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THE SCALING AND POTENTIAL IMPORTANCE OF CUTANEOUS AND BRANCHIAL SURFACES IN RESPIRATORY GAS EXCHANGE IN LARVAL AND JUVENILE WALLEYE *STIZOSTEDION VITREUM*

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Summary

Measurements were made of the surface areas (A_s) of the skin and gills of larval and juvenile walleye *Stizostedion vitreum* with a body mass (M) of between 2 mg (1 day post hatch) and 2.3 g (98 days post hatch). The skin, with a relative surface area (A_s/M) of approximately 8500 $mm^2 g^{-1}$, accounted for more than 99.9 % of the total surface area (skin + gills) at 1 day post hatch. The relative area of the skin decreased as fish grew at an allometric rate of $b-1=-0.32\pm0.01$ (mean ± S.E.M., where $b-1$ is the specific-mass exponent in the allometric equation $Y\times M^{-1}=aM^{b-1}$, in which Y is surface area and a is a constant). The relative surface area of the gills (filaments + lamellae) increased in a hyperbolic fashion from very low levels (approximately 5 $mm^2 g^{-1}$) at 1 day post hatch to reach a maximum of approximately 1100 $mm^2 g^{-1}$ at a body mass of approximately 200 mg. Thereafter, relative gill area declined at an allometric rate of $b-1=-0.19\pm0.10$ (mean ± S.E.M.). Gill area, because it declined at a slower relative rate, finally exceeded skin area at a body mass of approximately 700 mg. The relative surface area of the skin and gills combined (total surface area) decreased at a more-or-less constant allometric rate of $b-1=-0.21\pm0.01$ (mean ± S.E.M.) throughout the experimental period. On the basis of the allometric rates of expansion, the structural capacity to supply oxygen ($b-1=-0.19$; total gill area, this study) and metabolic demand for oxygen ($b-1\approx-0.13$; mean literature value for routine and resting metabolism) appear to remain fairly closely matched in postlarval walleye (>300 mg). The two parameters do not display the same degree of concordance during larval development. In larvae, total respiratory surface area declines on a mass-specific basis at roughly the same rate ($b-1=-0.21$) as gill area does in older fish but, unlike in older fish, metabolic demand for oxygen does not change ($b-1\approx0.0$). This results in a progressive decline in effective respiratory surface area ($A_s/\dot{M}_{O_2}$) but does not affect O_2 uptake, probably because larvae are so small that surface area is not the limiting factor in gas exchange. Analysis of data from the literature suggests that surface area typically becomes limiting at a body mass of approximately 100 mg. The major function of gills in smaller larvae (<100 mg) appears to involve ionoregulation or related aspects of acid–base balance rather than respiratory gas exchange.

Key words: scaling, skin, gill, oxygen consumption, respiratory gas exchange, ionoregulation, walleye, fish, larva, *Stizostedion vitreum*.

Introduction

The concept of symmorphosis, the idea that the capacity of structural elements should match but not exceed the functional requirements of the organism, has been advanced as a testable hypothesis for examining structure–function relationships (Weibel *et al.* 1991). While this concept was developed initially in the context of the mammalian respiratory system (Taylor and Weibel, 1981), it has been used as a framework for examining structure–function relationships in other taxa, including fish. In general, it appears that respiratory surface area and metabolic demand for oxygen are closely matched in juvenile and adult fish (Goolish, 1995). With most fish, the gill is the primary site of gas exchange during adult life. Adults of active species tend to have proportionately larger gills than sluggish species (Gray, 1954; Hughes, 1966), just as one would predict if symmorphosis applied. Also as predicted, gill area and oxygen usage expand at roughly the same rate as fish grow (Pauly, 1981; Hughes, 1984*a*), while surgical procedures that effectively reduce gill area result in an immediate and proportionate decrease in aerobic capacity (Duthie and Hughes, 1987).

At present, it is not clear whether respiratory surface area and metabolic demand for oxygen are as closely matched in fish larvae as they are in later life. This uncertainty arises, in part, because of the difficulty investigators have had in

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defining and accurately measuring respiratory surface area in larvae. Gas exchange in larvae, unlike that in adult fish, is not effectively restricted to the gills. Most larvae hatch with only rudimentary gills and as a consequence the skin plays a much more important role than it does in older fish (Rombough, 1988*a*). Even salmonid larvae, which hatch with relatively well-developed gills compared with those of most species, initially obtain over 80 % their oxygen across the skin (Rombough and Ure, 1991; Wells and Pinder, 1996*b*). This means that to obtain a meaningful estimate of exchange capacity in larvae one must measure the surface area of the skin as well as the gills, something that few investigators have done. Matters are further complicated by the fact that the relative size and importance of various subregions within the skin and the gills change as development proceeds. Subregions that play little role in gas exchange in adults may be much more important in larvae. For example, gill filaments play only a very minor role in gas exchange in adult fish but appear to be a major site of branchial gas exchange during much of larval development (Rombough and Ure, 1991; Wells and Pinder, 1996*a*). Unfortunately, the tendency has been to assume that if a structure is not important in adults it is probably not important in larvae, so filament area has seldom been taken into account. To confuse matters further, the results of the two most complete studies to date are contradictory. Rombough and Moroz (1990) reported a poor correlation between respiratory surface area and metabolic demand for oxygen in larvae of chinook salmon *Oncorhynchus tshawytscha*. The specific-mass exponent (b–1 in the allometric equation $Y \times M^{-1} = aM^{b-1}$, where Y is the parameter under consideration and M is tissue mass) for total surface area in chinook larvae was approximately –0.61, while that for the rate of oxygen consumption was only approximately –0.05 (Rombough and Moroz, 1990). Wells and Pinder (1996*a*,*b*), in contrast, reported specific-mass exponents for total surface area (b–1=–0.05; Wells and Pinder, 1996*a*) and metabolic rate (b–1=–0.06; Wells and Pinder, 1996*b*) for larvae of Atlantic salmon *Salmo salar* that are virtually identical, suggesting that, as in adults, the two parameters are closely linked.

