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THE RESPIRATORY DEVELOPMENT OF ATLANTIC SALMON

II. PARTITIONING OF OXYGEN UPTAKE AMONG GILLS, YOLK SAC AND BODY SURFACES

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Summary

During post-hatch development of Atlantic salmon (*Salmo salar* L.), O_2 uptake partitioning changes from primarily cutaneous to primarily branchial. Over 80 % of post-hatch O_2 uptake was cutaneous, with the yolk sac responsible for 33 % of total O_2 uptake. The well-vascularized yolk sac was a less effective gas exchanger than the unperfused skin of the body, suggesting that oxygen delivery is by direct diffusion to the tissues. Branchial O_2 uptake increased quickly as gill lamellae developed, contributing 60 % of total O_2 uptake before the completion of yolk resorption (body mass 0.2 g) and increasing to 69–81 % in fish weighing over 0.3 g. The area-specific O_2 uptake of the skin decreased through development as skin thickness increased, while that of the gills increased from 0.10 $\mu g\,h^{-1}\,mm^{-2}$ to 0.23 $\mu g\,h^{-1}\,mm^{-2}$. Partitioning of O_2 uptake of the skin and gills changed in concert with changes in the partitioning of the anatomical diffusion factor (ADF, mass-specific surface area per unit diffusion distance) between skin and gills, which changed from more than 95 % to less than 10 % cutaneous; thus, ADF is a useful rough indicator of oxygen uptake potential. Caution should be used in predicting oxygen uptake potential from ADF, however, because O_2 uptake per unit diffusion barrier of the yolk sac was less than half that of the general body surface, and O_2 uptake per unit diffusion barrier of the gills changed dramatically over development.

Key words: respiration, development, fish, oxygen uptake partitioning, Atlantic salmon, *Salmo salar*, yolk sac, gills.

Introduction

One of the most important physiological transformations during fish development from larva to juvenile is the transfer of the primary site of O_2 uptake from the skin to the gills (Rombough, 1988; Burggren and Pinder, 1991). At hatch, larvae of most species of fish have under-developed or non-functional gills (Rombough, 1988; Blaxter, 1988; Wells and Pinder, 1996). Thus, it is generally accepted that O_2 uptake in larval fishes initially takes place across cutaneous surfaces (Balon, 1975; Blaxter, 1988; Rombough, 1988; Burggren and Pinder, 1991). In newly hatched chinook salmon (*Oncorhynchus tshawytscha*), cutaneous O_2 uptake was 80 % of total O_2 uptake (Rombough and Ure, 1991). As the gills develop lamellae and increase in surface area, they assume an increasing amount of O_2 uptake in juveniles (Rombough and Ure, 1991) and adults (Kirsch and Nonnotte, 1977; Nonnotte, 1981; Nonnotte and Kirsch, 1978).

The skin can be further partitioned into yolk sac and body surface. Although it has been hypothesized that the yolk sac of newly hatched salmonids is the principal site of O_2 uptake (Holeton, 1971; Balon, 1975; Peterson, 1975; Blaxter, 1988; Rombough, 1988), partitioning of O_2 uptake between the yolk sac and body surface has never been measured. The yolk sac of salmonids is highly vascularized (Garside, 1959; Holeton, 1971; Balon, 1975; Peterson, 1975; Blaxter, 1988; Rombough, 1988), has a large surface area (Rombough and Moroz, 1990) and a thin epithelium (Wells and Pinder 1996), suggesting a high morphological potential for O_2 uptake. Recent O_2 uptake (Rombough and Ure, 1991) and intravascular P_{O_2} measurements (Rombough, 1992), however, suggest that the yolk sac of salmonids is no more effective in taking up O_2 than the thicker, less vascularized skin of the body.

In the companion paper (Wells and Pinder, 1996) we analysed the anatomical diffusion factor (ADF, mass-specific surface area per unit diffusion distance) as a measure of the morphological gas exchange potential of the yolk sac, body skin and gills. In the present study, we analyse the actual partitioning of O_2 uptake among these surfaces. If ADF is a good measure of gas exchange potential, then the partitioning of O_2 uptake should match the partitioning of ADF. Differences between O_2 uptake and ADF partitioning may reflect physiological differences, for example in ventilation,

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perfusion, P_{O_2} difference across the gas exchanger or oxygen consumption by the gas exchanger.

Materials and methods

Spawning and fish rearing

Fish (*Salmo salar* L.) were reared as decribed by Wells and Pinder (1996) except that they were initially maintained at low temperatures (2±1 °C) to slow development and control hatching. Fish development and hatching were stimulated by moving larvae to 5±0.5 °C for 24 h, then to 10±0.5 °C. Prior to respirometry studies, fish were acclimated to 10 °C for at least 48 h.

