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A METHOD OF ESTIMATING THE RELATIVE VOLUMES OF WATER FLOWING OVER THE DIFFERENT GILLS OF A FRESHWATER FISH

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INTRODUCTION

Recent research into the respiratory mechanisms of fishes has been dominated by the work of Hughes and his collaborators (Hughes, 1960*a*, *b*, 1966; Hughes & Shelton, 1957, 1958; Hughes & Ballintijn, 1965*a*, *b*). However, it appears that there has been no previous study of the relative water flow over the four pairs of gills of teleosts. This information is of more than academic importance for the results obtained by the proposed method may be of relevance in investigating the manner in which certain gill parasites infect their fish hosts (Paling, 1968).

The way in which the present study fits into the framework of our existing knowledge is best illustrated with the aid of a diagram. A horizontal longitudinal section through a trout head (Fig. 1) shows the relative positions of the mouth, the buccal cavity, the gills, the opercular cavities and the opercula. Anterior to the gills on each side, there is a small lamellate structure, the pseudobranch, but this is thought to have a negligible respiratory function (Fry, 1957). The gill filaments (primary gill lamellae) of the four gills on each side of the head together form a sieve-like network in a living fish. Gaseous exchange takes place over the secondary lamellar surfaces of this network (Hughes & Grimstone, 1965).

The most relevant of the published research on teleost respiration has concerned itself with the varying pressures occurring in the buccal and opercular cavities and the consequent respiratory current over the gills as a whole (Hughes & Shelton, 1958). No attempt has been made previously to determine whether all of the four gills on each side play an equal part in gaseous exchange or whether more of the respiratory current passes over some gills than others. Considerations of size alone may lead one to suspect that at least in most freshwater fishes the more posterior gills (3 and 4, Fig. 1) have less water flowing over them than the anterior ones (1 and 2, Fig. 1).

Studies on the dogfish (Hughes, 1960*b*) have provided records which show that in some elasmobranchs the more anterior gill pouches draw water through the gills under greater negative pressures than occur in the more posterior pouches. Hence, as both the pressure in the buccal cavity and the time-course of the respiratory movements is very similar for the different gills, it is probable that larger volumes of water pass across the more anterior gills than across those located posteriorly.

This communication describes a simple method of estimating the relative volumes of water flowing over the different gills of freshwater fishes.

MATERIALS AND METHODS

All of this work was carried out on the brown trout, *Salmo trutta*. These fish were obtained from two sources. Hatchery trout were used initially in order to experiment with the techniques involved and to ensure the accuracy of the basic assumptions upon which the method is based. Wild brown trout from Lake Windermere were used for most of the experiments which provided the results shown later. These fish were obtained by netting carried out by the staff of the Freshwater Biological Association

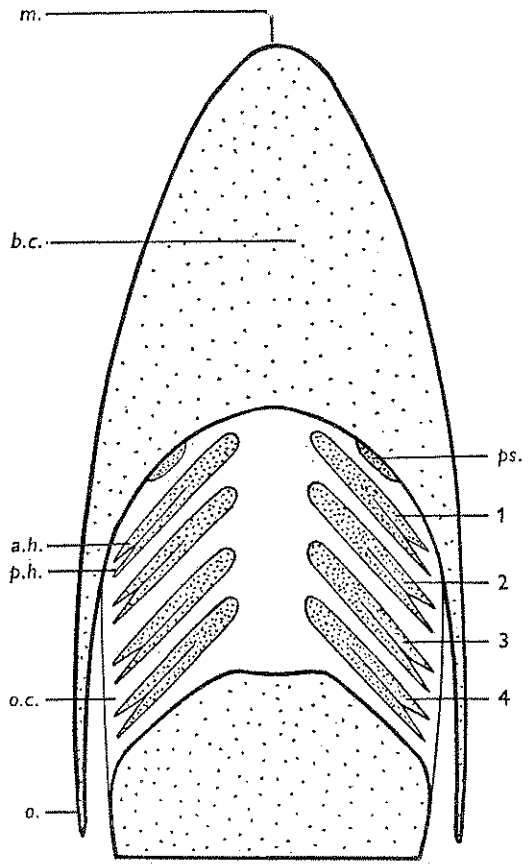

Fig. 1. A diagrammatic horizontal longitudinal section through the head of a trout. *a.h.*, anterior hemibranch; *b.c.*, buccal cavity; *m.*, mouth; *o.*, operculum; *o.c.*, opercular cavity; *p.h.*, posterior hemibranch; *ps.*, pseudobranch; 1, 2, 3 and 4, gills on the right-hand side of the head.