The aim of this study was to try to determine which pattern, the apparent match between respiratory surface area and metabolic oxygen demand seen in Atlantic salmon or the mismatch observed in chinook, is the norm for fish larvae by looking at how respiratory surface area changes during the course of larval development in a third, unrelated species. The study did not look at oxygen demand as such because the evidence is now pretty convincing that for most of larval development mass-specific oxygen uptake is independent of body mass (i.e. b–1=0.0; reviewed by Giguere *et al.* 1988; Rombough 1988*a*; Post and Lee, 1996). The species chosen for the study was the walleye *Stizostedion vitreum*. The larvae of walleye are, in a number of respects, more typical of teleost larvae generally than are the larvae of salmonids. Like most species, they are considerably smaller (3 mg *versus* 20–30 mg wet mass at hatch) and, because of their pelagic life style, more active than salmonids. Perhaps most importantly in terms of respiratory surface area, the yolk sac is relatively much smaller in walleye. The yolk sac is different from other potential respiratory surfaces in that it declines in absolute, not just relative, size as larvae grow. This tends to depress the overall rate of surface area expansion. The impact is especially important in species, such as the chinook, with large yolk sacs. In chinook, the yolk sac accounts for approximately 60 % of skin area at hatch and takes approximately a month to be absorbed into the body wall. As a result, relative skin area in chinook declines at a rapid rate (b–1=–0.74; Rombough and Moroz, 1990) even though the relative areas of subregions of the skin other than the yolk sac decrease much more slowly (i.e. b–1≈–0.33; Rombough and Moroz, 1990). In walleye, the yolk sac accounts for only approximately 10–15 % of skin area at hatch and is rapidly absorbed (4–6 days at 20 °C). The influence of the yolk sac on total skin area, therefore, should be minimal which should make it easier to recognize and interpret any patterns that arise.

Materials and methods

Newly hatched walleye *Stizostedion vitreum* (Mitchill) were obtained from the Freshwater Institute, Department of Fisheries and Oceans, Winnipeg, Manitoba, Canada. One group of fish was transferred to Brandon University and raised in the laboratory on a mixed diet of live *Artemia* sp. and commercial fish flakes, while another group was transferred to outdoor ponds where they fed on natural foods. The laboratory-reared fish were held at a constant mean temperature of 20.6 °C and exposed to a 16 h:8 h light:dark artificial photoperiod. The fish raised in the outdoor ponds were subject to natural variations in temperature and photoperiod.

The laboratory-reared fish were sampled 1, 4, 8, 12, 16 and 31 days post hatch. The pond-reared fish were sampled 29, 42, 54 and 98 days post hatch. Fish were killed and, with one exception, immediately preserved in Bouin's fixative (one fish in the 16 days post hatch sample was fixed in 4 % neutral buffered formalin; measurements for this fish fell within the range of those for Bouin's-fixed fish of the same age, so it was included in the analysis). Fish were kept in the preservative for a minimum of 30 days to ensure that any fixative-induced shrinkage was complete before measurements were taken. Body shrinkage for young walleye preserved in formalin-based fixatives such as Bouin's has been shown to be in the range 2.0–7.6 % (Glenn and Mathias, 1987). Once fixation was complete, the fish were weighed and lightly stained with Grenacher's borax carmine (Humason, 1972) to facilitate handling and to make it easier to take measurements of surface area. Measurements were made of skin area in 56 fish. Forty fish were used for gill measurements.

Slightly different measurement techniques were used for small (<60 mg wet mass) and large (>60 mg) fish. It was difficult to avoid damaging the gills while measuring the skin area of fish smaller than 60 mg, so different individuals taken from the same sample group were used for skin and gill measurements. The surface areas of the head, trunk and

unpaired fins of the small fish were estimated by projecting the image of the fish through a Bausch and Lomb trisimplex microprojector onto a digitizing tablet (Jandel Scientific, model 2210). Projected images were calibrated using a stage micrometer (Bausch and Lomb). The various surfaces were traced and their areas determined using a computerized measurement system (Sigma Scan 3.0, Jandel Scientific). The surface area of the paired fins was estimated by removing them from the body, mounting them on glass slides and projecting their images onto the digitizing tablet. All four gill arches were removed from the right side of the small fish and carefully mounted on glass slides in glycerin jelly. Images of the gills were projected through a tracing device attached to a Leitz Dialux 20 microscope onto the digitizing tablet. Measurements were made of the total length of each arch and the length occupied by filaments. The number of filaments on each hemibranch of the arch was counted, and the length and surface area of each filament were measured. Counts were made of the total number of secondary lamellae. The surface areas of all the lamellae oriented at right angles to the plane of view (generally approximately 50 % of the total number) were measured, and the mean value was used to estimate total lamellar area.

For fish weighing more than 60 mg, it was possible to measure both skin and gill surface areas of the same individual. The trunk area of the larger fish was determined by removing the skin in small pieces, flattening the pieces between a glass slide and coverslip, and projecting the images of the pieces onto the digitizing tablet. The surface area of the head was estimated from a projection of the intact head. Paired as well as unpaired fins were removed from the body and projected onto the digitizing tablet. All four gill arches were removed from the right side of the fish and dissected into anterior and posterior hemibranchs. Each hemibranch was divided into four segments so they would fit on glass slides. The segments of the posterior hemibranch were left intact. Filaments were carefully removed from the segments of the anterior hemibranch and mounted in glycerin jelly. Images of the posterior hemibranch and the filaments from the anterior hemibranch were projected onto the digitizing tablet, and the length and area of each filament were recorded. The number of secondary lamellae on each filament was recorded. Mean lamellar area was estimated using a sampling procedure similar to that proposed by Hughes (1984*b*). Measurements were made of three lamellae from each of twelve filaments taken at roughly equal intervals along the length of the anterior hemibranch of the second right arch. Representative lamellae were chosen from the tip, middle and base sections of each filament. Estimates of total lamellar area were based on the mean value.