Respirometry

Three types of respirometer were used. Two respirometers were divided into two chambers each for measuring gas exchange partitioning, and the other was a single chamber used to test whether the methods used to partition respiration affected total O_2 uptake. The partitioning respirometers used a thin rubber membrane of dental dam latex as a septum that formed a seal around the fish to separate (1) O_2 uptake of the head and gills from that of the body and yolk sac (Fig. 1A), and (2) O_2 uptake of the yolk sac from that of the body and gills (Fig. 1B). Because of changes in the shape of the yolk sac, fish more than 2 days post-hatch (20 ATUs, accumulated thermal units) were able to escape from the yolk sac partitioning respirometer. Thus, all measurements of yolk sac O_2 uptake (N=13) were on fish less than 15 ATUs post-hatch, weighing 0.033±0.05 g (mean ± S.E.M.). Partitioning between gills and skin was measured in fish from hatch up to 700–800 ATUs (0.45 g; N=47).

Both respirometers were made from acrylic plastic (10 ml total volume) and were held in insulated water baths in which the water temperature was maintained at 10±0.1 °C. The water baths were covered with black plastic to reduce light entry. A Radiometer E-5046-D oxygen electrode in each isolated chamber of the respirometers was connected to a Cameron model 100 dual-channel oxygen meter and measured the decline in P_{O_2} after the respirometers were sealed. Water in the respirometer chambers was stirred with magnetic stir bars.

Before each experiment, the oxygen electrodes were calibrated with aerated water; correction was made for water vapour pressure and barometric pressure. Electrode zero was checked less frequently owing to high stability. Zero was calibrated using water saturated with nitrogen or a sealed anoxic respirometer; this calibration was checked against electronic zero by unplugging the electrode.

The potential for diffusion of O_2 through the septum was tested by measuring the apparent rate of electrode O_2 consumption in an empty respirometer with and without a 13.3 kPa P_{O_2} difference across the septum. Oxygen uptake rates were not measurably different in two tests with a large P_{O_2} difference from the regularly measured blank O_2 uptake. Thus, diffusion of oxygen through the septum was negligible even with a large P_{O_2} difference. During experiments, the P_{O_2} difference between chambers was usually less than 5.0 kPa.

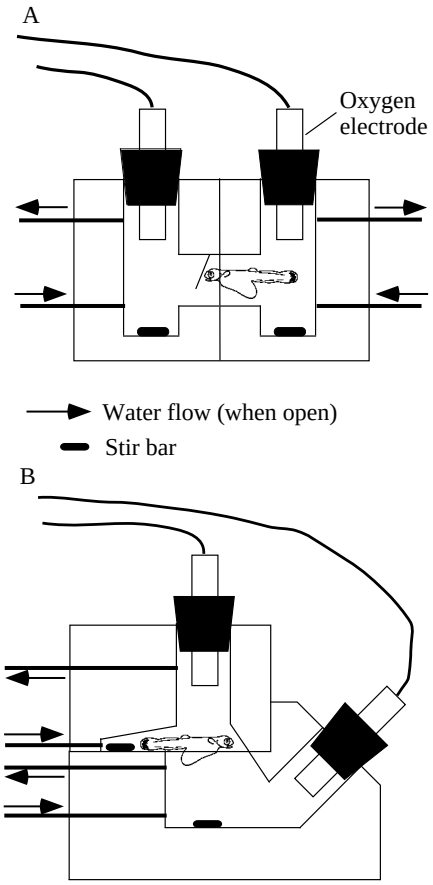

Fig. 1. Diagrams of the respirometers used to partition O_2 uptake among gills, body skin and yolk sac. (A) Respirometer used for partitioning O_2 uptake of the head and gills from the bulk of the cutaneous surfaces (the body and yolk sac). Fish were pushed through a hole in a latex rubber membrane up to the pectoral fins so that the head and gills were in one chamber and the body and yolk sac were in another. The total inner volume of the respirometer was 10.1 ml. (B) Respirometer used for separating yolk sac O_2 uptake from that of the body and gills. The yolk sac was pushed through a hole in the membrane so that the yolk sac was in the lower chamber and the body and gills were in the upper chamber. The total inner volume of the respirometer was 9.6 ml.

Experimental protocol

Unanaesthetized fish were placed in the partitioning respirometer by pushing either the head or the yolk sac through a hole in the septum. The size of the hole in the membrane was critical; if the hole was too large, the fish could escape or leaks could occur. Too small a hole caused the fish to struggle and cut off blood circulation. Fish that struggled continuously when they were placed through the membrane were discarded.

