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Metabolic rate of pre-smolt growth-enhanced transgenic Atlantic salmon (*Salmo salar*)

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Abstract

The rates of routine oxygen consumption of growth-enhanced transgenic Atlantic salmon were compared with that of non-transgenic salmon, over a pre-smolt body interval of 8–55 g to determine whether or not the transgenic salmon also expressed a greater metabolic rate. Routine oxygen consumption rates (mg O_2/h), inclusive of the heat increment associated with feeding, were 1.54- to 1.70-fold higher for transgenic fish compared to the controls. However, integrated over time from first feeding to smolt size, the transgenic salmon actually consumed 42% less total oxygen than the non-genetically modified controls to reach smolt size. In a post-absorptive state (24 h starvation), corresponding oxygen consumption rates of transgenic fish were 1.58- to 2.30-fold greater than that of regular salmon. The added cost to smolt producers for the short-term delivery of more water or oxygen to support the elevated metabolism of such growth-enhanced fish would appear to be justified in light of the benefits in reducing smolt production time.

Keywords: Transgenic; Atlantic salmon; Oxygen consumption; Metabolic rate; Growth enhancement; Growth hormone

1. Introduction

Considering the relatively high cost of pumping water (or generating oxygen) in land-based hatchery facilities, an understanding of the oxygen requirements for fast-

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growing transgenic salmon is required. Exogenous growth hormone treatment has been reported to significantly increase metabolism in parr and pre-smolt Atlantic salmon in relation to non-treated controls (Seddiki et al., 1996). Transgenic fish have greater daily feed consumption in comparison to control fish (Cook, 1999; Cook et al., 2000a) and Forsberg (1997) determined that the oxygen consumption of post-smolt Atlantic salmon increased proportionally with increased feed intake. Stevens et al. (1998) established that transgenic Atlantic salmon have a routine oxygen consumption 1.70-fold greater than non-transgenic salmon. Moreover, transgenic salmon exhibit higher activity levels than non-transgenic fish (Abrahams and Sutterlin, 1999), which might elevate the rate of oxygen consumption as is the case in unmanipulated salmonids (Fry, 1971). Consequently, the rapid growth exhibited by growth-enhanced transgenic fish could be associated with some additional metabolic cost to the fish and financial cost to the farmer. The objective of the present study was to compare the routine metabolism of transgenic salmon relative to that of non-genetically modified salmon reared under simulated aquaculture conditions.

2. Methods

2.1. Experimental fish

The experimental transgenic fish as well as the non-genetically modified control fish were Atlantic salmon (*Salmo salar*) bred from Saint John River stock, New Brunswick, Canada and reared at AquaBounty Farms in Prince Edward Island, a government-inspected hatchery designed with the required containment measures to prevent the escape of genetically modified organisms into the natural environment.

In fall of 1989, the transgene consisting of a chinook salmon growth hormone gene spliced to an ocean pout antifreeze gene promoter (Du et al., 1992) was microinjected (approximately 10^6 copies/egg) through the micropyle into the cytoplasm of fertilized, non-water activated salmon eggs (Shears et al., 1992). Milt from one of the fast-growing transgenic males arising from the injected eggs (P_1 — parental generation), which sexually matured in the fall of 1991, was crossed with a non-transgenic female. A fast-growing, transgenic female (F_1) resulting from this mating was crossed with a non-transgenic male in the fall of 1996 resulting in the F_2 transgenic fish used in the present study. Also in the fall of 1996, pooled non-transgenic milt and eggs from the same Saint John River stock were used to generate non-transgenic control fish.

Transgenic and control embryos and alevins were incubated separately in flowthrough heath incubators. To facilitate having transgenic and control fish of approximately the same weight at the start of the experiment, the lot of eggs giving rise to the transgenic fish was incubated at a lower water temperature (4°C) relative to control eggs (7°C). Consequently, time at first feeding was approximately 17 days later for the transgenic fry than for control fry.