Results

The area available for respiratory gas exchange across the skin surface greatly exceeded that across the gills throughout larval development (Fig. 1). At hatch, the skin was estimated to account for more than 99.9 % of the combined surface area

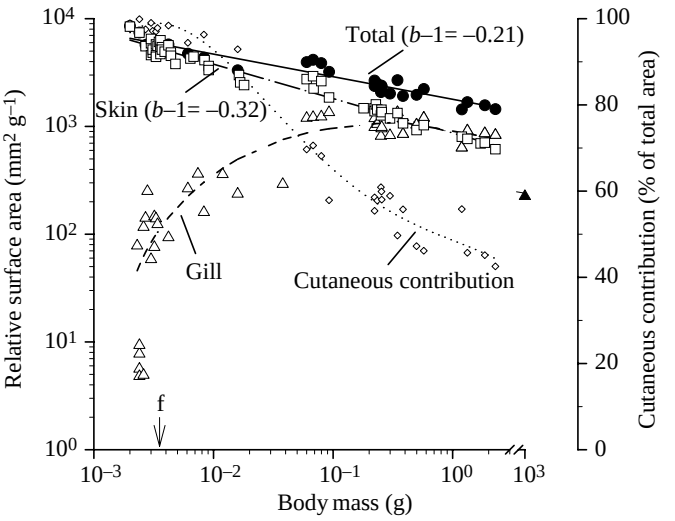

Fig. 1. Changes with growth in the relative surface areas of the skin and gills of young walleye *Stizostedion vitreum*. Values for the combined area of the skin plus the gills (Total) are plotted for individual fish weighing more than 60 mg (individual values are not shown for fish weighing less than 60 mg because different individuals were used for the skin and gill measurements; see text). Values for the fractional contribution of the skin to total area are for individuals weighing more than 60 mg. Values for fish weighing less than 60 mg are estimates from paired values for skin and gill areas of similarly sized, but different, individuals from the same sample. The filled triangle indicates the relative surface area of the gills for a 1 kg adult (Niimi and Morgan, 1980). The line leading into the triangle is the projected gill area at 1 kg based on the allometric relationship between relative gill area and body mass of fish weighing between 300 mg and 2.3 g. f indicates mass at first feeding; *b*–1 is the specific-mass exponent for the allometric growth relationship $Y \times M^{-1} = aM^{b-1}$, where Y is surface area, M is body mass and a is a constant.

of the skin and gills. The relative contribution of the skin declined as development proceeded but it was not until the fish reached a mass of approximately 700 mg, by which time they were well into the juvenile stage, that branchial surface area finally exceeded cutaneous surface area. The relative surface area (surface area per unit body mass) of the skin declined at a constant allometric rate of *b*–1=–0.32±0.01 (mean ± S.E.M., *N*=56) throughout larval and early juvenile development. The relative surface area of the gills varied in a more complex fashion. During larval development, the relative surface area of the gills increased in a roughly hyperbolic manner to reach a maximum of approximately 1100 $mm^2\,g^{-1}$ at a body mass of approximately 200 mg (Fig. 1). Thereafter (0.3–2.3 g), relative gill area declined at an allometric rate of *b*–1=–0.19±0.10 (mean ± S.E.M., *N*=10). The relative surface area of the skin and gills together (total area) declined at a more-or-less constant allometric rate of *b*–1=–0.21±0.01 (mean ± S.E.M., *N*=30) throughout larval and early juvenile development.

The head and trunk account for the bulk of cutaneous surface area throughout larval and early juvenile development (Fig. 2). The fins are somewhat more important in small larvae, where they account for approximately 35 % of total skin surface.

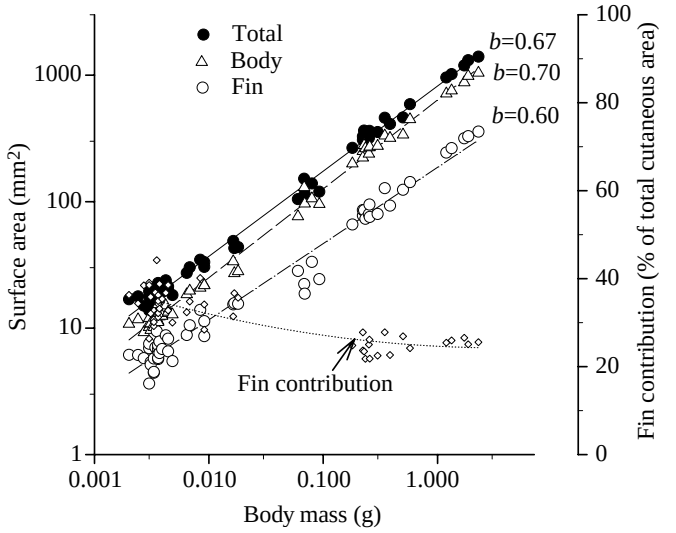

Fig. 2. Changes with growth in the absolute areas of various cutaneous surfaces. Allometric mass exponents (*b*) derived from the slopes of the double logarithmic plots were all highly significant ($P<0.01$).

However, once the finfold is resorbed (at approximately 30 mg), the fraction of skin area represented by the fins stabilizes at approximately 25 % of total skin area.

The gills of walleye are poorly developed at hatch. In 1-day-old larvae, no secondary lamellae and only a few primary filaments on the posterior hemibranch of the third gill arch are present (Fig. 3). Development of respiratory surfaces on the various gill arches proceeds asynchronously. Filaments begin to form on the anterior hemibranchs of arches III and IV and on the posterior hemibranchs of arches I and II approximately 4 days post hatch at an incubation temperature of 20 °C (Fig. 3). Filaments appear on the posterior hemibranch of arch IV approximately 12 days post hatch and on the anterior hemibranch of arch I at approximately 16 days post hatch. Filaments initially form near the epibranchial–ceratobranchial junction and are added sequentially in both directions until the arch is fully occupied. Arch III is filled (filaments on more than 90 % of the arch) first, followed by arches II, IV and finally I. It takes until approximately 32 days post hatch before arch I is completely filled. Lamellae first appear on filaments of the anterior and posterior hemibranchs of arch III approximately 4 days post hatch (Fig. 3). They next appear on both hemibranchs of arch II (8 days post hatch), followed by the posterior hemibranch of arch I and the anterior hemibranch of arch IV (12 days post hatch). Lamellae are present by 16 days post hatch on the anterior hemibranch of arch I, but do not appear on the posterior hemibranch of arch IV until approximately 32 days post hatch.