The respirometer was closed around the fish and aerated water was pulled through both chambers using a peristaltic pump. In the gill/body respirometer, an angled piece of plastic prevented fish from moving forwards in the membrane yet

allowed normal opercular respiration (Fig. 1A). No restraint was needed to maintain fish in position in the yolk sac/body respirometer (Fig. 1B). Total oxygen uptake in each of the partitioning respirometers was the same as in a respirometer in which the embryos were not restrained by a membrane (see below).

Fish were allowed to acclimate for 2 h, chamber ventilation was then stopped and the respirometer water inlets and outlets were sealed. The decline in P_{O_2} of each chamber was recorded on a chart recorder. The first 5 min of recordings were not used for calculation of oxygen consumption because the rate of P_{O_2} decrease was not constant during this period. Measurement periods for larger fish studied in the gills/body respirometer (>0.2 g) were 15–30 min or more; smaller fish (<0.2 g) were measured for 30–60 min. Measurement periods for yolk sac/body respirometry were 30–45 min. The P_{O_2} in the respirometer chambers was not allowed to drop below 13.3 kPa (65 % of air saturation) during O_2 uptake measurements, above the critical oxygen pressure for salmonids (8 kPa or less; Rombough and Ure, 1991).

After the O_2 uptake measurements, the respirometer water inlets and outlets were opened and the chambers were flushed with aerated water. When the P_{O_2} in the chambers was near air saturation, the fish were removed and the respirometers were resealed immediately for a 'blank' measurement of bacterial respiration and electrode O_2 consumption. A septum with no hole partitioned the respirometers. Electrodes were recalibrated after the blank O_2 uptake measurement. The change in calibration was usually 1–3 %. For calculations of O_2 uptake, electrode drift was assumed to be linear.

While still in the partitioning membrane, fish were examined microscopically for injury or gross alteration in blood flow resulting from the membrane. Data from fish with skin damage or interrupted blood flow were discarded. Uninjured fish were removed from the partitioning membrane and placed in a 10 °C waterbath for 1 h after the O_2 uptake measurement. Data from fish that died or did not behave normally after 1 h were discarded. Fish that behaved normally were then killed by MS222 overdose, weighed and fixed in buffered formalin for measurement of tissue and yolk wet masses.

To test for changes in O_2 uptake due to the stress of being placed in the rubber septum, total O_2 uptake of eight fish (mass 0.032–0.40 g) was measured in a single-chambered respirometer. Conditions and procedures for these measurements were identical to those used for partitioning measurements except for the absence of the restraint imposed by the septum.

Calculations of O_2 uptake partitioning

The contribution of cutaneous O_2 uptake (body + head) was calculated by adding an estimate of cutaneous O_2 uptake from the head chamber to the O_2 uptake measured in the chamber with the body and yolk sac. The surface area-specific O_2 uptake measured in the body chamber was multiplied by the estimated cutaneous surface area in the head chamber. Estimates of surface area were made using relationships between body mass and surface areas presented in Wells and Pinder (1996). This calculation assumed that all cutaneous surfaces were equally efficient at taking up O_2. Calculated cutaneous O_2 uptake in the head chamber was subtracted from total O_2 uptake in the head chamber to give branchial O_2 uptake.

Measurements of yolk sac O_2 uptake were also corrected because some of the yolk sac was in the chamber with the general body surface and gills. O_2 uptake of the whole yolk sac was estimated by adding the measured O_2 uptake of the yolk sac isolated in the yolk sac chamber to the estimated O_2 uptake of the yolk sac in the body chamber. The surface area-specific O_2 uptake in the yolk sac chamber was multiplied by the estimated surface area of yolk sac in the body chamber to estimate the O_2 uptake of the part of the yolk sac in the body chamber.

Statistical analysis

Least-squares linear regressions and tests of significance were performed with Systat v. 5.2. Second-order polynomial regressions were used in some figures to describe the data, but were not tested statistically. Data are presented as mean ± standard error.