during several periods of study at their Windermere Laboratory. Trout of both sexes and of sizes ranging from 27 to 79 cm. were used at random as they were brought in to the laboratory.

The basis of the method finally adopted to assess the relative water flows across the gill arches is very simple. It depends on the use of marker parasites which are allowed to enter the mouth passively with the respiratory current and thence to attach themselves to the gill filaments as the water flows across them. If suitable parasites are used,

they will then be distributed over the eight gills in proportions which reflect the actual volumes of water per unit time passing over the different gills.

Ideal marker parasites for this purpose are the small larvae, glochidia, of certain freshwater mussels such as species of *Anodonta*, *Unio*, and other genera (see Ellis, 1962). In nature, during the breeding season of the mussels, these larvae are released into the water in masses comprising hundreds of individuals (Latter, 1891). Each glochidium has a small shell having two valves with inwardly directed points at their outer tips (Fig. 2) by means of which the larva can attach itself to a passing fish. If an infective glochidium comes into contact with a fin or the gill tissue of a fish, the shells will snap shut and embed their spines into the fish tissue. Thus begins a short parasitic phase in the life cycle of these molluscs.

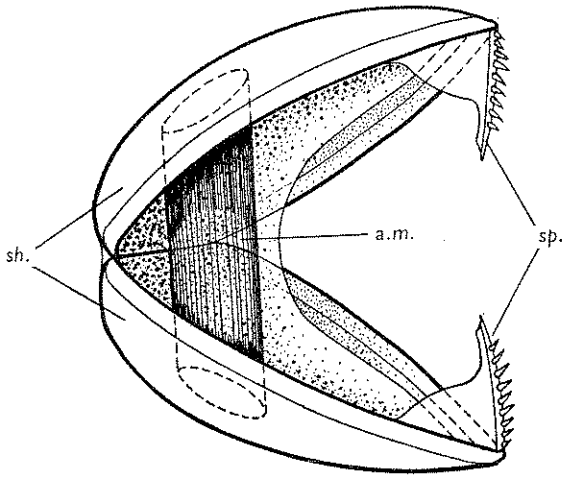

Fig. 2. A stereogram of a single glochidium showing the major organs of attachment. *a.m.*, adductor muscle; *sh.*, bivalved shell; *sp.*, barbed spines (hinged at base).

The glochidia used in the present study were obtained from specimens of *Anodonta cygnea* which were collected from the Lancashire Canal near Carnforth, England. This species was found to contain glochidia during November, December and January and it is possible that they may be ripe also in the adjacent months. Most of the large specimens of *Anodonta* sp. were in fact females and about half of these were found on dissection to contain glochidia during the above months of the year. However, it was not convenient to wait for them to shed their glochidia naturally. Accordingly, the mussels were opened and it was possible to see immediately if the outer gills were swollen with glochidia (see Bullough, 1966, p. 394). If glochidia are present, they can readily be removed from the parent by making a longitudinal cut into the wall of the outer ctenidium and scraping the viscous masses of glochidia into a glass dish. When water is added to these larvae, they will open and shut their shells spasmodically if they are sufficiently mature to infect a fish. If the glochidia are too young to be capable of infection, then the addition of water will not elicit the active clamping movements.

The method outlined above has two major limitations inherent in the choice of glochidia as marker animals. Neither the glochidia-producing mussels nor the glochidia themselves can tolerate sea water and so the method is restricted to investigations on

freshwater fishes. Furthermore, until such time as it becomes possible to induce breeding in *Anodonta* sp. or its allies during any month of the year, the method is limited to those months when ripe mussels can be obtained from the field.