In 1996, the above progeny resulting from the cross between a transgenic female (F_1) and a non-transgenic male exhibited a bimodal size distribution at the fingerling stage in June, a phenomenon not usually seen until the first autumn of growth (Thorpe, 1977;

Thorpe et al., 1980). Consequently, the two modes could be separated into two groups based on fork length above and below 8.0 cm, with 50% of the total population in each mode, which is typical of Mendelian segregation of an allelic insert on a single chromosome. This separation was later confirmed by the exclusive presence of the transgene in the upper modal group as revealed using polymerase chain reaction. The transgenic fish used in the present experiment were from the upper modal group of fish from the 1996 spawning.

Well water was used at all stages of the experiment with properties as follows: hardness as $CaCO_3$ was 150 mg/l, pH 7.6, and salinity 4‰; water temperature was maintained at 12.6°C ± 0.03 (SEM) and lighting within the hatchery mimicked the natural photoperiod (46.2°N, 62.3°W).The experiment lasted for 21 weeks and was conducted between July 18, 1997 and December 12, 1998.

2.2. Protocol

Six-hundred sixty transgenic salmon, average weight 9.42 ± 0.09 g, were randomly distributed to 12 tanks for a total of 55 fish/tank. Six-hundred sixty control salmon, average weight 6.62 ± 0.05 g, were randomly assigned to 12 additional tanks for a total of 55 fish/tank. The fish were acclimated for 3 weeks in the experimental tanks, and during the experiment were fed by hand to satiation (at least 20% excess ration) three times per day on a commercial feed of the following composition: 92.40% dry matter, 55.69% crude protein, 18.57% crude lipid and 8.17% ash. Mean weights at the start of the experiment were 13.72 ± 0.21 and 6.98 ± 0.07 g for transgenic and control fish, respectively. As part of ongoing growth and food deprivation experiments (Cook et al., 2000a,b), the fish in a minimum of three, and up to a maximum of 12 replicate tanks for each of the two experimental groups were used in measuring routine oxygen consumption. A non-invasive protocol was developed whereby the fishrearing tanks were temporarily converted to metabolic respiration chambers.

2.3. Respirometer design

Prior to entering the respirometer, incoming water was heated to approximately 13°C, stripped of excess nitrogen using oxygen injectors, run through a packed column with upwelling air to remove excess oxygen, and finally cooled by approximately 0.5°C to prevent bubble formation on the tank surfaces. Employing a single-pass, flowthrough system, water entered the 92-l tanks at the periphery and circulated towards the centre drain.

At the start of the experiment and every 2 weeks thereafter, the experimental growth tanks were converted to flowthrough respiration chambers and the oxygen consumption of the contained fish was measured. In each respirometer, a rubber stopper was placed in the external standpipe to cause the water level to rise above the tank upper rim. A foam rubber gasket on the rim of the tank allowed for the plexiglass cover to be sealed airtight with clamps after any air remaining inside the respirometer was bled out. Water exited from the tank at a higher point on the external standpipe resulting in a slightly positive

pressure in the sealed tank. Water flow rates to each individual tank were individually adjusted to facilitate a decline in oxygen concentration (due to fish respiration) to approximately 60% water saturation, a level well above the critical concentration-limiting oxygen uptake (Stevens et al., 1998). Oxygen levels were measured using an Oxyguard Handy Mark 4 oxygen sensor (Point Four Systems, Port Moody, BC, Canada). The entire process of converting a growth tank into a respiration chamber generally took less than 5 min with minimal stress to the fish. Each respirometer contained a small submersible pump to maintain a slow circular current. After each tank had been sealed, an acclimation period of approximately 24 h commenced before the first oxygen consumption measurements (Brett and Zala, 1975). Fish were continually fed to satiation three times per day including the days in which the tanks were sealed and oxygen measurement made. Feed was introduced into each respirometer through a tube fixed to the plexiglass cover, which prevented water from escaping or air being introduced to the tank. Water flow rates to each tank and dissolved oxygen (DO) concentrations in the inflow and outflow water were measured every 4 h over a 24-h period and the means were used to calculate the rate of oxygen consumption. The respiration tanks were unsealed after the last measurements were taken and the fish biomass was measured. At approximately 10 g weight intervals, oxygen consumption rates of fish in a post-absorptive state were also determined. Subsequent to the metabolic measurements in which the fish were fed, the respirometers were kept sealed and the fish fasted for 24 h (Brett and Zala, 1975) in order to determine the fish metabolism in a post-absorptive state. Inflow and outflow oxygen concentrations and flow rates were measured every 4 h over the next 24-h period. Each tank was then unsealed and the fish biomass measured.