Results

Oxygen uptake by the yolk sac

The yolk sac contributed approximately 33 % of the total O_2 uptake just post-hatch. O_2 uptake by the yolk sac was $1.93\pm0.09\,\mu g\,h^{-1}$ compared with $3.95\pm0.21\,\mu g\,h^{-1}$ for the skin and gills combined. The estimated surface area of the partitioned yolk sac ($74\pm2.2\,mm^2$) was less than that of the gills and cutaneous surfaces ($99\pm3.4\,mm^2$), but the calculated ADF of the isolated yolk sac ($1.64\pm0.07\,cm^2\,g^{-1}\,\mu m^{-1}$) was slightly higher than that of the body skin ($1.51\pm0.07\,cm^2\,g^{-1}\,\mu m^{-1}$) because of the difference in diffusion distance (13.9 μm for yolk sac, 20.3 μm for body skin; Wells and Pinder, 1996). Thus, O_2 uptake ($\mu g\,h^{-1}$) per unit diffusion barrier ($cm^2\,\mu m^{-1}$) of the vascular yolk sac was less than half that of the surfaces of the rest of the body ($37\pm2.4\,\mu g\,h^{-1}\,cm^{-2}\,\mu m$ compared with $82\pm4.9\,\mu g\,h^{-1}\,cm^{-2}\,\mu m$). This measure is the same as mass-specific oxygen uptake/ADF [$(\mu g\,h^{-1}\,g^{-1})/(cm^2\,\mu m^{-1}\,g^{-1})$].

Interestingly, as a function of mass in each partitioned chamber, the yolk sac tissue consumed three times the amount of O_2 as did the skin and gills ($425\pm27\,\mu g\,h^{-1}\,g^{-1}$ compared with $139\pm7\,\mu g\,h^{-1}\,g^{-1}$). These estimates assume the O_2 to be consumed locally.

Partitioning of O_2 uptake between skin and gills

Just post-hatch, O_2 uptake across the skin (body + yolk sac) accounted for 80 % of the total O_2 uptake for the fish (Figs 2, 3). The branchial contribution to total O_2 uptake quickly increased to about 60 % of total O_2 uptake at the end of yolk resorption (body mass 0.2 g) and to about 69–81 % in fish weighing more than 0.3 g. Area-specific cutaneous O_2 uptake decreased through development with a mass exponent of –0.233 (Fig. 4; regression equation in Table 1; areas calculated

Table 1. *Regression line equations for oxygen uptake as a function of body mass*

	a	*b*	S.E.M. of *b*	*N*	r^2	*P*
$\dot{M}_{O_2}$ (μg h^{-1})						
Total	200	0.94	0.05	47	0.90	<0.001
Branchial	254	1.41	0.06	47	0.92	<0.001
Cutaneous	36	0.48	0.07	47	0.50	<0.001
Surface area-specific $\dot{M}_{O_2}$ (μg h^{-1} mm^{-2})						
Branchial	0.33	0.37	0.06	47	0.47	<0.001
Cutaneous	0.015	–0.23	0.07	47	0.19	0.002
Oxygen uptake per unit diffusion barrier (μg h^{-1} cm^{-2} μm)						
Branchial						
m=0.032–0.180 g	8	–1.22	0.16	38	0.62	<0.001
m=0.180–0.450 g	198	0.94	0.18	9	0.79	0.001
Cutaneous	98	0.09	0.07	47	0.04	NS

$\dot{M}_{O_2}$, rate of oxygen uptake; *m*, body mass.
The regression lines take the form $\dot{M}_{O_2}=am^b$.
NS, not significant.

from the regressions presented in Wells and Pinder, 1996), so that area-specific O_2 uptake decreased by almost 50 % from 0.032 to 0.017 μg h^{-1} mm^{-2} between hatch and 0.45 g body mass. Branchial area-specific uptake was much higher (0.095 μg h^{-1} mm^{-2} at hatch) and increased with a mass exponent of 0.37.

Cutaneous oxygen uptake per unit diffusion barrier (diffusion barrier dimensions were calculated from regressions presented in Wells and Pinder, 1996) remained constant during development (Fig. 5; Table 1). Branchial O_2 uptake per unit diffusion barrier, however, changed dramatically over the course of development. At hatch, branchial O_2 uptake per unit diffusion barrier was very high and dropped rapidly until metamorphosis. After metamorphosis, O_2 uptake per unit diffusion barrier increased again with body mass with a mass exponent of 0.935.

Restraining the fish to measure partitioning of O_2 uptake did not significantly alter O_2 uptake. Total O_2 uptakes measured in both partitioned respirometers were not significantly different from measurements in the single-chambered respirometer (Wilcoxon signed-rank test).

