through gills of a tench of this size (150 g.) is 1–2 c.c./sec.

A similar calculation for a 50 g. *Callionymus* gave values of about 20 c.c./sec., whereas the usual volume pumped is about 0·5 c.c./sec. (Hughes & Umezawa, 1967).

Similar calculations of the flow of water through the gills of different sizes of *Micropterus* during its development are given in Table 2. It can be seen that although the gill area increases about 18 times there is only a two-fold increase in the volume of water, q, which could be expected to flow through a single pore for the same differential pressure. It appears that this important feature of the sieve, and hence the resistance per unit cross-section of the whole sieve, remains relatively constant during development. There is, however, some variation in the smaller fish.

In all cases, then, the results of applying the equation (Table 4) have given larger ventilation volumes than those measured experimentally. At first this was an agreeable surprise because the calculations were initiated when it was thought that such small pressures might be inadequate to produce the passage of a sufficient volume of water between the secondary folds. However, the difference—an order of magnitude —between the calculated and expected result demands some consideration of the possible reasons for such a discrepancy.

There are many possibilities, but the most obvious is that a part of the gill sieve is not of the type shown diagrammatically in Fig. 2*a*. This is because of the presence of an interbranchial septum which extends between the filaments of the two hemibranchs attached to a given arch and hinders the flow of water through a significant portion of the sieve. The actual proportion varies in different teleosts and is related to the type of intrinsic gill musculature. In some fish there is a well-defined 'diaphragm'

joining the hemibranchs (Duvernoy, 1839; Bijtel, 1949) so that only the distal half or third of the filaments are sufficiently free to allow the unhindered flow of water across the secondary lamellae. A further point is that the main flow of water is not necessarily at right angles to the sieve because of the orientation of the filaments in the respiratory chambers. This will add to the resistance to flow, as will the presence of gill rakers between the individual arches. Another important feature of the sieve to be considered is the way in which it may modify its position during an individual respiratory cycle. Some of these modifications may be produced passively by the pressure gradient

Table 4. *Total gill areas of the fish used in the present work together with calculations of the flow through the sieve based upon the modified Poiseuille equation*

Fish	Wt. (g.)	Total pores in gill sieve (N) ($\times 10^4$)	Water flow/ pore/cm. water/sec. (q)	Total flow (c.c./sec.) across sieve/cm. water/sec. (Nq)	Total surface area of sec. lamellae (mm.^{2}) $\times 10^4$
Trachurus trachurus	12	14·90	0·80	11·92	0·89
	40	27·44	0·82	22·50	3·30
	125	44·75	0·56	25·06	13·43
	135	44·09	0·67	29·54	15·00
Clupea harengus	85	18·12	0·69	12·50	5·49
Gadus merlangus	51	7·25	3·76	26·26	2·17
Onos mustela	20♂	3·71	1·69	6·27	0·61
	80♀	5·03	5·60	28·17	2·01
Crenilabrus melops	65	4·96	9·41	46·67	2·18
Lophius piscatorius	1550	22·55	5·20	117·26	22·1
Pleuronectes platessa	86	8·85	7·1	62·84	3·72
Zeus faber	300	10·61	2·75	29·18	5·31
Trigla gurnardus	17·8	4·99	0·57	2·85	0·40
Cottus bubalis	40	2·75	10·0	27·5	1·81
	52	2·94	8·33	24·5	1·30
Callionymus lyra	64♂	2·13	22·7	48·35	1·87
	46♂	1·87	4·2	7·85	0·91
	24♀	1·43	10·8	15·44	0·40
Chaenocephalus sp.	750	20·85	19·4	404·5	33·4
	790	18·46	28·2	520·6	31·1
Salmo trutta sp.	175	14·8	0·95	14·1	5·93
Tinca tinca	140	26·8	0·76	20·2	5·36

and the flow of water across the sieve. But it is equally clear that other changes are produced actively by the branchial arch musculature and also the adductor and abductor muscles of the individual filaments. The precise nature of the movements of the gills and of the individual filaments during the respiratory cycle is complex and difficult to investigate under normal conditions. Observations on transparent species of *Gobius* have shown (1) that the gill curtain tends to be maintained despite the abduction of the operculum, and (2) that there is also a rhythmic adduction of the filaments (Hughes, 1961). Similar conclusions were reached for other teleosts by Pasztor & Kleerekoper (1962) who regarded the first type of activity as a mechanism ensuring even ventilation of the whole sieve and the second as a regulatory mechanism during periods of increased ventilation.