The validity of the proposed method

Certain basic assumptions are made in using the above technique. First, the glochidia larvae must be taken in passively with the fish's respiratory current. This assumes that the glochidia cannot swim, unlike, for example, the marine lamellibranch *Pecten maximus* which can propel itself by rapidly opening and closing its shells. In order to verify this, new observations were made on glochidia and these confirmed the views of Latter (1891). When placed in still water glochidia descend slowly to the bottom and this vertical movement is unaffected by any snapping movements of the shells. However, relatively small disturbances in the medium cause the larvae to be washed up into mid-water again. Thus if water containing free glochidia is vigorously stirred and a fish is introduced, those glochidia that attach to the gills will have been taken there passively along with the respiratory current.

A second assumption is made in using the number of attached glochidia as a measure of the different volumes of water flowing over the various gills and between the separate gill slits. This is that the glochidia passing through different regions in the gill network are equally successful in achieving attachment. This may be questioned at first sight on the grounds that even if equal volumes of water were to pass through all the gill slits, those gills having a larger 'catchment area' may be thought to retain more glochidia than those gills which expose a smaller surface to the infective mussels. Similarly, it may be thought that because the posterior-most gill slit is bounded by a lamellar surface on one side only, the number of glochidia on that hemibranch could not provide a reliable estimate, even for comparative purposes, of the total volume of water passing through that slit. These apparent complications merit consideration in some detail at this juncture.

It has been established that the gills of fishes form an efficient partition separating the buccal and opercular cavities and requiring a coordinated series of pumps in order to force and pull water through the sieve network of secondary gill lamellae (Hughes & Shelton, 1958). In normal respiration the tips of adjacent gills touch one another so that virtually all the respiratory current is made to pass through the fine secondary lamellar network of the gills (Hughes, 1966). In view of this strikingly efficient anatomical arrangement it is highly probable that all of the water entering into the most anterior and most posterior gill slits will also pass through the gill network, even though there is an effective gill surface on one side only of those particular gill slits. This could easily be achieved by the lateral extremities of the two hemibranchs concerned being pressed against the anterior and posterior walls of the gill cavity on each side.

It thus seems probable that in the context of the present technique all the glochidia in the respiratory current will be brought in contact with the gill network. It follows that for practical purposes the 'catchment area' presented to the infective larvae by a particular gill is unimportant provided that the 'mesh size' (of the pores between the secondary lamellae) is constant. Hughes (1966) records that there is very little difference in the spacing of the secondary lamellae on the different gills of a

given species of fish and so this satisfies the above requirement. When measurements are taken of the glochidia and the spacing of the secondary lamellae, it becomes increasingly apparent that all the marker parasites would readily be caught by the gill sieve and hence would stand a high chance of attachment. The distance between two adjacent secondary lamellae of specimens of *Salmo trutta* is approximately 0·037 mm. (Hughes, 1966, using sea trout), whereas glochidia measure approximately 0·19 mm. at their smallest diameter.

It is thus reasonable to conclude from the above discussion that the numbers of glochidia on the gills do provide a valid measure of the different volumes of water flowing over the various gills and between the separate gill slits.

Next it is necessary to consider whether it is possible for a glochidium, once attached, to move its position on to a different gill or on to a different part of the same gill. But the glochidia possess no organs which would appear to permit active locomotion over the gill tissue and so it seems highly probable that once they clamp into the host tissue, they do not move from their first position of attachment. This view was confirmed by simples tatistical analyses of a type used later (see p. 542). These techniques showed conclusively that the distribution of glochidia over the gills of a freshly infected fish did not differ significantly from that on the gills of fish which had been infected known periods previously. Other glochidia were observed to attach themselves on fins of fishes and these parasites also showed no movement following the moment of attachment.

