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A FIRST LOOK AT HOW FISH GILLS WORK

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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 (Byrtkowska-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 c. 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

* Byrtkowska-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.

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Steven Perry discusses G. M. Hughes' 1966 paper entitled: The dimension of fish gills in relation to their function. A copy of the paper can be obtained from http://jeb.biologists.org/cgi/content/abstract/45/1/177.

Forty-two years ago Lyndon B. Johnson was US President, a first class postage stamp cost 5 cents, the first 'Star Trek' episode was broadcast and England defeated West Germany in the Soccer World Cup. Major changes were in the wind. The Beatles recorded 'Eleanor Rigby', 'Yellow Submarine' and 'Good Day Sunshine', marking the beginning of their metamorphosis. The War in Indochina intensified, with 400,000 US ground troops in South Vietnam. Worldwide student unrest began.

In respiratory science, many now-famous names in comparative respiratory physiology such as Pierre Dejours, Hans-Rainer Duncker, Kjell Johansen, Johannes Piiper, C. Ladd Prosser, Hermann Rahn, Knut Schmidt-Nielsen, S. Marsh Tenney and Ewald Weibel were well into their careers. Physiological ecology was emerging.

Among the major players was also George M. Hughes. After focusing on control of respiration and locomotion in invertebrates such as dragonfly larvae, crabs and marine snails early in his career, Hughes began studying fish respiration in the late 1950s, and it became his strength over the following three decades. In addition to numerous reviews, book chapters and a popular book on comparative respiratory physiology (Hughes, 1963), Hughes has published over 200 peer-reviewed papers, two-thirds of them on fish respiration. Of these publications three have been cited more than 200 times (Hughes, 1966; Hughes, 1972; Hughes and Morgan, 1973).

In his classic 1966 paper, 'The dimensions of fish gills in relation to their function' (Hughes, 1966), Hughes founded the modern age of gill biophysics, by measuring various gill tissue dimensions in fish ranging from 12 g mackerel (*Trachurus trachurus*) to 1.5 kg angler fish (*Lophius piscatorius*), to analyse gill resistance to water flow. The paper is presented in the IMRD (introduction, methods, results, discussion) format, typical of experimental studies, although it is actually an integrative and analytical review of factors involved in gill resistances supplemented with new morphological data.

In the Introduction, Hughes focuses on the rationale for the paper, stating that 'The present investigation was undertaken because earlier work (Hughes and Shelton, 1958) suggested that the nature of the gill resistance is an important variable in the ventilation mechanism.' In that paper, Hughes and Shelton had suggested that the secondary lamellae, the microscopic flap structures on gill filaments where gas exchange occurs, might be so closely spaced and water so viscous that water may not penetrate between the lamellae: that is, they may not be ventilated directly (Fig. 1). In that case, the diffusing capacity of the gill, which is inversely proportional to the distance that oxygen must travel by diffusion from the gill surface to the nearest blood space, would be vastly overestimated by earlier morphological studies, which had assumed that diffusion begins at the lamellar surface. If water cannot flow between the lamellae, then the dimensions of the interlamellar spaces must be added to the total distance that gas molecules must move on the basis of diffusion alone. But how much of the interlamellar space must be included? In addition, Hughes doubted that the pressure differential of 0.5 cm H_2O measured across the gills would be sufficient to overcome the resistance of a fine gill sieve and ventilate the interlamellar spaces (Hughes, 1985). Hughes realised that a biophysical model that directly verified fluid flow through the gill would clear up these doubts.

The Methods section presents a description of morphometric techniques developed by I. E. Gray 12 years earlier (Gray, 1954) and further refined by Hughes to measure various gill morphology parameters, including filament length, secondary lamellae spacing and surface area. Many of these methods were later to become

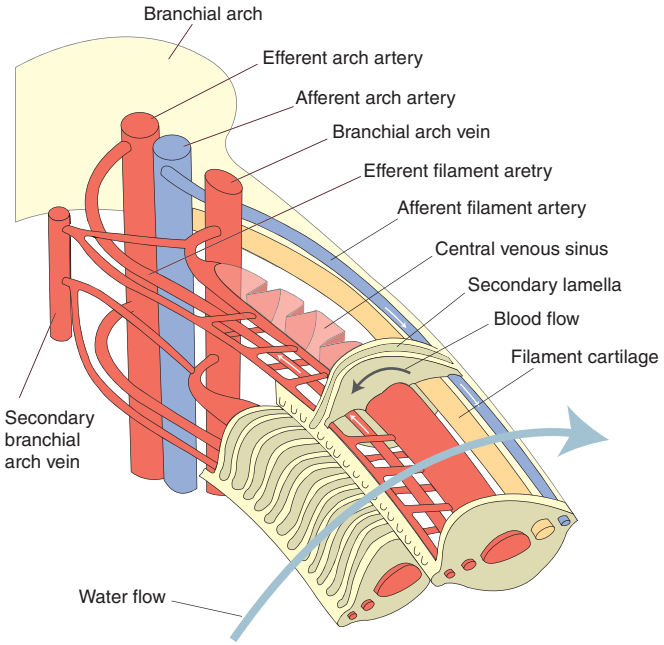

Fig. 1. Schematic representation of a teleost gill showing portions of two filaments and their secondary lamellae. The direction of blood flow in the secondary lamellae is opposite to that of the water flow between them (countercurrent system). Note the extensive secondary circulatory system, including the central venous sinus and the secondary branchial arch vein. The afferent branchial vessels (in blue) carry oxygen-poor blood into the gill, whereas oxygen-rich blood (in red) is either returned to the branchial arch for distribution to the body or stored in the central venous sinus and eventually returned to the heart in the branchial veins.

standard in gill morphometry. The technique for secondary lamellar surface area measurement was particularly laborious and it is perhaps fortunate that modern methods for measuring gill surface area (Costa et al., 2007) did not exist in 1954 and 1966, otherwise the parameters necessary to construct the biophysical model, such as filament number and length, and secondary lamellar height, length and frequency, might not have been measured.