This folding-up of the gill fan produces marked increases in the pressure between

the filaments (G. M. Hughes and C. M. Ballintijn, unpublished) and probably assists in the ventilation of the gills.

However, consideration of the modified Poiseuille equation suggests that the adjustments which might be most important are those which affect the thickness, d, of the channels through which the water passes. In this connexion it must be remembered that the presence of a film of mucus is bound to reduce the effective size of these pores. In the calculation given above it has been assumed that the channels are as shown in Fig. 2*a*, but in life the secondary lamellae alternate with one another and may even interdigitate as in Fig. 2*b*. In the extreme case figured, the channels will be reduced in height, b, by half and by more than half in thickness, d. Calculations based upon such a configuration of the gill sieve gives figures for the flow through the gills of the tench of about 4·5–5·0 c.c./min. This minute volume is far less than what has been measured. It is certain that adjacent filaments never interdigitate as completely as has been assumed in this modified calculation. These conditions for which the flow has been calculated represent the two extremes and assume that all the water passes between the secondary lamellae. Hence the minute volume of a 175 g. tench would be expected to fall within the range 5·0–600 c.c. which is confirmed by the direct measurements. If the gill resistance changes during the respiratory cycle by variation in the degree of interdigitation of the secondary lamellae it would provide a very effective means of control. Varying degrees of interdigitation may occur as a result of alterations in the curvature of the branchial arches produced by the adductor branchialis and other gill muscles.

There are, then, many ways in which the gill resistance can vary during the respiratory cycle. The number and dimensions of the channels through which water passes are such that the measured differential pressures are sufficient to maintain the flow of water across the sieve and all of it is brought into close contact with the respiratory epithelium.

(4) *The relationship between gill area and resistance*

We now have two equations based upon the parameters of the gill sieve in relation to (*a*) the total gill area and (*b*) the resistance to the flow of water. They are:

$$\text{Total area, } A = (2L/d')bl = L(2bl/d'). \tag{1}$$

$$\text{Total flow, } Q = \frac{L}{d'}\,\frac{p_1-p_2}{\eta}\,\frac{5d^3b}{24l} = LK\frac{d^3b}{d'l}. \tag{2}$$

The only measurement of the gill sieve which is different in the two equations is that of d' and d, but these two are clearly related to one another so that equation (2) may be approximated to

$$Q = LK(d^2b/l) \quad \text{where} \quad K = \frac{5(p_1-p_2)}{24\eta}\,\frac{d}{d'}.$$

It is apparent that when one of these parameters is altered the effects upon the flow and upon the area are not always in the same direction. For example, if there is an

increase in the total number of pores ($N = L/d'$) there will be an increase in the gill area but the flow will only be greater if the increased gill area is produced by a greater total filament length, L. An increase of N, while keeping L constant, can only be achieved by decreasing d' which will increase the gill resistance and hence decrease the flow.

We may summarize the effect of the main parameters on the area, A, and on the total flow, Q, as follows:

(a) increase in $L \rightarrow$ increase of A and Q;
(b) increase $b \rightarrow$ increase of A and Q;
(c) increase of $l \rightarrow$ increase A, decrease of Q;
(d) increased $d \rightarrow$ decrease A, increase of Q.
(or decrease $1/d'$)

From the respiratory point of view any increase in the gill area should not be accompanied by too great an increase in the resistance to flow as this would further load the respiratory muscles. Evidently it can best be achieved by having longer filaments, each having longer secondary lamellae, i.e. increase of L and b. Both of these increases in dimension require greater space within the head of the fish and no doubt this places some limit upon them.

(5) *Conditions for diffusion in fish gills*

As the oxygen entering a fish from the water must do so through the gills (except in species where cutaneous respiration plays a large part), it is clear that the gill epithelium and its relationship to the water provides the first site in the respiratory chain which limits the capacity for gaseous exchange, and this factor ultimately determines the fish's ability to provide energy for its activity. The morphology of the gills strongly suggests that the conditions for exchange are extremely good, for not only is there a very large area but it is so organized that the water is brought into very close contact with the whole respiratory surface. The diffusion pathway from the water to the haemoglobin in the blood corpuscles is made up of the following parts:

(1) Diffusion in the water to the gill surface.

(2) Diffusion across the respiratory interface (i.e. water-blood pathway made up of epithelial layer, basement membrane, and flange of pillar cell (Hughes & Grimstone, 1965).