Finally, mention must be made of the slender possibility that a few glochidia may land on the gills as a result of entry under the operculum. It has already been established that glochidia are unable to swim actively against the respiratory current, but it is theoretically possible that some may be drawn momentarily into the opercular cavity during the brief interval in the respiratory cycle during which the current across the gills is reversed (see Hughes & Shelton, 1958). Accordingly attempts were made to infect trout artificially via the operculum. This involved inserting the front of a resting fish's head through a slit in a rubber partition which was arranged so that the water around the opercula did not mix with that surrounding the mouth. Suspensions of glochidia were then pipetted repeatedly over the opercula and towards the opercular apertures. After about 5 min., the trout was killed and the gills were examined but on no occasion did any of the gills harbour glochidia. It was thus possible to show by this technique that in practice infection of the gills via the operculum does not take place.

Experimental procedure

A shallow aquarium of size 4 ft. × 3 ft. × 1 ft. was used as a convenient vessel in which to carry out the oral infection of the trout at Windermere. It was filled with lake water and selected glochidia from ripe mussels were added from a pipette. Special precautions were required to ensure that the glochidia were added as a suspension of single individuals. The manner of collection of these marker animals results in there being clumps of glochidia still invested in viscous gill material. As it is obviously undesirable for the present purposes if the glochidia were to enter the mouth in clumps the freshly extracted glochidia and the added water were vigorously agitated in a separate vessel by means of repeated suction and expulsion from a large bulbed pipette. Any remaining aggregations of the larvae were discarded and separated individuals were selected for the infection vessel.

The number of *Anodonta* used to provide a suitably concentrated suspension depends, of course, on the size of the infection vessel and the time for which the fish is to be left in the suspension. In the circumstances of the present study, glochidia from three mussels enabled a trout to acquire a convenient number of glochidia on the gills (about 20/gill) in the space of about 5 min.

The Windermere trout were caught in gill nets and kept alive in holding tanks until required. They were then caught singly and transferred to another holding tank to await infection. The suspension of glochidia in the infection vessel was then stirred up vigorously in order to randomize the marker parasites in the water and the fish was introduced. Commonly it lay quietly or swam around slowly during the 4–5 min. which were allowed for infection. During this time the glochidia were kept in suspension by agitation with the bulb of a pipette.

If the trout threshed around at any stage in the proceedings, it was assumed that the 'depth' of its respiration would be atypical and so the glochidia counts from such fish were omitted from the final results. Later work (see p. 540) has shown that 'depth' of respiration may have an effect on the distribution of the marker parasites over the different gills.

After removal from the infection tank the experimental fish was placed in a sink containing clear water and left for about 15 min. in order to allow any glochidia temporarily entangled in the gills (but not attached) to be washed free. The trout was then killed, the four gills on each side of the buccal cavity were excised and placed separately in Petri dishes containing water. The gills were placed in such a way that the anterior and posterior faces of each gill could be identified. In addition, the pseudobranchs were also removed from some of the fish and these also were examined for glochidia.

The gills and pseudobranchs were examined with incident and transmitted light and the numbers of glochidia on each gill were carefully recorded. In the case of about half of the experimental fish the glochidia were scored separately for the anterior and posterior hemibranchs of each gill, and also for the pseudobranchs (see Fig. 1).

It was thought inadvisable to direct the suspension of glochidia towards the mouth of the trout (say, by allowing a suspension of glochidia to fall just in front of a stationary trout) as it was possible that this might lead to a distribution of glochidia over the gills which would not accurately reflect the relative water flows over the different gills. If there were a linear flow of water through the buccal cavity, then it may be that glochidia dropped immediately in front of the mouth may get carried to particular gills. There is some evidence from studies on dogfish (Hughes, 1960*b*) that this is so at least in some types of fishes and hence in these experiments unless the contrary is explicitly stated, the glochidia were randomized in the water and not in any way directed towards the centre of the fish's mouth.

RESULTS

The relative rates of flow across the four pairs of gills

In all of the results which follow, the numbers of glochidia on each pair of gills are aggregated as it is assumed that there is no significant difference between volumes of water flowing out of the left and right sides of the buccal cavity. The total number of

glochidia recorded on the first, second, third and fourth pair of gills of brown trout is shown in Table 1.

It can be seen that, under the conditions of the experiment, most of the water flows over the second and third pairs of gills, less flows over the first pair on each side and least of all across the most posterior pair of gills.