At hatch, the surface area of the gills is very small (approximately 0.01 mm^2) and, because there are no secondary lamellae, the only surfaces available for respiratory gas exchange are on the primary filaments and the gill arches. The combined surface area of the filaments expands throughout larval and early juvenile development, although the rate of expansion decreases as the fish get older (Fig. 4). Secondary lamellae first appear approximately 4 days post hatch but, because there are so few, their combined area initially accounts for only a minor fraction of the total surface area of the gill. The rate of lamellar expansion, however, is more rapid than that of filaments so that as development proceeds the lamellae come to account for a progressively greater fraction of total gill area (Fig.

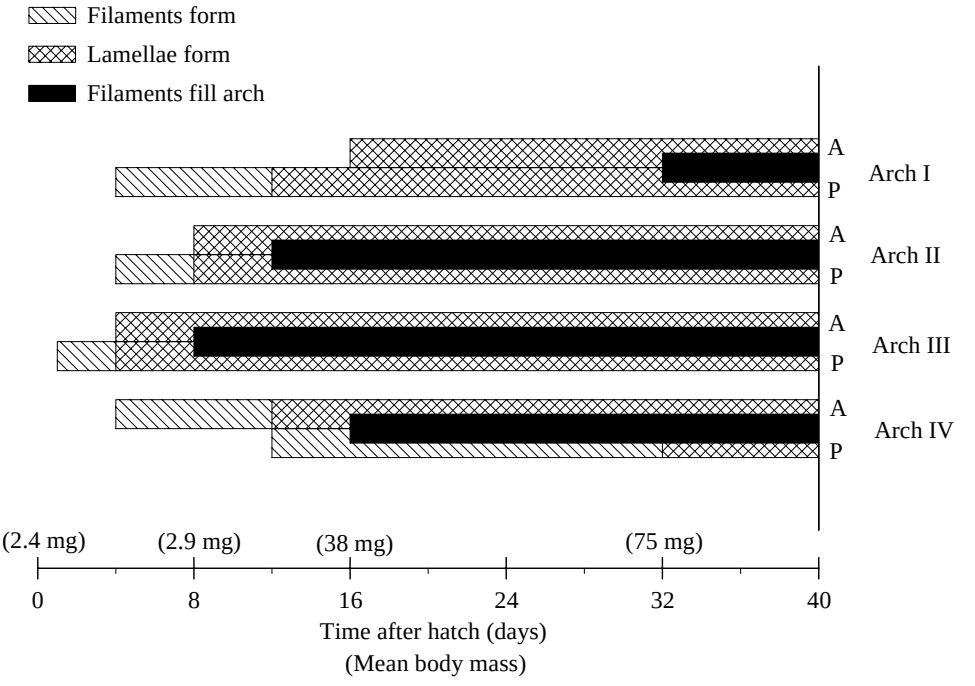

Fig. 3. Stages at which filaments (hatched bars) and lamellae (cross-hatched bars) first form on the anterior (A) and posterior (P) hemibranchs of each of the four gill arches in walleye at an incubation temperature of 20 °C. The filled bars indicate when filaments first occupy 90 % of the available space on both hemibranchs.

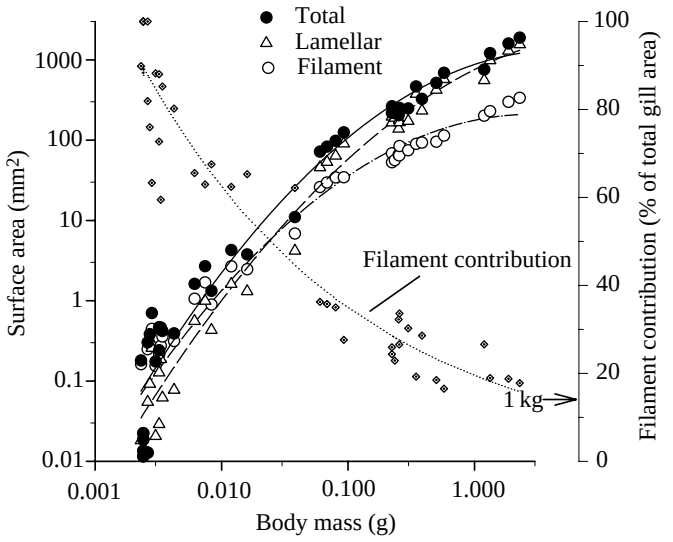

Fig. 4. The patterns of expansion of combined filament surface area, combined lamellar surface area and total gill surface area (filaments plus lamellae) with growth. Lines are best-fitted second-order polynomials. The arrow shows the contribution of filaments to total gill area for a 1 kg adult walleye (Niimi and Morgan, 1980).

4). Lamellar surface area finally exceeds filament surface area when fish reach a mass of approximately 23 mg (13 days post hatch). As was found for filaments, the rate of lamellar expansion tends to decrease as development proceeds. In both cases, the shift to slower rates of expansion appears to occur gradually. No transition points were identified when the log-transformed data relating the various branchial surface areas (filamental, lamellar and total) and body mass were tested for discontinuities using the algorithm of Yeager and Ultsch (1989).

The expansion of filament area is initially due mainly to an increase in the number of filaments. Growth in the size of individual filaments becomes progressively more important as development proceeds, and it is the major determinant of total filament area in fish larger than approximately 200 mg (Fig. 5). The increase in the number of filaments (F_n) did not follow the standard allometric relationship, $Y=aM^b$, but was better described by the simple semilogarithmic relationship $F_n=1027+374\log M$ ($r^2=0.985$). The standard allometric model, $F_a=0.23M^{0.80}$, did provide a reasonably good fit ($r^2=0.965$) for the data relating mean filament area (F_a) and body mass. The distance between adjacent filaments did not change significantly ($P>0.05$) as the number of filaments increased during the process of filling the gill arch. The mean spacing of filaments on the occupied portion of the arch was 21.5±4.1 mm^{-1} (mean ± S.D., $N=20$).