DO readings (measured to the nearest ± 0.05 mg O_2/l), water flow rate, and the number of fish in the tank were used to calculate the oxygen consumption rate of a fish of known weight, according to the following equation:

$$VO_2[MO_2]^* = \frac{(\Delta[O_2] \times Vw)_{\text{fishtank}} - (BOD \times Vw)_{\text{blanktank}}}{N[W]^*},$$

where VO_2 is the routine oxygen consumption rate of the fish (mg O_2/h), $\Delta[O_2]$ is the DO concentration change from the inflowing water supply (mg O_2/l), $[O_2]_{\text{inflow}}$ and the outflowing drain water, $[O_2]_{\text{outflow}}$, Vw is the flow rate (l/h), N is the number of fish in the tank, and BOD is the biological oxygen demand (mg O_2/l) of a tank without fish. To standardize for changes in body weight, where MO_2 is the routine weight-specific oxygen consumption rate (mg O_2 kg fish^{-1} h^{-1}), the above equation was divided by the total fish biomass (W) in the respirometer instead of the number of fish.

To correct for possible microbial oxygen consumption, oxygen measurements were made concurrently on 'blank' tanks that normally had fish in them prior to the experiment and were therefore representative as containing the same film of microbial flora resulting from nutrient enrichment associated with daily inputs of fish feed and fish defecation. This rate of oxygen consumption (less than 4% positive error) was subtracted from the oxygen consumption of tanks containing fish to obtain a true representation of the fish metabolism.

The rate of oxygen consumption (mg O_2/h) was related to fish body weight according to the power relationship in the following equation:

$$VO_2 = aBW^b,$$

where BW denotes the body weight (g) of the fish, b is the weight exponent, and a is the weight coefficient.

The relationship between oxygen consumption and weight was demonstrated by regression analysis, using 95% confidence interval as the critical level for determining significance.

3. Results

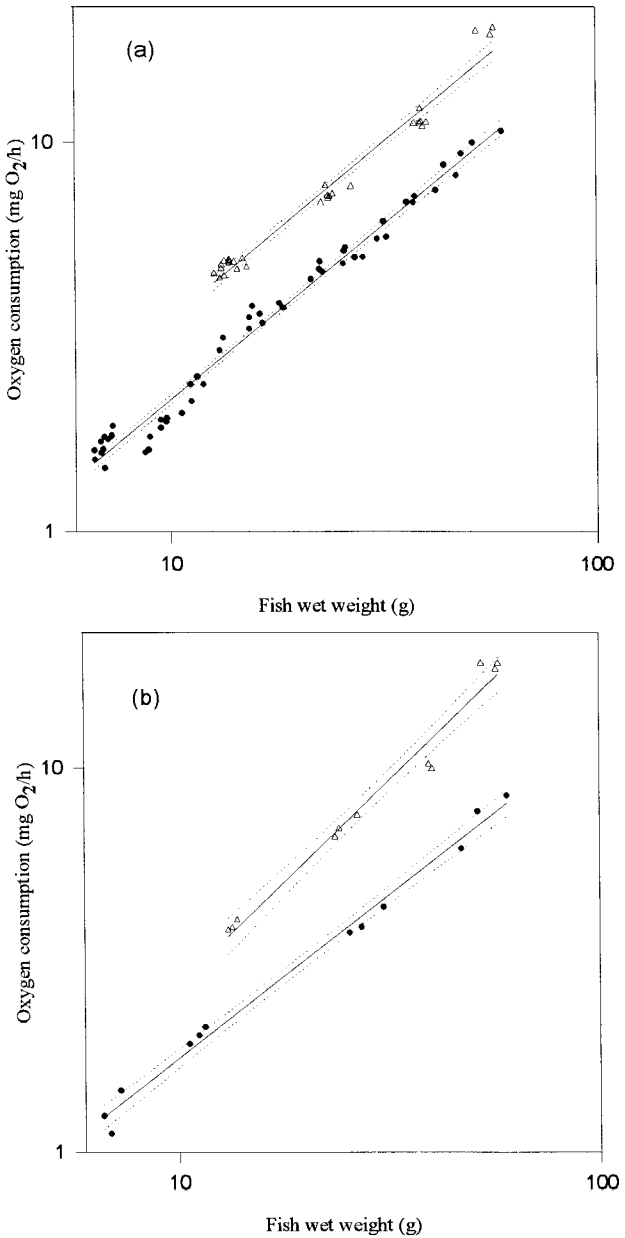
3.1. Routine oxygen consumption and weight exponent