Table 1. *The numbers of glochidia recorded on each of the four pairs of gills of Windermere brown trout infected orally*

(Total number of trout used 47. Total number of glochidia recovered 14,537. Distribution over the four pairs of gills.)

	1st pair of gills (most anterior)	2nd pair of gills	3rd pair of gills	4th pair of gills (most posterior)
No. of glochidia	3517	4350	4109	2561
Percentage	24·2%	30·0%	28·2%	17·6%

Table 2. *The number of glochidia recorded on the gill lamellae bounding the five pairs of gill slits of Windermere brown trout, infected orally*

(Total number of trout used 29. Total number of glochidia recovered 8516. Distribution between the five pairs of gill slits.*)

	1st pair	2nd pair	3rd pair	4th pair	5th pair
No. of glochidia	807	2369	2753	1936	651
Percentage	9·5%	27·7%	32·2%	23·0%	7·6%

* 1st gill slit is between the pseudobranch and the anterior hemibranch of the first gill. 2nd gill slit is between the posterior hemibranch of the first gill and the anterior hemibranch of the second gill. 3rd gill slit is between the posterior hemibranch of the second gill and the anterior hemibranch of the third gill. 4th gill slit is between the posterior hemibranch of the third gill and the anterior hemibranch of the fourth gill. 5th gill slit is between the posterior hemibranch of the fourth gill and rear of the buccal cavity.

The relative rates of flow between the different gill arches

Reference to Fig. 1 will make clear that there are five distinct gill slits on each side of the buccal cavity. The most anterior lies between the pseudobranch and the anterior side (hemibranch) of the first gill, while the second lies between the posterior hemibranch of the first gill and the anterior hemibranch of the second gill, and so on. The fifth slit is bounded by the posterior hemibranch of the fourth gill and the rear of the buccal cavity. Table 2 shows the relative volumes of water flowing between these slits as measured by the numbers of glochidia that attached themselves following entry via the mouth.

It can be seen that more of the respiratory current flows through the central pair of gill slits than through any of the other pairs. Lesser though considerable volumes flow through the second and fourth pairs of gill slits, whereas the first and fifth pairs of gill slits together appear to carry only about one-sixth of the total respiratory current.

It is of interest to record that out of a total of 8516 glochidia which successfully attached to the gill tissue, only two fastened themselves to the pseudobranchs.

The effect of different rates of respiration on the distrubtion of glochidia

Marker parasites can be used to assess the effect on the pattern of respiratory flow of such variables as different ages or different physiological conditions of a fish species

In view of the possible applications of the above technique to other experimental studies of parasites, it was of particular interest to examine what effect stress might have on the respiratory pattern of the fish. It was thought possible that, when a fish breathes more rapidly and deeply, the relative volumes of water passing over the different gills may alter. Accordingly, a simple experiment was conducted to test this as follows. Seventeen brown trout were selected from a stock as being as nearly identical as possible (all hatchery-bred females of length 26·5–29·8 cm.). These fish were transferred singly into a separate tank where they were each infected with glochidia. The manner of infection was identical for all of the fish, namely the free glochidia (see p. 537) were dropped from a pipette placed immediately in front of and about 2 in. above the mouth of the stationary trout. The marker parasites could be seen to enter the buccal cavity with the respiratory current.

Table 3. *The effect of an increased respiration rate on the flow of water over the different gills of hatchery trout, using glochidia as markers*

Fish	Rate of respiration (breaths/min)			No. of glochidia on each pair of gills			
	Before infection	After infection	Average	1st pair	2nd pair	3rd pair	4th pair
			A. Fish respiring normally				
1	42	49	45·5	13	21	20	0
2	40	36	38·0	63	111	104	29
3	30	40	35·0	11	15	19	5
4	51	47	49·5	115	158	123	63
5	41	39	40·0	59	90	59	45
6	37	37	37·0	74	98	71	39
7	41	41	41·0	54	86	61	21
8	36	38	37·0	92	106	83	53
9	35	35	35·0	61	96	87	35
	Average rate of respiration ≏ 40·0			Total 542	781	627	290
			B. Fish respiring rapidly				
1	76	78	77·0	193	260	204	129
2	73	74	73·5	137	175	184	110
3	74	66	70·0	176	153	169	72
4	79	84	81·5	122	117	93	53
5	79	84	81·5	69	101	95	63
6	71	69	70·0	110	127	102	57
7	69	67	68·0	205	296	224	156
8	79	81	80·0	48	60	49	22
	Average rate of respiration ≏ 75·0			Total 1060	1289	1120	662