The major determinant of total lamellar area in small larvae was the number of lamellae (Fig. 6). However, as with filaments, the rate of proliferation of lamellae tended to decline and the increase in the size of individual lamellae became progressively more important as development proceeded. A second-order polynomial relationship $\log L_n=4.66+0.333\log M-0.286\log M^2$ (Fig. 6) gave the best fit to the data relating lamellar number (L_n) and body mass ($r^2=0.977$). As with filament area, the standard

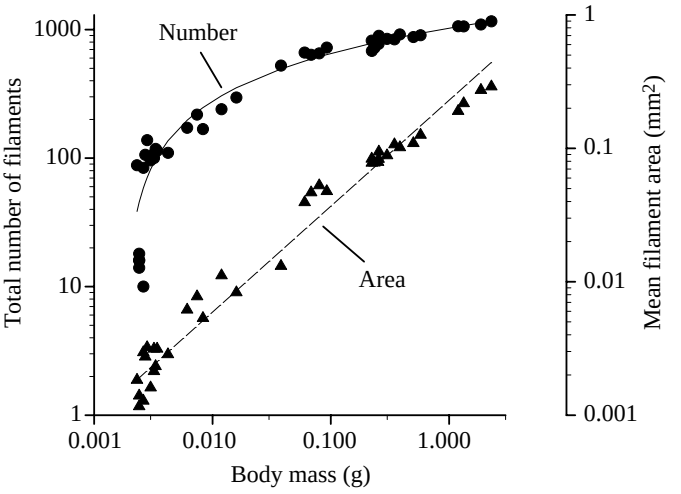

Fig. 5. The patterns of expansion in the total number of filaments on all four gill arches and the mean surface area of individual filaments with growth. Equations for the plotted lines of best fit are given in the text.

allometric equation, $L_a=0.018M^{0.56}$, provided a reasonably good fit to the data relating bilateral lamellar area (L_a) and body mass ($r^2=0.947$). The spacing of lamellae on the filament decreased in a hyperbolic fashion from approximately 20 mm^{-1} in the youngest larvae towards an asymptotic value of approximately 60 mm^{-1} in juvenile fish (Fig. 6).

The relative contribution of the various gill arches to total gill area (filaments + lamellae) was quite different in larvae

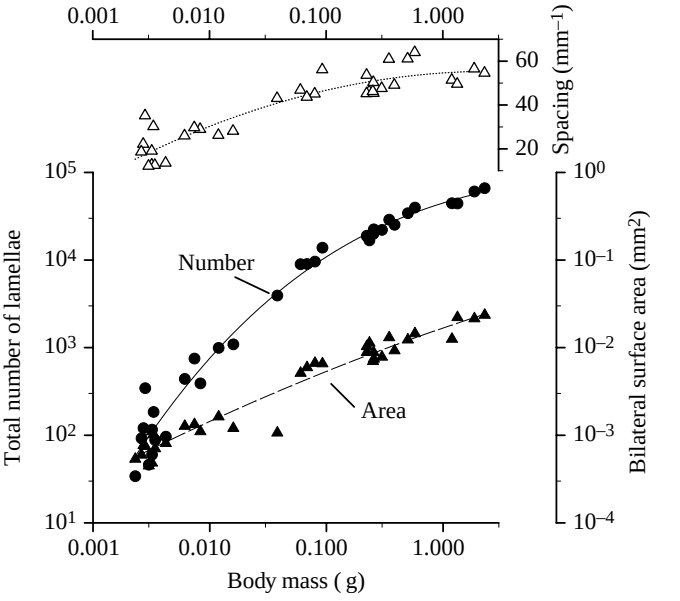

Fig. 6. The patterns of expansion in the total number of lamellae on all four gill arches and the change in the mean surface area of individual lamellae with growth. The spacing of lamellae on the filament gradually decreased from approximately 20 mm^{-1} at 4 days post hatch to an asymptotic value of approximately 60 mm^{-1} in a 1 g fish. Equations for the plotted lines for lamellar number and mean area are given in the text.

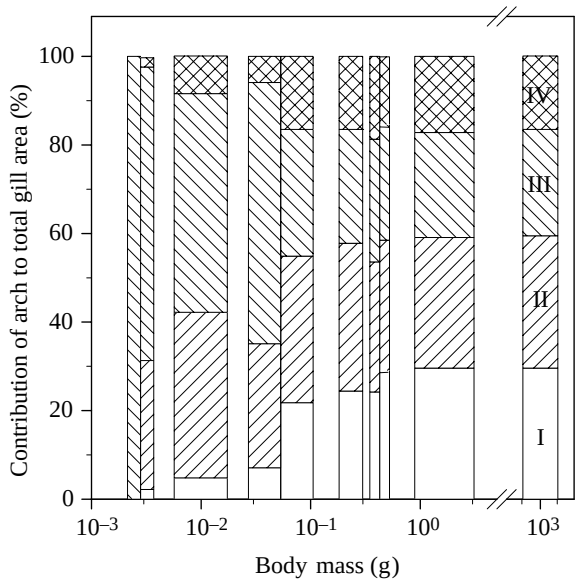

Fig. 7. The fraction of total gill area represented by each gill arch for walleye of different sizes. The proportions for the 1 kg fish are taken from from Niimi and Morgan (1980). Roman numerals indicate gill arches I to IV.

from that in adult fish (Fig. 7). Arch III provided 100 % of available surface area in newly hatched larvae and remained the largest single contributor in fish weighing less than 75 mg. Arch II was also important in small larvae and eventually surpassed arch III as the major contributor to total gill area. Arches I and IV remained minor contributors until the fish reached approximately 38 mg, after which they underwent fairly rapid expansion. It was not until the fish weighed approximately 230 mg, however, that the relative contributions of the various arches approached that observed in adult fish.

Discussion

Respiratory surface area and metabolic demand for oxygen do not appear to be closely matched during larval development in walleye. As mentioned in the Introduction, the mass-specific rate of oxygen consumption in fish larvae is typically independent of body mass (i.e. $b-1=0.0$; Giguere *et al.* 1988; Rombough 1988*a*; Post and Lee, 1996). Preliminary experiments in our laboratory confirmed that this holds true for walleye larvae for at least the first 12 days post hatch at 20 °C (I. Manns, Brandon University, unpublished data). Total respiratory surface area, in contrast, declines on a mass-specific basis at a rate equivalent to $b-1=-0.21$ (Fig. 1). This means that respiratory surface area per unit oxygen uptake effectively declines as walleye larvae grow. A progressive decline in effective respiratory surface area with growth as a result of a mismatch between rates of expansion of respiratory surface area and metabolic demand for oxygen appears to be the norm for fish larvae. A survey of the literature found that, on a mass-specific basis, respiratory surface area decreased at a significantly faster rate than metabolic demand for oxygen, not only in walleye but also in chinook salmon *Oncorhynchus tshawytscha*, herring *Clupea harengus* and plaice *Pleuronectes platessa* (Table 1). Indeed, to date, the only species in which rates of change appear to be even approximately matched is Atlantic salmon (Table 1).