(3) Diffusion within the blood plasma;

(4) Diffusion across the red blood cell membranes and to the haemoglobin molecules.

Because of the nature of its flow across the gill there is a continuous renewal of the water between the secondary lamellae which provides a more or less constant head of oxygen pressure. The maximum length of the diffusion pathway may therefore be taken as from the centre of the space between the two secondary lamellae to the centre of a blood channel between the pillar cells. Because of the counter flow between the blood and water across the surface of the secondary lamellae, the gradient of partial pressure will tend to remain more nearly constant across the whole exchanger surface and will be approximately the same as that between the water when it reaches the leading edge and the blood leaving in the efferent branchial vessel. In most gills

this leading edge is higher than the rest of the secondary lamella, an adaptation which ensures that the blood in the marginal vessel remains in contact with well oxygenated water for a relatively long time. Furthermore, the remainder of the secondary lamellar surface is not uniform. Byczkowska-Smyk (1957), in estimating the respiratory surface of teleost gills, made allowance for the fact that part of the surface of the lamellae lies above the ends of the pillar cells and cannot be involved in gaseous exchange. In the perch she found that the area of the blood channels in the secondary lamellae formed only 72% of their total area, and in the flounder 63·5%. Estimates for the gills studied in the present work gave figures in the range of 60–70%. These regional differences in the secondary lamellar surface are further complicated because of the more rapid flow of blood in the blood channel along the free edge of the secondary lamella.

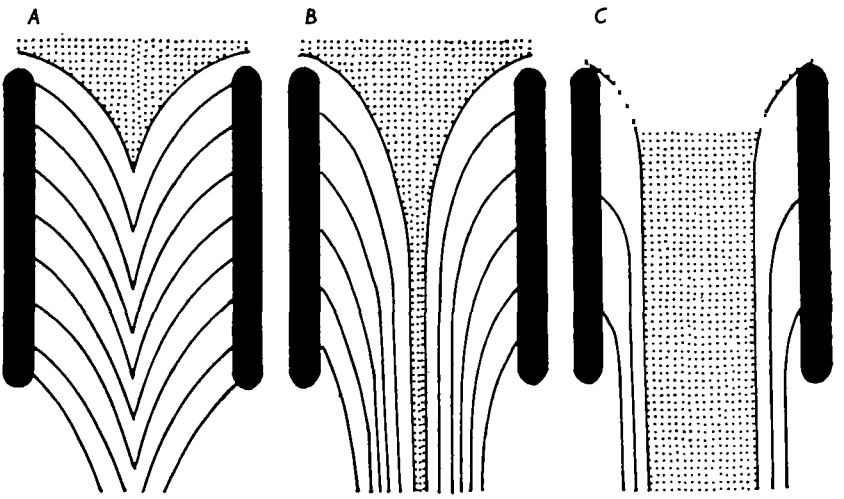

Fig. 3. Diagrams of the flow of water between two adjacent secondary lamellae. The contours of water containing different amounts of oxygen are indicated. The dotted regions represent water from which no oxygen has been removed. *A*, *B* and *C* show the effect of increasing the velocity of water flow, assuming constant conditions in the blood circulation. Alternatively the three diagrams can be regarded as showing the effect of different spacings between the secondary lamellae ($A < B < C$) with a constant velocity of water flow. In the latter case, of course, the scale is not constant.

The relationship between oxygen consumption (R_{O_2} in ml./min.), total respiratory area (A cm.^{2}), oxygen pressure gradient (Δp_{O_2} mm Hg.), diffusion distance (d cm.), and coefficient for diffusion (D) in the water and across the respiratory interface is given below:

D = ml. O_2/min. through 1 cm.^{2} and 1 cm. distance if $\Delta p_{O_2} = 760$ mm. Hg.

Therefore
$$R_{O_2} = \frac{DA\Delta p_{O_2}}{d.760}$$

or
$$d = \frac{DA}{R_{O_2}} \frac{\Delta p_{O_2}}{760}.$$

Values for the constant D given by Krogh (1941) are 0·000034 for water, and 0·000011 for connective tissue. About one-third of the diffusion pathway is in tissue, and so a reasonable approximation for the whole pathway is 0·000025.