About half of the trout were left undisturbed for at least a day prior to infection. Each of the remainder was vigorously chased around its tank for about 5 min. with a rubber-ended rod before the infection with glochidia. In every case the rate of respiration was recorded for each fish immediately before and after the infection. The trout were then removed to a holding tank and after 15 min. they were killed, the gills removed and the numbers of attached glochidia were scored. The results of this experiment are shown in Table 3 and can be summarized as follows:

Distribution of glochidia over the four pairs of gills during normal respiration

1st pair	2nd pair	3rd pair	4th pair	Total
542	781	627	290	2240
24·2 %	34·9 %	28·0 %	12·9 %	

Distribution of glochidia over the four pairs of gills during rapid respiration

1st pair	2nd pair	3rd pair	4th pair	Total
1060	1289	1120	662	4131
25·7 %	31·2 %	27·1 %	16·0 %	

It is clear that the considerable alteration in the rate of respiration produced relatively small changes in the pattern of the respiratory flow. So slight do the changes of the respiratory pattern appear that it is desirable to question whether the two distributions recorded above are in fact significantly different. As a basis for calculation, it is convenient to take as a null hypothesis that there is *no* significant difference between the two sets of figures, that is to say that, in the range covered by the experiment, the rate of respiration does not affect the pattern of water movement over the different gills.

If the two sets of figures show no significant difference, the best estimated distribution reflecting the use of the different gills is given by adding the numbers from the two experiments:

Pairs of gills

1st pair	2nd pair	3rd pair	4th pair	Total
542 + 1060	781 + 1289	627 + 1120	290 + 662	
1602	2070	1747	952	6371
25·1 %	32·5 %	27·5 %	14·9 %	

On this basis, the expected distributions in the two experiments involving normally and rapidly respiring fish would be as follows:

Normal respiration

1st pair	2nd pair	3rd pair	4th pair	Total
25·1 % × 2240	32·5 % × 2240	27·5 % × 2240	14·9 % × 2240	2240
562·22	728·00	616·00	333·76	

Rapid respiration

1st pair	2nd pair	3rd pair	4th pair	Total
25·1 % × 4131	32·5 % × 4131	27·5 % × 4131	14·9 % × 4131	4131
1036·88	1342·57	1136·02	615·52	

Now we can apply

$$\chi_3^2 = \sum_{i=1}^{4}\left\{\frac{(O_i - E_i)^2}{E_i}\right\}, \quad \chi_3^2 = \sum_{i=1}^{4}\left\{\frac{O_i^2}{E_i}\right\} - T,$$

where T is the total number of marker parasites used in both experiments.

$$\chi^2_3 = \left(\frac{542^2}{562{\cdot}22} + \frac{781^2}{728{\cdot}00} + \frac{627^2}{616{\cdot}00} + \frac{290^2}{333{\cdot}76} + \frac{1060^2}{1036{\cdot}88} + \frac{1289^2}{1342{\cdot}57} + \frac{1120^2}{1136{\cdot}02} + \frac{662^2}{615{\cdot}52}\right) - 6371$$

$$= (522{\cdot}51 + 837{\cdot}86 + 638{\cdot}20 + 251{\cdot}98 + 1083{\cdot}63 + 1237{\cdot}57 + 1104{\cdot}21 + 711{\cdot}99) - 6371$$

$$= 6387{\cdot}95 - 6371{\cdot}00$$

$$= 16{\cdot}95,$$

therefore probability is $\simeq 0{\cdot}001$.