There appears to be a shift in the relationship between respiratory surface area and oxygen demand near the end of larval development. In walleye, as in most other fish species (Hughes, 1984*a*; Hughes and Al-Kadhomiy, 1988; Rombough and Moroz, 1990), changes in respiratory surface area appears to be closely linked to changes in metabolic demand for oxygen once larval development is completed. Relative gill area in walleye weighing more than 300 mg declined at a rate equivalent to an allometric specific-mass exponent (mean ± S.E.M.) of $b-1=-0.19\pm0.10$ (Fig. 1). This is not significantly different from the rate at which mass-specific metabolic rate measured under resting ($b-1=-0.12$; Tarby, 1981) or routine ($b-1=-0.14$; Cai and Summerfelt, 1992) conditions is reported to decrease with growth in juvenile and adult walleye. The fact that our results for small juvenile walleye are consistent with the results of similar studies involving adults of walleye and other species suggests that our methods for measuring surface

Table 1. *Literature values for specific-mass exponents (*b*–1) for respiratory surface areas and metabolic rates of fish larvae derived from the allometric equation* $Y\times M^{-1}=aM^{b-1}$, *where* Y *is either surface area or metabolic rate,* M *is body mass and* a *is a constant*

	Mass exponent				
Species	Skin area	Gill area	Skin + gill area	Metabolic rate	Reference
Walleye (*Stizostedion vitreum*)	–0.32	2.60	–0.21	≈0.0	This study (preliminary data)
Herring (*Clupea harengus*)	–0.42	2.36*	–0.41*	–0.18	De Silva, 1974 (De Silva and Tytler, 1973)
Plaice (*Pleuronectes platessa*)	–0.50	0.59*	–0.49*	–0.35	De Silva, 1974 (De Silva and Tytler, 1973)
Carp (*Cyprinus carpio*)	–0.33	6.07*		–0.02	Oikawa and Itazawa, 1985 (Kamler, 1972)
Flounder (*Platichthys flesus*)		1.21*		–0.15	Hughes and Al-Kadhomiy, 1988
Rainbow trout (*Oncorhynchus mykiss*)		2.44*		0.05	Hughes and Al-Kadhomiy, 1988 (Rombough, 1988*b*)
Chinook salmon (*Oncorhynchus tshawytscha*)	–0.74	1.50	–0.61	–0.05	Rombough and Moroz, 1990
Atlantic salmon (*Salmo salar*)	–0.19	0.97	–0.05	–0.06	Wells and Pinder, 1996*a* (Wells and Pinder, 1996*b*)

*Filament area not measured.

References for metabolic rate are in parentheses if different from those for surface area.

area were reasonably accurate and that the mismatch observed in larvae is probably real. The question that needs to be answered then is not whether, but rather why, there is a mismatch between respiratory surface area and oxygen demand in larvae.

One possibility is that surface area, on its own, simply is not a sufficient indicator of overall respiratory gas exchange capacity in a complex, changing system such as that encountered during larval development (Hughes and Al-Kadhomiy, 1988; Rombough and Moroz, 1990). Surface area is only one of the parameters that determines gas exchange capacity; the thickness of the diffusive barrier, the degree to which the gas exchanger is perfused and the extent to which it is ventilated are also important. In adult fish, the functional anatomy of the gills and the manner in which they are ventilated remain more or less constant. The only parameter to change significantly with growth is the surface area of the gill. Changes in gill surface area, thus, can be used as a surrogate for changes in gas exchange capacity. The situation is more complex in larvae; not only are there ontogenetic changes in the relative areas of the various surfaces involved in respiratory gas exchange, but there are also major changes in some of the other diffusive parameters. To our knowledge, Wells and Pinder (1996*b*) have made the only attempt to quantify the impact of any of these changes. In particular, they noted that the thickness of the blood–water barrier (δ), as well as the surface area (A_s), appears to be an important descriptor of the exchange capacity of larval respiratory surfaces. In their study of Atlantic salmon, they found that the anatomical diffusion factor (ADF=A_s/δ), which takes both variables into account, was a better predictor of the measured exchange capacities of the gills and skin than was surface area alone (Wells and Pinder, 1996*b*). The anatomical diffusion factor, however, did not appear to be a complete descriptor of aerobic capacity in Atlantic salmon larvae, especially during the early stages of larval development. Wells and Pinder (1996*b*) speculated that, for early larval stages, changes in physiological variables such as ventilation and perfusion rates, P_{O_2} gradients and differences in the thickness of the boundary layer may also be important. Unfortunately, these variables are difficult to quantify, so it will probably be some time before they can be incorporated into a useful indicator.

An alternative explanation for the poor correlation between surface area and aerobic metabolism in larvae is that, unlike in adult fish, surface area is not a limiting factor in respiratory gas exchange (Wells and Pinder, 1996*a*; Rombough, 1997). Larvae have larger surface areas relative to their body mass than do adult fish. They also have relatively higher metabolic rates but, because mass-specific metabolic rates are independent of body mass during much of larval development, larval rates are not as high as one would predict on the basis of the allometric relationship between metabolic rate and body mass seen in juvenile and adult fish (Fig. 8). The net result of this difference in scaling is that larvae may have 'excess' diffusive exchange capacity compared with adult fish. For example, the relative respiratory surface area of a newly hatched walleye larva