Discussion

The yolk sac as a respiratory organ

In the present study, oxygen uptake through the yolk sac has been measured for the first time in any fish larva. Yolk sac O_2 uptake, 33 % of total O_2 uptake (41 % of cutaneous O_2 uptake),

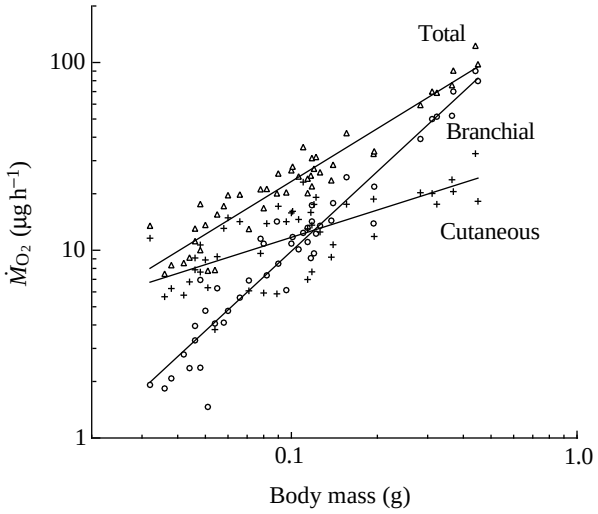

Fig. 2. Partitioning of O_2 uptake between gills and cutaneous surfaces: total O_2 uptake (△), cutaneous O_2 uptake (+) and branchial O_2 uptake (○). Linear regression equations are in presented in Table 1. All regressions are highly statistically significant (P<0.001).

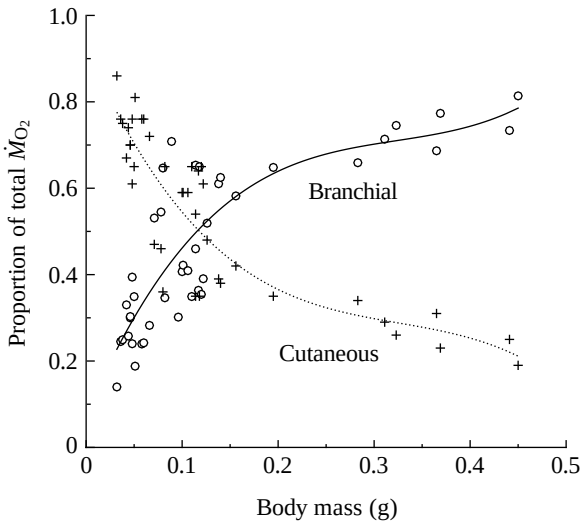

Fig. 3. Proportions of branchial (○) and cutaneous (+) contributions to total O_2 uptake ($\dot{M}_{O_2}$). The lines are best fitted by second-order polynomial regressions.

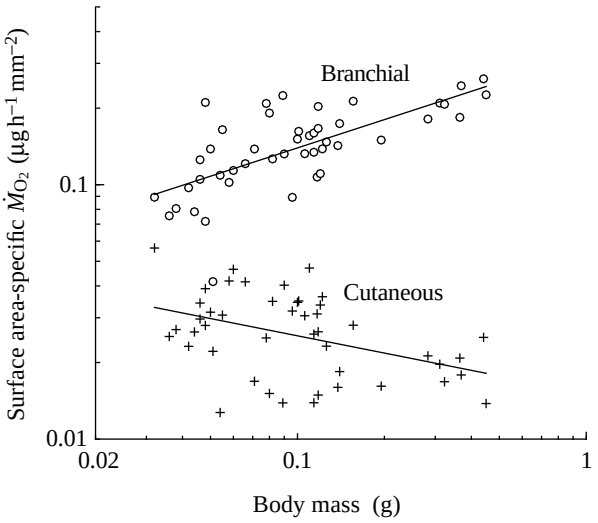

Fig. 4. Surface area-specific O_2 uptake of the gills (○) and cutaneous (+) surfaces as a function of mass during the early developmental phases of Atlantic salmon (*Salmo salar*). Oxygen uptake per unit surface area (mm^{-2}) of the gills more than doubled during the sampling period, whereas cutaneous area-specific O_2 uptake decreased. Areas were calculated from the regressions of surface areas on body mass presented in Wells and Pinder (1996). Equations for the linear regressions are presented in Table 1. Both regressions are highly significant (P<0.002).