The culture methods and growth rates of the two experimental groups of fish for which the oxygen consumption rates are presented here are described by Cook (1999) and Cook et al. (2000a). The specific growth rates (% wet body weight/day) averaged 3.61%/day and 1.50%/day for transgenic and control fish, respectively, over the body weight interval measured. There was a significant difference ($P < 0.05$) between the routine rates of oxygen consumption of transgenic and control fish, inclusive of the heat increment associated with being fed to satiation three times per day (Fig. 1a) at all measured body weights. The regression equations accounted for between 97% and 98% of the data's total variance for transgenic and control fish, respectively. The slope of the regression line was significantly ($P < 0.05$) steeper for the transgenic fish compared to the control fish, indicating that the transgenics had a more rapid increase in oxygen consumption with increase in body weight. A comparison of rates of oxygen consumption of transgenic and control fish over a weight interval of 14–52 g revealed that the rates of oxygen consumption of transgenic salmon were 1.68- to 1.69-fold greater than those of control salmon. However, the total quantity of oxygen consumed by transgenic fish, when integrated over the time to grow from 14 to 52 g, was 10.18 g, approximately 37% less (due to their higher growth rates) than the 16.06 g of oxygen consumed by their non-transgenic counterparts. Extrapolation of the data to the weight of first feeding fry (0.12 g) suggests that the transgenic fish would have consumed approximately 42% less total oxygen to reach a smolt size of 52 g.

At 14 g, the daily feed intakes of transgenic and control fish (as a percentage of body weight) were 3.53% and 1.35%, respectively, representing a 2.61-fold difference. Upon reaching a wet body mass of 52 g, the daily feed consumption by transgenic and control fish were 1.67% and 0.78%, respectively, representing a 2.14-fold difference (Cook, 1999; Cook et al., 2000a).

There was also a significant difference ($P < 0.05$) between the rates of oxygen consumption of transgenic and control fish (in a post-absorptive state) at all measured body weights (Fig. 1b), and the regression equations accounted for 98–99% of total variances for transgenic and control fish, respectively. The slope of the regression line was significantly ($P < 0.05$) steeper for the transgenic fish compared to the control fish,

indicating that the transgenics had a more rapid increase in oxygen consumption, with increase in body weight possibly indicating that the influence of the transgene was also increasing. The difference in routine post-absorptive oxygen consumption between the two groups at 14 g was 1.67-fold, with transgenic and control oxygen consumption rates



3.94 and 2.36 mg O_2/h, respectively. The magnitude of difference increased to 2.20-fold at 52 g, with transgenic and control oxygen consumption rates 15.82 and 7.19 mg O_2/h, respectively.

Double logarithmic plots of metabolic rate in relation to body weight revealed weight exponents of 0.91 and 0.90 for feeding transgenic and control fish, respectively (Fig. 1a). Consequently, when the body weight of transgenic and control fish doubled, their oxygen consumption increased by approximately 90%. The weight exponents of transgenic and control fish in a post-absorptive state were 1.06 and 0.85, respectively (Fig. 1b). The weight exponents in both the fed and post-absorptive state were significantly different ($P < 0.05$) between the two experimental groups of fish.

Control salmon, with respect to the transgenic group, exhibited a significantly greater reduction in oxygen consumption when measured from a fed to a fasting state. For the transgenic fish, this reduction in oxygen consumption from a fed to a fasted state was 22% at 14 g and declined to 1% at 52 g; values for controls at comparable body weights were 23% and increasing to 32%, respectively.