There will be some variation in Δp_{O_2} across the exchanger despite the counterflow, but a reasonable average figure is 40 mm Hg. Calculations for fish such as the trout and *Callionymus* give values for the oxygen consumption (108 c.c. and 52 c.c./kg./hr. respectively) which are in the range that have been measured. Similar data may also be used to calculate the diffusion distances when the oxygen consumption is known. This distance is almost exactly that from halfway between two secondary lamellae to the centre of the blood space. Evidently the spacing of the secondary folds is ideal for the conditions of ventilation in a given fish for it enables the water to be almost completely depleted of its oxygen. If the spacing were wider relative to the flow of water (Fig. 3*c*) then the utilization would fall. The higher oxygen consumption of a fish must be accompanied by the passage of a larger volume of water across its gills for a given utilization. For this to occur it is necessary for the distance between the secondary lamellae to be shorter in order to reduce the diffusion distance, as is generally found in active fish. As the spacing between the secondary lamellae is wider in sluggish fish, then for the same gradient of oxygen pressure and utilization, the rate of water flow can be less because of the lowered metabolic rate. These observations suggest that it would be of interest to investigate the effect of changing the rate of water flow on the utilization of active and sluggish fish. One would expect a more marked fall in utilization with a widely spaced sieve, assuming that the rate of blood flow remained constant.

(6) *Volume of blood in the gills and its rate of flow*

From the measurements of the total surface area of the secondary lamellae it is possible to estimate the volume of blood contained within these structures and hence the proportion of the total blood volume in the gills at any one time. If we suppose that only about one half of the total surface area of each secondary fold has blood beneath it, and that the thickness of the passages or lacunae through which the blood circulates is the same as the minor axis of the red blood cells, then the volume of blood in a secondary lamella $= \frac{1}{4}blx$, where x is the diameter of a red blood cell. The total blood volume in the gills can therefore be estimated from the measurements of total gill area as the latter equals $L.2bl/d'$. Therefore the total blood volume in the gills is $Lblx/d'2$, because only one side of each secondary lamella is to be taken as the area occupied by the blood, i.e. half the total gill area.

Applying these equations to a mackerel with a gill area of 1000 mm.^{2}/g. and a minimum blood corpuscle diameter of 10 μ we obtain figures of about 0·3 c.c./100 g. fish. Total blood volume measurements of such fish have given figures of about 2·5 c.c./100 g. This leads us to the conclusion that about $\frac{1}{8}$th to $\frac{1}{10}$th of the total blood volume is contained within the gills at any one time.

Estimates of the cardiac output of the mackerel can be made knowing the oxygen capacity of the blood and assuming that during its passage through the gills the blood changes from 15% saturation to 85% (Hall, 1930), i.e. from 2·5 vol. O_2 % to 13·5 vol. O_2 %. Figures obtained for the oxygen consumption of the mackerel are 360–760 c.c./kg./hr. (Baldwin, 1924; Hall, 1930) or 6–12 c.c./kg./min. Hence during this time it uses 6–12 c.c. of oxygen per kg. Therefore to obtain the latter it needs 54–109 ml. of cardiac output/kg./min. This is a surprisingly high figure when it is remembered that the cardiac output of man is 80 ml./kg./min., and it must be remembered that the

mackerel is a very active fish. Using this figure for the cardiac output and the fact that the blood volume is about 25 ml./kg. then the mean total circulation time is about $\frac{1}{2}$–$\frac{1}{4}$ min. It is also possible to estimate the mean velocity of the blood through the gill capillaries from the above estimate of the total cross-sectional area of the gill capillaries of 2·5 mm.^{2}/g. and the minimal velocity is about 0·036–0·073 cm./sec. Thus it must take about 1·0–1·9 sec. for the blood to circulate through the gill lamellar capillaries. This is of the same order as the only measurements available, made by Mott (1950) on the anaesthetized eel, which gave values of $5{\cdot}6 \pm 1{\cdot}9$ sec.

DISCUSSION

The measurements on fish gills have confirmed previous observations that more active fish have larger gill areas per unit of body weight. In the present paper, however, these results and those of some previous workers have been analysed in relation to the ways in which the areas are distributed. This centres upon a consideration of the resistance of the gills to the flow of water and conditions for gaseous exchange. These two features are particularly important because the amount of oxygen removed from the water depends upon (*a*) the ventilation volume, and (*b*) the percentage utilization. With large ventilation volumes it is important that the resistance should not be too great because the work done by the respiratory muscles in pumping water through the sieve forms a significant part of the resting oxygen consumption. Calculations have shown that even where the pores of the sieve are small the total resistance is not too high because of their large number and there seems little doubt that most of the water passing through the gill system normally flows between the secondary lamellae. With respect to utilization, however, the small pores are advantageous because they reduce the diffusion distance although the gill resistance will be increased. Evidently the structure and morphology of the gills in a given species is a compromise between these two demands in relation to the mode of life and metabolic requirements of the fish.