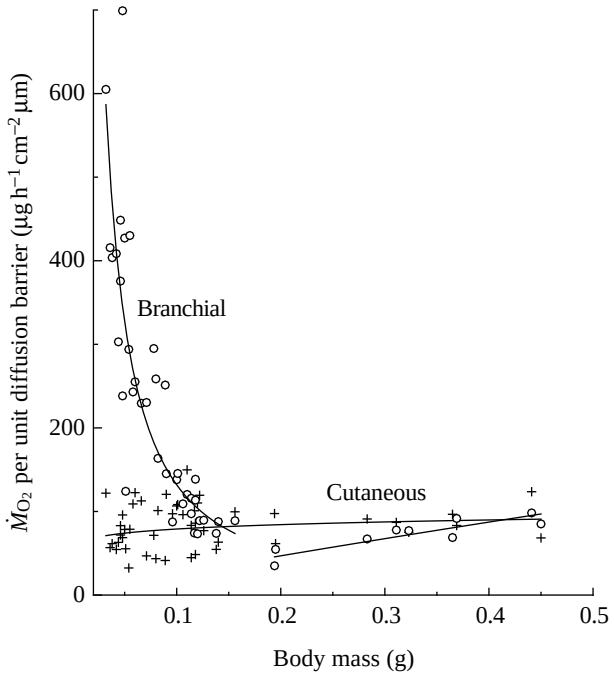

Fig. 5. O_2 uptake per unit diffusion barrier (mass-specific $\dot{M}_{O_2}$/ADF) for the gills (○) and skin (+). Dimensions of diffusion barriers were calculated from the regressions of ADF on body mass presented in Wells and Pinder (1996). The lines are linear regressions on log-transformed data; equations are presented in Table 1. There was no significant change in cutaneous O_2 uptake per unit diffusion barrier, but branchial O_2 uptake per unit diffusion barrier changed significantly both before and after metamorphosis. The break point between the two lines (at a body mass of 0.18 g) is the break point for ADF from Wells and Pinder (1996).

is significant, but the yolk sac is not the main surface for O_2 uptake as was previously hypothesized (Holeton, 1971; Balon, 1975; Peterson, 1975; Blaxter, 1988; Rombough, 1988). Oxygen uptake was lower than predicted from the ADF of the yolk sac (54 % of cutaneous ADF) compared with that of the skin (46 %). The yolk sac is also better vascularized than the general body surface and blood flow appears to be efficiently arranged for countercurrent gas exchange (Olko, 1955; Liem, 1981; Rombough, 1992). Given the morphological potential of the yolk sac for O_2 uptake, it was somewhat surprising that the yolk sac was responsible for less than half of total O_2 uptake just post-hatch, at least in well-oxygenated water.

Larvae of many other fish species also have large well-vascularized yolk sacs, which have also been suggested to be used for O_2 uptake, e.g. lake herring (*Coregonus artedii*, Brooke and Colby, 1980), white sucker (*Catostomus commersoni*, McElman, 1983), brown trout (*Salmo trutta*; Olko, 1955) and *Monopterus albus* (Liem, 1981). Several authors have suggested a correlation between environmental O_2 concentrations and yolk sac vascularization. Blaxter (1988) suggested that salmonids are too large for cutaneous respiration to supply sufficient O_2 by diffusion across the body surfaces; thus, they need pigmented blood and respiratory vessels on the yolk sac to support metabolic O_2 requirements. McElman (1983) suggested that vascularization in larval walleye (*Stizostedion vitreum*) was less than that of larval white sucker (*C. commersoni*) because of differences in dissolved oxygen in lotic (walleye) *versus* benthic (sucker) environments. Larval lake trout (*Salvelinus naymaycush*) reared in hypoxic conditions increased yolk sac vascularization (Garside, 1959), as did larval lake herring (*Coregonus artedii*) (Brooke and Colby, 1980). Balon (1975) suggested that larvae spawned in oxygen-poor habitats have increased vascularization on both the fins and yolk sac. These hypotheses should be tested, given the relatively low contribution of the yolk sac to overall O_2 uptake in the present study and the apparently small influence of yolk sac vascularization on yolk sac O_2 uptake. The yolk sac and circulatory oxygen transport may be more important for oxygen uptake during environmental hypoxia, however, because of the shorter diffusion distances and extensive vascularization of the yolk sac relative to the general body surface.

The higher O_2 uptake per unit diffusion barrier through the body skin compared with the yolk sac (82 compared with 37 μg h^{-1} cm^{-2} μm), despite the much higher vascularization of the yolk sac, suggests that much of the oxygen delivery in just post-hatch salmon embryos is by direct diffusion to tissues rather than by blood circulation. The dense vascularization of the yolk sac is more likely to be for yolk uptake and transport than for gas exchange. Some of the most metabolically active tissues of the fish are distributed peripherally, while less active tissues such as white muscle are more central. The skin itself may consume much of the O_2