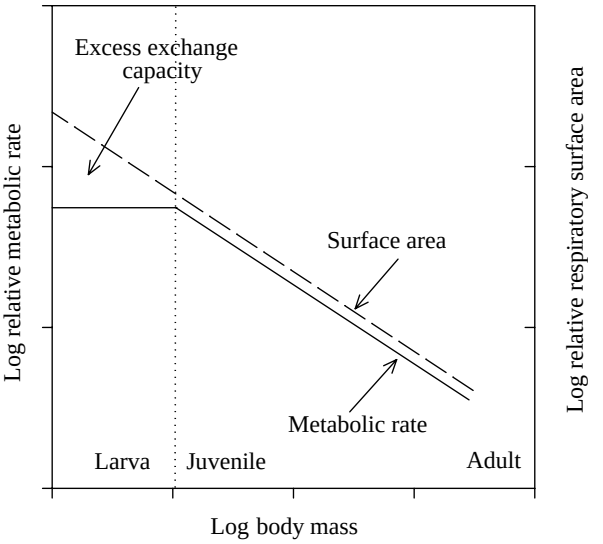

Fig. 8. Schematic diagram illustrating differences in scaling patterns for metabolic intensity and relative respiratory surface area in larvae and in juvenile and adult fish. Larvae appear to have excess exchange capacity compared with older fish.

(approximately 8500 mm^2 skin area g^{-1}; this study) is roughly 38 times that of a 1 kg adult (approximately 225 mm^2 gill area g^{-1}; Niimi and Morgan, 1980). The relative rate of oxygen uptake of newly hatched walleye larvae at 20 °C (approximately 1200 $\mu g\, O_2\, g^{-1}\, h^{-1}$; I. Manns, Brandon University, unpublished data), in contrast, is only approximately 8.5 times that of a 1 kg adult (approximately 140 $\mu g\, O_2\, g^{-1}\, h^{-1}$; Cai and Summerfelt, 1992). Walleye larvae, thus, have approximately 4.4 times the effective surface area ($A_s/\dot{M}_{O_2}$) of adult fish.

Larvae of other species also appear to have relatively larger respiratory surfaces in relation to their metabolic demand for oxygen than do adult fish (Fig. 9). This is particularly true of smaller larvae which, at 10 °C, can have up to 14 times the effective surface area (A_s per unit O_2 uptake) of adult fish (Fig. 9). Gas exchange in adult fish is primarily branchial, while in larvae it is primarily cutaneous. One could argue that, since cutaneous exchange is generally less efficient than branchial exchange, larvae of necessity must have relatively larger exchange surfaces. The additional surface area available to small larvae, however, appears to be greater than is required to compensate for the lower efficiency of cutaneous gas exchange. The effective surface area ($A_s/\dot{M}_{O_2}$) available to small larvae (<10 mg) is 2.5–6.0 times what would be required if gas exchange took place as efficiently in larvae as it does across the skin of adult fish (Fig. 9). There is no reason to think that gas exchange is any less efficient in larvae. Indeed, there is good experimental evidence that the efficiency of cutaneous gas exchange is virtually identical in larvae and adults (Rombough and Ure, 1991; Wells and Pinder, 1996*b*). All other factors being equal, the relatively larger surface area available for respiratory gas exchange in small larvae should result in 'excess' overall diffusive exchange capacity

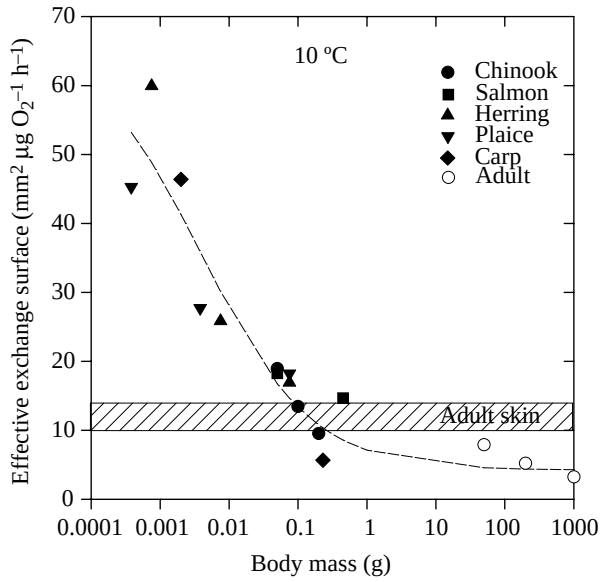

Fig. 9. Available respiratory surface area per unit oxygen uptake as a function of body mass for larval (filled symbols) and adult (open symbols) fish. The data were fitted to a logistic curve. The hatched band shows the range of values reported in the literature for cutaneous oxygen uptake in adult fish (data compiled by Rombough and Ure, 1991). Plotted values were calculated using published data on surface area and oxygen uptake from the following sources: chinook, Rombough and Moroz, 1990; Rombough and Ure, 1991; salmon, Wells and Pinder, 1996*a*,*b*; herring and plaice, De Silva and Tytler, 1973; De Silva, 1974; carp, Oikawa and Itazawa, 1985; Kamler, 1972; adult fish, rainbow trout (1000 g); Niimi and Morgan, 1980; Kiceniuk and Jones, 1977; rainbow trout (200 g) and catfish (50 g), Randall and Daxboeck, 1984.

compared with adult fish. One of the logical consequences of 'excess' exchange capacity is that larvae should be less affected than adult fish by changes in environmental conditions that interfere with diffusive gas exchange. While the empirical evidence is rather sketchy at present, this appears to be the case. Small larvae show very little in the way of compensatory physiological or metabolic responses to relatively profound levels of hypoxia (Colmorgen and Paul, 1995) or to changes in the thickness of the diffusive boundary layer (Rombough, 1997).

Although the diffusive capacity of the skin is initially sufficient, and perhaps in some cases more than sufficient, to meet the metabolic demand of larvae for oxygen, the surface area of the skin must eventually become limiting. As larvae grow, their body surface-to-mass ratio becomes progressively smaller while their metabolic demand for oxygen per unit mass remains approximately the same (Fig. 8). Larvae escape this dilemma by forming gills first to supplement and ultimately to replace the skin as the primary organ of gas exchange. In general, it appears that in cool water (≈10 °C) larvae must begin to supplement cutaneous uptake at approximately 100 mg. As mentioned in the preceding paragraph, very small larvae effectively have larger exchange surfaces than adult fish. The available surface area per unit uptake of oxygen, however, declines as larvae grow, and by the time larvae reach approximately 100 mg values are similar to those for uptake across adult skin (Fig. 9). Given that the inherent efficiency of cutaneous gas exchange appears to be roughly the same in larval and adult fish (Rombough and Ure, 1991; Wells and Pinder, 1996*b*), it seems reasonable to assume that larvae do not require gills until values for respiratory surface area per unit oxygen uptake fall below those for adult skin (i.e. at ≈100 mg wet mass; Fig. 9).