3.2. Routine weight-specific oxygen consumption

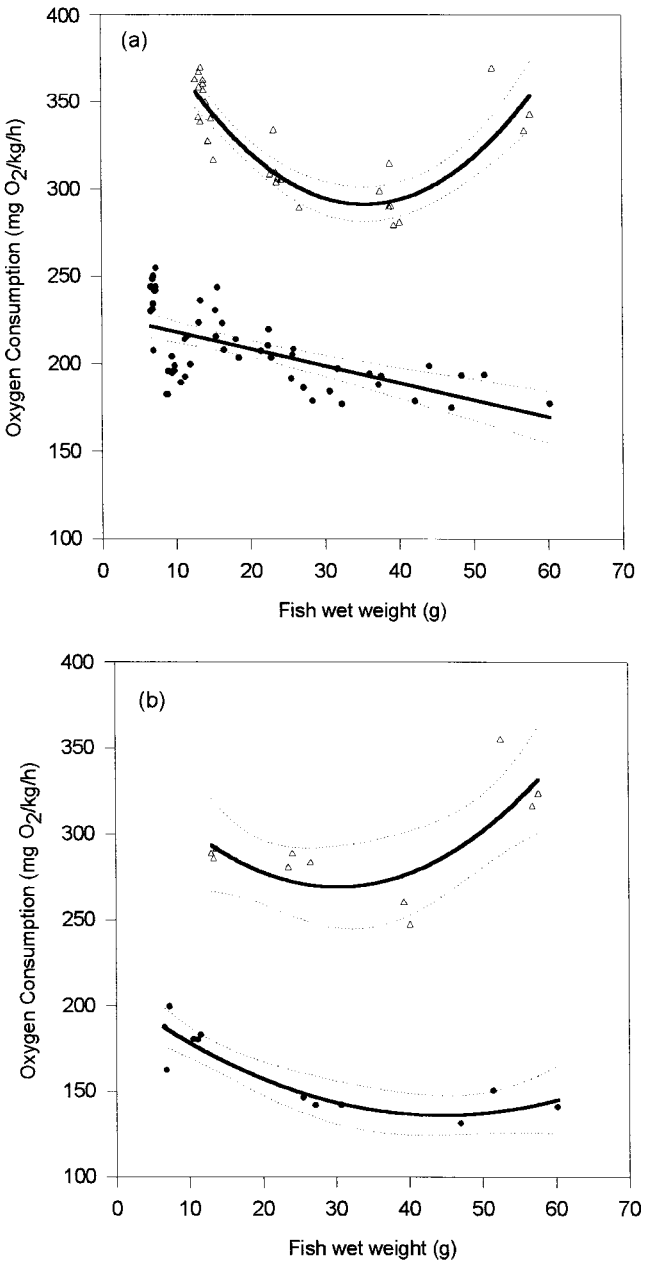
On a quantitative basis, the larger fish (be it transgenic or control) consumed more oxygen than smaller fish; however, on a unit weight basis, oxygen consumption decreased as body mass increased. The weight-specific, routine oxygen consumption of transgenic fish (Fig. 2a), inclusive of the heat increment associated with being fed, was best explained by a quadratic regression (table in Fig. 2). A linear regression was adopted for control fish as a second degree polynomial was found to produce no better fit.

There was a significant difference ($P < 0.05$) in oxygen consumption of fed transgenic and control fish at all weights measured. At a body weight of 14 g, the oxygen consumption rates of transgenic and control fish were 348 and 214 mg O_2 kg^{-1} h^{-1}, respectively, representing a 1.63-fold greater rate of weight-specific oxygen consumption by the transgenic fish. Transgenic fish exhibited a parabolic oxygen response in relation to body size (Fig. 2a), reaching a minimum metabolic rate at approximately

Fig. 1. (a) Routine oxygen consumption in relation to body weight of transgenic Atlantic salmon (open triangles) and controls (solid circles) at 12.6°C, with inclusion of the heat increment associated with feeding. Each data point represents mean values from fish in one tank. Data are presented with fitted regression lines (solid lines) surrounded by 95% confidence intervals (dashed lines). (b) Routine oxygen consumption, in relation to body weight, of transgenic Atlantic salmon and controls in a post-absorptive state (fasted for 24 h). The following equation represents the relationship between VO_2 (mg O_2 h^{-1} $fish^{-1}$) and body weight, BW (g), of transgenic Atlantic salmon and controls: $VO_2 = a\,BW^b$ where a is the regression coefficient and b is the weight exponent.