taken up. The epidermis of a newly hatched fish, rich with mucous and chloride cells, makes up about 10 % of the total body mass (calculated from the total cutaneous surface area, 156 mm^2, and skin thickness, 20 μm, of a 30 mg newly hatched fish; data from Wells and Pinder, 1996). The metabolic rate of the skin of adult plaice (*Pleuronectes platessa*), which has a much greater proportion of metabolically inactive connective tissue, was almost double the average rate of O_2 consumption of the fish as a whole (Steffensen and Lomholt, 1985). The density of cutaneous capillaries of adult fish does not reflect the cutaneous contribution to total O_2 uptake (Kirsch and Nonnotte, 1977; Nonnotte and Kirsch, 1978; Jakubowski, 1989), again suggesting that oxygen taken up cutaneously is consumed locally and is delivered by direct diffusion. Just under the skin over most of the body in newly hatched larvae there is a layer of red muscle (Wells and Pinder, 1996). Because the red muscle layer has few blood vessels at this stage, it is unlikely that this layer supplies O_2 to other tissues, but the metabolism of the red muscle itself could be supplied by direct diffusion from the exterior. The width of the entire body is less than about 3 mm, so that even some internal organs might be supplied by direct diffusion. The partitioning of oxygen uptake between yolk sac and body surface may shift towards the yolk sac in hypoxia as the driving pressure for direct diffusion to the tissues is reduced. Our measurements were made in well-aerated, stirred water, which would maximize the P_{O_2} adjacent to the skin and favour direct diffusion.

Although the ADF-specific O_2 uptake of the yolk sac was low, the mass-specific O_2 uptake of the yolk sac (not including the mass of the metabolically inactive yolk) was three times higher than that of the body. Three non-exclusive explanations for this high mass-specific O_2 uptake are (1) that there is some transport of O_2 away from the yolk sac, (2) that the yolk sac tissue has a large proportion of epidermis which probably has a high metabolic rate, and (3) that the yolk sac endothelium may have a high metabolic rate associated with yolk resorption. Although the majority of oxygen uptake seems to be by diffusion from the nearest external surface directly to the tissues, the yolk sac may be a gas exchange organ supplying oxygen *via* the circulation to internal organs and the central nervous system in the first few days post-hatch.

Transition from cutaneous to branchial respiration

The transition of O_2 uptake from primarily cutaneous to primarily branchial in larval Atlantic salmon correlates well with a dramatic increase in the branchial surface area (Rombough and Moroz, 1990; Wells and Pinder, 1996) and anatomical diffusion factor (ADF) of the gills (Fig. 6). At hatch, the gills of Atlantic salmon are poorly developed, representing 5 % of total surface area, with an ADF of 8 % of the total (Wells and Pinder, 1996). Branchial surface area, ADF and O_2 uptake increase in concert as the gills develop their mature morphology. There must also be increases in gill perfusion; the gills cannot act as a gas exchange organ without circulatory transport of oxygen to other tissues.

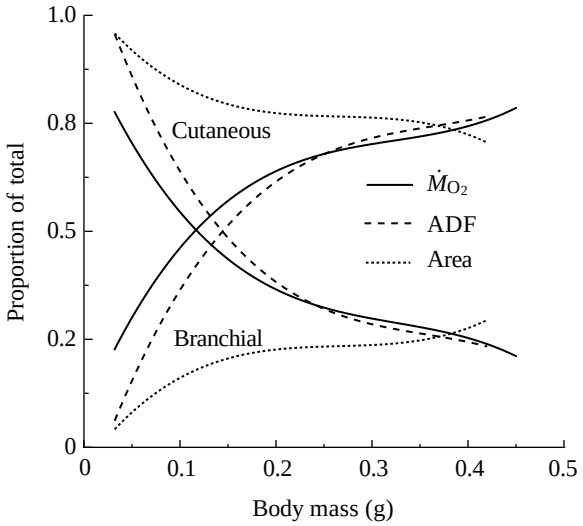

Fig. 6. Changes in O_2 uptake partitioning between skin and gills compared with changes in surface area and ADF. Lines are best-fitting second-order polynomial regressions. Surface area data are taken from Wells and Pinder (1996).

Branchial O_2 uptake per unit surface area doubles between hatch and the end of yolk resorption, similar to the approximately threefold increase reported in larval chinook salmon (Rombough and Ure, 1991). This increase in the efficiency of branchial O_2 uptake (O_2 uptake per unit surface area) is associated with a decrease in branchial diffusion distances. Just after hatch, the 'gills' are filaments without lamellae; lamellae appear and start to dominate the branchial surface area by the end of yolk resorption (Wells and Pinder, 1996). Because the filaments have a diffusion distance of 14 μm compared with 3 μm in lamellae, the average diffusion distance across the gills decreases dramatically as the lamellae proliferate, and ADF increases much faster than surface area.