In the Results section, Hughes reviewed gill parameters in the context of fish lifestyle while also analysing the biophysical implications of secondary lamellar dimensions and interlamellar distance for ventilation. To this end Hughes employed a modified version of the Poiseuille equation to calculate flows through a rectangular aperture and estimate the resistance to fluid flowing between secondary lamellae. He came to two conclusions. First, the gill surface area correlates with lifestyle in marine fishes, as Gray had already stated (Gray, 1954), as well as for hemoglobin-free icefish (*Chaenocephalus* sp.) and two fresh-water species; the sea trout (*Salmo trutta*) and the tench (*Tinca tinca*). Second, not only was the mesh of the gill sieve formed by the interlamellar spaces calculated to be large enough to allow direct ventilation, but also the maximum calculated ventilatory rate based on the morphometric data was an order of magnitude greater than that measured physiologically. Taking a closer look at different anatomical variables, such as increasing gill surface area by increasing the mean length of filaments (thereby increasing the number of secondary lamellae) or by increasing mean lamellar height, resulted in an increase both in gill surface area and in water flow over the surface. However, Hughes also calculated that increasing the area by increasing the mean lamellar length, or decreasing the interlamellar distance, would be accompanied by a large decrease in water flow across the respiratory surfaces.

The Results section merges imperceptibly with the Discussion, in which Hughes discusses the tradeoffs in gill structure and function and identifies and analyses dead space (i.e. the water volume that lies outside the interlamellar spaces), as well as illustrating how the fish might actively control the use of dead space. Particularly interesting is the implication of the possibilities of blood movement within the filaments. Most of the blood flows through the secondary lamellae, returns through efferent filamentar arteries to the gill arch and is distributed then to the body (Fig. 1). In 1966 alternative pathways of blood flow within the filaments had just been recognised by other researchers but the significance was not clear. Hughes suggested that the mechanisms for controlling the flow of blood to these other vessels (central venous sinus and branchial vein; Fig. 1) within the filament might be under humoral and nervous control, just like pulmonary blood flow in the mammalian lung. The significance of this albeit speculative statement should not be underestimated, as it highlights the importance of comparative studies in the recognition of overarching principles. Such observations, which can only be made by a person with broad experience, ranging from the molecular level right up to the whole animal in its environment, can stimulate basic research for generations to come.

During the course of this study, Hughes was meticulous in his measurements. For example, gill structure is not uniform; most teleost fish have four pairs of functional gill arches that are bilaterally symmetrical but differ from anterior to posterior. Also, the filaments within a given arch have different lengths and the structure of the secondary lamellae differs along the length of the filament. In order to take into account all of these deviations from uniform structure, Hughes took samples from the base, middle and tip of every tenth filament in all arches from one side of the fish, and fixed the tissue in Bouin's solution before transferring it to 70% ethanol and measuring the dimensions of the filaments and lamellae. Based on his experiences, Hughes was able to simplify the procedure later, by taking only the second arch on one side of the fish and multiplying by eight to represent the whole gill apparatus in subsequent publications. To measure the secondary lamellar surface area, Hughes dissected individual or pairs of secondary lamellae, traced them onto graph paper using a camera lucida and determined the surface area of these projections by point counting. In addition, he determined the number of secondary lamellae per millimeter of filament (secondary lamellar frequency). From all this information it was possible to reproducibly give an estimate of gill surface area.

However, even if measurements are reproducible it does not necessarily mean that they are correct, and not all of Hughes' assumptions have stood the test of time. For example, secondary lamellae are only rarely flat, and the height of secondary lamellae is related to the oxygen tension in the water (see Nilsson, 2007; Ong et al., 2007) and/or temperature. In addition the surface area of the lamellae is certainly underestimated by removing them manually: a very demanding procedure that can only err in

the direction of underestimation. Preliminary studies on trout gills have indicated that the underestimate may be approximately a factor of two in this species (Höller, 2004). A further limitation of Hughes' method is that it only applies to fish with secondary lamellae that can be manually removed. Thus extremely small species or those that lack secondary lamellae (e.g. lungfish of the genera *Protopterus* and *Lepidosiren*) would not be measurable.

All of this, however, does not change the basic assumptions, models and conclusions of Hughes' 1966 paper, and that contributes to its timelessness. The paper's main impact, and the reason it is still cited, is that it is the first study to apply engineering principles to gills. The paper has been cited 212 times, and continues to be cited regularly as it enters its fifth decade. The interest in the self-regulatory mechanisms and how gills work continues (Nilsson, 2007) and Hughes' paper is where this interest began.

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