		a	b	r^2
Fed	Control	0.27	0.91	0.98
	Transgenic	0.44	0.91	0.97
Fasted	Control	0.25	0.85	0.99
	Transgenic	0.24	1.06	0.98

35 g. The magnitude of difference between fish in the two experimental groups decreased to 1.48-fold at approximately 30 g where oxygen consumption rates were 295 and 199 mg O_2 kg^{-1} h^{-1} for transgenic and control fish, respectively. A subsequent



increase of oxygen consumption by the transgenic salmon to 326 mg O_2 kg^{-1} h^{-1} resulted in an 1.83-fold greater rate than the control fish (178 mg O_2 kg^{-1} h^{-1}) at the final body weight of 52 g.

The weight-specific oxygen consumption of transgenic and control fish in a post-absorptive state was best described by a quadratic regression (Fig. 2b) . Fasting control fish had significantly lower ($P < 0.05$) rates of oxygen consumption than fasting transgenic fish at all measured body weight. At a body weight of 14 g, the transgenic and control oxygen consumption rates were 291 and 169 mg O_2 kg^{-1} h^{-1}, respectively, representing a 1.72-fold difference. The divergence in metabolic rate between the two experimental groups increased to 2.23-fold at 52 g, where the oxygen consumption rates were 308 and 138 mg O_2 kg^{-1} h^{-1} for transgenic and control fish, respectively.

4. Discussion

4.1. Routine oxygen consumption and weight exponent

The routine metabolism of F_2 generation growth-enhanced transgenic Atlantic salmon was significantly greater than that of non-transgenic controls over a weight range representative of smolt production. The heat release associated with metabolism has generally been accepted as representing a loss of energy to fish. The higher oxygen consumption of transgenic fish at first glance might be attributed to their larger daily feed intake compared to that of control fish (Cook, 1999; Cook et al., 2000a). Forsberg (1997) established that oxygen consumption increased proportionally with increased feed intake in post-smolt Atlantic salmon reared in commercial-scale fish tanks. However, the decline in oxygen consumption when the experimental fish in the present study were starved for 24 h was larger for the control group even though they had a lower daily feed intake than the transgenic group. If the difference between transgenic and control rate of oxygen consumption was only a function of differences in their daily feed intake, both experimental groups should have had the same oxygen consumption when deprived of feed. The transgenic fish, however, maintained a significantly higher

Fig. 2. (a) Weight-specific routine oxygen consumption in relation to body weight of transgenic Atlantic salmon (open triangles) and controls (solid circles), with inclusion of the heat increment associated with feeding. Each data point represents mean values of one tank of fish. Data are presented with fitted regression lines (solid lines) surrounded by 95% confidence intervals (dashed lines). (b) Weight-specific routine oxygen consumption, in relation to body weight, of transgenic Atlantic salmon and controls in a post-absorptive state (fasted for 24 h). The following equation relates the weight-specific routine oxygen consumption, MO_2 (mg O_2 kg $fish^{-1}$ h^{-1}) and body weight, BW (g), of transgenic Atlantic salmon and controls : $MO_2 = b_0 + b_1 BW + b_2 BW^2$ where b_0, b_1, and b_2 are regression coefficients.

		b_0	b_1	b_2	r^2
Fed	Control	228	−0.96	n.a.	0.37
	Transgenic	448	−0.84	0.13	0.75
Fasted	Control	207	−3.18	0.04	0.81
	Transgenic	334	−5.01	0.08	0.56

oxygen consumption than control fish after 24 h feed deprivation and, in fact, continued to maintain higher metabolic rates than control fish after extended periods of feed deprivation lasting 8 weeks (Cook, 1999; Cook et al., 2000b). This higher oxygen consumption rate by transgenic fish relative to non-genetically modified salmon might be attributed to a greater activity level (Abrahams and Sutterlin, 1999) and/or a greater basal metabolism, possibly indicative of a higher maintenance requirement.