It has been shown (Van Dam, 1938; Hughes & Shelton, 1962; Saunders, 1964; Hughes, 1966) that an increased ventilation volume is usually associated with a fall in utilization, although the total amount of oxygen extracted may increase. There are several reasons for this, one of which may be an increase in the volume shunted between the tips of the filaments (Fig. 4*b*) but another is simply due to the increased rate of flow, as illustrated in Fig. 3. This diagram shows the approximate nature of the gradients of oxygen found in the water as it flows between a pair of secondary lamellae. Lloyd (1961) has deduced that the oxygen concentration, X, at a given distance from a secondary lamella is proportional to the square root of the velocity of water flow, V; i.e. $X = B\sqrt{V}$. Differences in the interlamellar distance will affect the constant B. With an increased flow the amount of water in the axial path from which little oxygen has been removed (equivalent to the physiological dead space of lung) will be greater. The presence of narrow channels is therefore advantageous in systems where the flow is relatively rapid because it will reduce the total size of this dead space.

From the comparative study of gills it appears that the distribution of total gill area in different species is adapted to meet the conflicting effects on resistance, diffusion distance, and space. In active pelagic fish a streamlined form is important but tends

to reduce the space available to accommodate the large gill area. In these fish the total cross-section of the sieve is not increased so much as in sluggish fish and the individual pores are small but large in number. In pelagic fish it is probable that a large proportion of the work of breathing is accomplished by the swimming muscles and in certain species, e.g. mackerel, they alone are responsible for gill ventilation. In these fish the large number of secondary lamellae per unit distance increases the resistance to flow but decreases the diffusion distances. Some restriction of the increase in gill resistance occurs if the length, *l*, of the lamellae is decreased. Little is known of the variation in

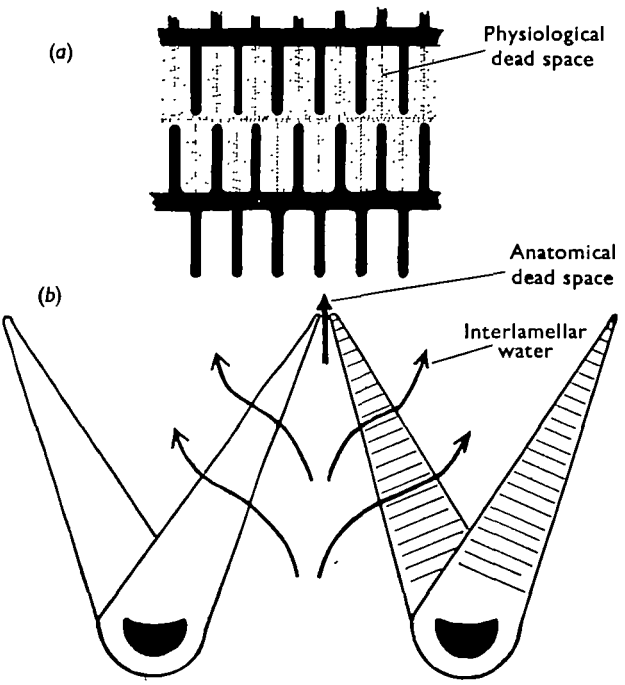

Fig. 4. Diagrams to show the main subdivisions of the water flowing through the gill sieve. (*a*) Longitudinal section of two filaments. The water flows at right angles to the page. The concentration of oxygen in the *interlamellar water* (equivalent to *alveolar air*) is indicated by the density of the stippling. The water which passes through without losing any oxygen is equivalent to the *physiological dead space*. (*b*) Diagram of transverse section through two adjacent gill arches with their rows of attached filaments. The filaments normally touch one another at their tips and most water passes between the secondary lamellae (interlamellar water). With excessive pressure gradients across the gills, the volume of water shunted between the tips of the filaments increases. This portion of the respiratory water is equivalent to the *anatomical dead space* of the lung.

utilization during swimming but it will almost certainly be less than in stationary fish. However, the animal obtains not only irrigation of the respiratory epithelium but also forward movement and perhaps food using the same muscles. If the red muscles of the trunk used in swimming are more efficient than the respiratory muscles at converting chemical energy to mechanical energy then this will lead to a net economy to the fish. In bottom-living forms the respiratory rhythm is usually slower and the opercular pump maintains a more or less constant differential pressure across the gills. Typically the sieve contains larger holes, each of greater length. These con-

ditions allow a longer time for the diffusion of oxygen from the centre of the interlamellar water (Fig. 3).