ADF gives a much better estimate of gas exchange potential than does surface area alone (Fig. 6) because it incorporates a measure of the thickness of the gas exchanger. Rombough and Ure (1991) related changes in partitioning of O_2 uptake to changes in surface areas of gills and skin. The proportion of branchial O_2 uptake was much higher, and cutaneous O_2 uptake lower, than would be predicted from the partitioning of surface area. This discrepancy between the partitioning of O_2 uptake between skin and gills can be explained by the difference in diffusion distances across the two surfaces.

If ADF is a good predictor of gas exchange capacity, mass-specific O_2 uptake per unit ADF (O_2 uptake per unit diffusion barrier) should remain constant as the animal grows, the cutaneous surface area to volume ratio decreases, and the diffusion distances across the skin and gills change. The ratio of O_2 uptake per unit diffusion barrier was, in fact, constant for skin throughout development (Fig. 5). Just post-hatch, however, branchial O_2 uptake per unit diffusion barrier was very high, decreasing markedly as lamellae develop. After metamorphosis, branchial O_2 uptake per unit diffusion barrier increased again.

The relatively high branchial O_2 uptake per unit diffusion barrier just after hatching might be explained by a combination of high local oxygen consumption and underestimating the true ADF. Our measure of branchial O_2 uptake included gills, buccal cavity and opercular cavity, whereas the ADF was estimated only for the gills. Before lamellae develop on the gills, a large proportion of the gas exchange surface in the branchial cavity may be the walls of the cavities themselves. Oxygen consumption of tissues in the head may also be higher than the average for the rest of the body. Rombough (1992) suggested that the gills of newly hatched fish may consume O_2 locally, reflecting the cost of ionoregulation. It has been estimated that adult fish may consume up to 20 % of metabolic O_2 for ionoregulation (e.g. Roa, 1968). Ionoregulatory costs have not been estimated, however, either in larval salmonids or when the main site of ionoregulation shifts from the body surface to the gills. The constantly contracting buccal ventilatory muscles are also immediately adjacent to the buccal cavity. The cost of branchial ventilation in rainbow trout, estimated from the decrease in metabolic rate during the transition from branchial pumping to ram ventilation, was approximately 10 % of resting metabolic rate (Steffensen, 1985). The cost of ventilation in larval fish is unknown. The heart and brain are also very close to the branchial cavity. Thus, local O_2 consumption may be quite high and, in combination with direct diffusion of O_2 to these tissues, may help explain the very high branchial O_2 uptake per unit ADF in just-hatched larvae.

The low branchial O_2 uptake per unit diffusion barrier at metamorphosis and its increase with further growth may be the result of increasingly effective gill ventilation. Unlike some fish, newly hatched salmonids do ventilate their gills (Peterson, 1975; Rombough, 1988; Rombough and Moroz, 1990). The regularity and coordination of ventilatory movements improve during early development (Peterson, 1975), however, and the orientation of branchial water flow changes from cross current to countercurrent (Rombough and Moroz, 1990).

If the main determinants of gas exchange capacity are surface area and diffusion distance, rather than physiological variables such as ventilation, perfusion or P_{O_2} differences across the surface, then O_2 uptake per unit diffusion barrier of different gas exchangers should be roughly similar. Skin and gills do have similar O_2 uptakes per unit diffusion barrier after metamorphosis (Fig. 5), despite obvious differences in ventilation, perfusion and structure. Thus, ventilation and perfusion may be grossly matched to the morphological gas exchange potential of the various surfaces. A close match between O_2 uptake partitioning and ADF would not be expected because ADF does not take into consideration changes in the effective surface area associated with changes in the perfusion of gills (Booth, 1979) or skin (Pinder *et al.* 1991) or differences in ventilation and boundary layer thickness. The difference in O_2 uptake per unit diffusion barrier between body skin and yolk sac also provides a caveat for using ADF as a predictor for O_2 uptake capacity; the higher O_2 uptake per unit diffusion barrier of body skin appears to be related to the metabolic rate of tissues underlying the body skin compared with the yolk sac, not to the gas exchange surfaces themselves.

The findings of the present study may be summarized as follows. (1) At hatch, O_2 uptake through the yolk sac is 33 % of total oxygen consumption. The yolk sac is never the main site of O_2 uptake. (2) Oxygen uptake per unit anatomical diffusion factor (ADF) is twice as high for the non-vascularized skin of the body as for the well-vascularized yolk sac; thus, O_2 delivery is probably by direct diffusion to tissues rather than by the circulation. (3) Changes in the partitioning of O_2 uptake between the gills and skin are related much more closely to ADF than to surface areas alone, but the distribution of ADF can give only a very rough approximation of the distribution of O_2 uptake.

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