Thus the null hypothesis is rejected and it is acknowledged that a significant difference does exist between the volumes of water flowing over the different gills of trout when resting fish are compared with vigorously exercised fish. This conclusion is discussed later (see p. 543).

(It should be pointed out that the detailed results in this section do not permit accurate comparisons to be drawn with the records for the infection of Windermere fish. Not only did the two batches of fish differ considerably in composition, but the detailed method of infection was not the same in the two cases.)

DISCUSSION

The technique described above uses marker parasites in order to estimate the different volumes of water flowing over the four pairs of gills of a freshwater fish. In the absence of more sophisticated methods producing more accurate results it serves a useful function in providing an estimate of a physical parameter which may interest ichthyologists as well as those studying gill parasites.

One of the striking findings of this study is that most of the respiratory current appears to flow through the third pair of gill slits, with smaller yet appreciable volumes flowing through the second and fourth pairs of slits. The first and last gill slits, on the other hand, carry relatively little of the water flow. This is not unexpected when it is recalled that the first and last pairs of gill slits are effectively bounded by only one lamellar surface. The pseudobranch is the vestigial remains of a more anterior gill which, presumably, would not have become reduced to such a degree if that particular gill slit still played a major part in respiration.

When the numbers of glochidia recovered from the different pairs of gills are compared (Table 1), it is clear that approximately equal volumes of water flow over the second and third pairs of gills while significantly less flows over the first pair on each side. This, of course, is a reflection of the water distribution passing through the different gill slits as recorded in Table 2.

Having established the general pattern of water movements over the gills, it is of further interest to compare the distribution of glochidia over the four pairs of gills with the relative surface areas of the corresponding gills. Unfortunately, only one set of figures for the surface area of the different gills of brown trout is available. Professor

G. M. Hughes (personal communication) has calculated the surface areas separately for the various pairs of gills of a 175 g. fish. These were as follows:

Pairs of gills	Surface area (mm^2)
1st	16,364
2nd	17,422
3rd	15,254
4th	10,164
	59,204

In the absence of similar calculations on other specimens of brown trout it is clearly unwise to use these figures for any detailed comparison. However, if this one fish were typical of the species, it would appear that the area of the respiratory surfaces of the different gills does not accurately reflect the relative volumes of water flowing over those particular gills.

Another experiment was performed which showed that a large increase in the rate of respiration affected the distribution of the marker parasites over the different gills. A further look at the summary of the relevant results (seep. 541) suggests that the first and fourth pairs of gills may be functioning below their full capacity in resting fish. It appears that when trout were exercised so that their respiration rate increased on average from 40 to 75 breaths per minute, the first and fourth pairs of gills took relatively more of the water thus reducing slightly the relative volumes passing over the second and third pairs.

An alternative explanation is that the tips of the primary gill lamellae may cease to meet in the face of the increased volume of the respiratory current (Hughes, 1966). This would mean that some ot the water flowing between the gill slits would pass directly into the opercular cavity without coming into contact with the secondary lamellae. As a consequence of this, the gill networks bordering the various gill slits would be sieving the marker parasites with different degrees of efficiency. Those gills over which most of the respiratory current flows (the second and third pairs) would be likely to become relatively less successful at entrapping glochidia.

Either of these two phenomena could explain the results observed when the trout's respiration rate was increased. However, it is notable that even under these conditions of severe stress, the general pattern of water flow over the gills alters only by a small degree.

SUMMARY

1. A technique is described which uses marker parasites to estimate the relative volumes of water flowing over the different gills of a freshwater fish.

2. It was found that in brown trout from Windermere most of the respiratory current flows over the second and third pairs of gills, less flows over the first pair on each side and least of all across the most posterior pairs of gill. Similarly, the median pair of gill slits carries more of the respiratory current than any of the other slits whereas first and fifth pairs of gill slits together carry only about one-sixth of the total water flow.

3. Hatchery-bred brown trout showed a slight but significant difference in pattern of water movement over their gills following vigorous exercise. Possible reasons for this are discussed.

I wish to acknowledge the cooperation of Professor Hughes in providing the figures for the surface areas of the different gills and Mr Philip Taylor for his technical assistance throughout the study.

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