It appears that this is, in fact, approximately the size at which many species make the transition to branchial respiration. There is empirical data from partitioning studies on the timing of the transition for only two species. In Atlantic salmon, the gills supplant the skin as the dominant site (more than 50 % of total exchange) of gas exchange at approximately 120 mg (Wells and Pinder, 1996*b*). In chinook salmon, the transition takes place at approximately 280 mg (Rombough and Ure, 1991). Circumstantial evidence suggests that the transition takes place at roughly the same size in other species. In both species of salmon, but particularly in chinook, there is a fairly close correlation between the timing of the transition to branchial respiration and peak relative gill area: relative lamellar surface area is maximal at approximately 210 mg in Atlantic salmon (Wells and Pinder, 1996*a*) and approximately 240 mg in chinook (Rombough and Moroz, 1990). Plots of relative lamellar area *versus* body mass for species for which we were able to find sufficient information in the literature show a high degree of convergence, both in terms of peak values and in terms of corresponding body masses (Fig. 10). The degree of convergence is especially striking when one considers that the gills begin to form at radically different sizes

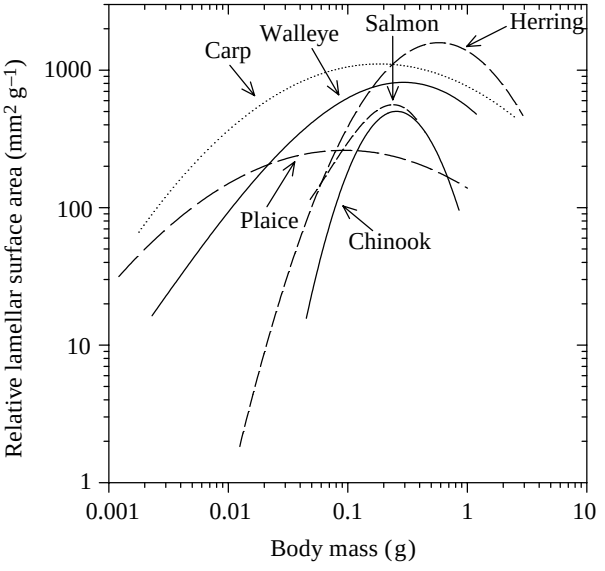

Fig. 10. Relative lamellar surface area as a function of body mass for larvae of various species of teleost fish. The sources of the data are as follows: walleye (this study), chinook (Rombough and Moroz, 1990), salmon (Wells and Pinder, 1996*a*), plaice and herring (De Silva, 1974) and carp (Oikawa and Itazawa, 1985).

in different species (e.g. approximately 1.6 mg in carp *versus* approximately 45 mg in chinook). The relatively narrow size range for peak relative gill area (100–500 mg) strongly suggests that surface-to-mass considerations constrain larvae from delaying gill expansion and, thus, the transition to branchial respiration much beyond the 100 mg body mass predicted on the basis of the surface area per unit oxygen uptake data.

Gill development begins in many species long before the gills would appear to be required for respiratory gas exchange. In walleye, for example, gill development begins within 2 days of hatch at a body mass of only 2.5 mg (Fig. 3). Carp begin to form gills at approximately 1.6 mg (Oikawa and Itazawa, 1985), while plaice already have gills with well-formed secondary lamellae at a body mass of 1.2 mg (De Silva, 1974). As argued in the preceding paragraph, most larvae probably do not require gills to supplement cutaneous gas exchange until they attain a mass of approximately 100 mg. At first glance, this might suggest that symmorphosis does not apply during larval development. However, one must remember that gas exchange is not the only function of the gills, and the concept of symmorphosis does not say anything about the nature of the functional requirement that structural elements are designed to satisfy. In adult fish, the gills play an important role in ionoregulation and related aspects of acid–base balance in addition to their role in gas exchange. The ontogeny of ionoregulation in general is poorly understood but, on the basis of numbers of mitochondrion-rich ('chloride') cells, it appears that the yolk sac is the major site of ionoregulatory activity during embryonic and the initial stages of larval development (Alderdice, 1988; Ayson *et al.* 1994). In tilapia *Oreochromis mossambicus*, for example, the density of mitochondrion-rich cells in the yolk sac membrane rises steadily during late embryonic development to reach a maximum at approximately 2 days post hatch before declining rapidly (Ayson *et al.* 1994). Small, rapidly developing larvae, such as the tilapia, quickly consume their yolk so that within a few days of hatch the yolk sac membrane is probably too small to support sufficient mitochondrion-rich cells to meet ionoregulatory requirements. Tilapia, and presumably other species with small larvae, appear to respond by shifting the site of ionoregulatory activity to the gills. Within 3 days of hatch (at approximately 4 mg wet mass), the epithelium of the gill filaments of tilapia larvae is almost entirely occupied by mitochondrion-rich cells, and by 10 days post hatch cell densities are not significantly different from that of adult fish (Li *et al.* 1995). The mitochondrion-rich cells in the larval gill appear to be very active metabolically, at least in tilapia where the mass-specific Na^+/K^+-ATPase activity of the gills is approximately 390 times greater in 10-day-old larvae than it is in adult fish (Li *et al.* 1995). The early appearance of large numbers of very active, mitochondrion-rich cells in the gills at a time when then skin still provides sufficient surface area (more than 99 % of total area) to satisfy respiratory requirements suggests that gill development may be driven, at least initially, by ionoregulatory rather than respiratory considerations. If this is true, the way we view gills in developing but also in adult fish would seem to merit re-examination. Aspects of gill structure that until now we have assumed to be purely respiratory adaptations, in fact, may turn out to represent solutions to ionoregulatory challenges.

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