During gill ventilation we may distinguish different portions of the respiratory water according to the path taken. The most important is the interlamellar water (Fig. 4) which is analogous to the alveolar air of a lung. The water in the axial stream which does not lose any of its oxygen may be regarded as a physiological dead space (Figs. 3 and 4*a*). Correspondingly, because of the morphology of the gills there is some water which spills over between the filament tips (Fig. 4*b*) either because of the water pressure against the elasticity of the filaments or because of their rhythmical adduction during the respiratory cycle. Such loss of water is perhaps analogous to the anatomical dead space of the mammalian lung. Nevertheless, it is not very large in volume because movements of the branchial arches and of the individual filaments tend to maintain the whole of the gill sieve as a curtain across the respiratory current in a way that is remarkably efficient despite the rhythmic movements of the whole pumping system. The volume of these different portions of the water current will vary in different fishes and according to the conditions of ventilation. As yet no data are available concerning their relative sizes. The anatomical dead space may be regarded as a protective shunt which comes into operation should the differential across the gills become excessive. It also provides a path through which water may be reversed in its direction for cleaning purposes.

On the blood side of the exchange mechanism it is of interest to consider the probable velocity profile of its flow through the secondary lamellae. From the sections and electronmicrographs (Hughes & Grimstone, 1965), it appears that the channel along the free edge of the secondary lamella is the only one through which the blood corpuscles have a clear pathway. Along the other pathways they must collide with the pillar cells. Therefore the velocity will probably be most rapid in the outer regions and will decrease progressively in those parts of the secondary lamellae closer to the axis of the filament. Such a distribution would parallel the velocity of flow in the water. As it is important that there should be some adjustment in the capacity flow rates of the blood and water (Hughes, 1964), these qualitative considerations suggest that at least they will be in the right direction. Steen & Kruysse (1964) have recently suggested that there is a blood shunt whereby blood need not circulate through the respiratory surfaces because of an alternative route within the filament axis. They showed that the proportion of the branchial circulation passing through the two systems can be varied by changing the concentration of adrenaline or acetyl choline. There is, then, an analogy between this system and the system of shunts which operates in the mammalian lung. In addition to these considerations it is necessary to add the complications which result from the counter flow between the water and blood. In general such a system will serve to emphasize the importance of factors involved in maintaining a high utilization and therefore it is of great importance in the functioning of fish gills.

SUMMARY

1. Measurements have been made of the gill areas of fourteen species of British fish, all marine except for the tench and trout. In addition the gill area of the Antarctic icefish, *Chaenocephalus* (lacking haemoglobin) has been measured.

2. The different parameters of the gill sieve have been considered quantitatively in relation to the size of the gill area, resistance to the passage of water, and the diffusion conditions at the gills.

3. From calculations of the expected flow through a sieve of the measured dimensions it has been concluded that a sufficient volume would pass in the inter-lamellar spaces despite their fine dimensions. Differences in the nature of the sieve relative to the theoretical one and changes during the respiratory cycle account for the ventilation volumes measured being less than those calculated.

4. The theoretical analysis of the operation of the gill sieve are discussed in relation to measurements of Gray (1954) and to the developmental studies of Price (1931).

5. It is concluded that more active fish not only have larger gill areas but that the conditions for gaseous exchange are better than for more sluggish forms and that the area is increased in such a way as to keep the resistance to flow to a low value. This is mainly achieved by having an increased total filament length and a large number of secondary folds. More sluggish fish have more widely spaced and higher lamellae and their total filament length is reduced. The resistance to flow relative to the area is less in these forms than in the more active species.

6. Consideration is given to the different pathways along which water can infiltrate past the respiratory epithelium and analogies are drawn with the alveolar air, and the physiological and anatomical dead-spaces of the mammalian lung.

I wish to thank the Director of the Plymouth laboratory for the facilities that were placed at my disposal, and his staff for collecting the fishes. The late Prof. D. Keilin, F.R.S., kindly allowed me to use the gills from his two specimens of *Chaenocephalus*. I also wish to thank Dr I. E. Gray of the Department of Zoology, Duke University, for letting me use some unpublished details of his extensive measurements on fish gills. Finally it is a pleasure to acknowledge my indebtedness to Dr K. E. Machin for having derived the modified Poiseuille equation.

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