The relationship of body weight to oxygen consumption, expressed as $VO_2 = a\,BW^b$, has an approximate weight exponent value of 0.8 for a variety of freshwater and marine fish species in a fasted state (Kazakov and Khakyapina, 1981; Sims, 1996). The weight exponents obtained in the current study were 0.91 and 0.90 for transgenic and control fish in a feeding state and 1.06 and 0.85, respectively, following 24-h feed deprivation. The increase in the weight exponent exhibited by the transgenic fish from a feeding to a fasting state has also been observed in non-salmonid fish. For example, Hogendoorn (1983), in studying African catfish (*Clarias lazera*), theorized that the reason fish do not grow indefinitely is that having a greater weight exponent value in a fasting state than in a feeding state will ultimately lead to a metabolic 'scope for growth' (maximum metabolic rate − standard metabolic rate) equal to zero. As a fish increases in weight, its maximum metabolic rate decreases at a faster rate than standard metabolic rate, resulting in convergence (Brett, 1979). In contrast, the weight exponent of control fish decreased from a feeding to a fasting state. Considerable intraspecies variation has been observed in Atlantic salmon, with weight exponents of fasted fish ranging from 0.67 up to 0.84 (Kazakov and Khakyapina, 1981; Grottum and Sigholt, 1998).

4.2. Weight-specific routine oxygen consumption

Direct intra- and interspecific comparisons of oxygen consumption are fraught with numerous uncertainties imposed by insufficient attention to variables such as fish weight and water temperature (Brown et al., 1984; Cech et al., 1985, 1994; Cai and Summerfelt, 1992), quantity and composition of feed intake (Beamish, 1974; Jobling and Davies, 1980; Forsberg, 1997), and stress preceding and during experimentation (Barton and Schreck, 1987; Davis and Schreck, 1997). A confounding variable observed in the present study was that the transgenic fish appeared to undergo precocious smoltification. Superficially, the transformation from parr to smolt can be monitored based upon several externally visible indices such as obscuring of the 'parr' marks by the silvery appearance of the scales, darker fin margins, and a lower condition factor (weight/length^3 × 100) (Komourdjian et al., 1976; Gorbman et al., 1982). This transformation typically occurs in the spring in preparation for migration from the natal fresh water environment to the sea, and is correlated with a rise in serum growth hormone levels and elevated growth. Using growth-enhanced transgenic Atlantic salmon sibling to those used in the present study, Stevens et al. (1998) reported that the transgenic fish took on a silver coloration and lost the dark vertical parr marks at a smaller size than did control fish. They also observed a decline in condition factor by the transgenic fish much earlier than typical non-transgenic pre-smolts cultured under hatchery conditions. Saunders et al. (1998) demonstrated that transgenic Atlantic salmon attained smolt status in early summer. Had the control fish been retained in fresh water until the following spring to

produce 1 + year smolts, the difference in total oxygen consumed would have become more profound than the 42% reported in the present study. Seddiki et al. (1996), while describing the positive effect of bovine growth hormone treatment on the seawater adaptability of salmon pre-smolts, noted that the treatment caused a 1.30-fold increase in routine oxygen consumption. This would explain why the transgenic weight-specific oxygen consumption decreased in a similar fashion as control fish up to a body weight of approximately 35 g but then gradually increased until the termination of the experiment at approximately 52 g. Wiggs et al. (1989) described an increase in the oxygen consumption for smolt over parr, but this normally occurs in the spring of the year with non-genetically altered salmon. This size-dependent early smoltification (which is quite insensitive to photoperiod) appears to be an obligatory consequence of using fast-growing transgenic salmonids (Saunders et al., 1998). Hence, studies using juvenile transgenic salmonids may be faced with the unavoidable and confounding physiological influence of smoltification.

Bergheim et al. (1991) illustrated the relationship between oxygen consumption and temperature in feeding non-transgenic Atlantic salmon. When the mean water temperature observed in the present study (12.6°C) was placed in their regression model, oxygen consumption rates of 246 mg O_2 kg $fish^{-1}$ h^{-1} for 20–75 g non-transgenic fish were obtained. This rate of oxygen consumption by the non-transgenic salmon is higher than observed in the current study and may be accounted for by the loss of oxygen from water supersaturated with oxygen as a result of the water-to-surface air exposure, thereby possibly overestimating the oxygen consumption of fish (Bergheim et al., 1991). A weight-specific oxygen consumption rate of 120 mg kg^{-1} h^{-1} for 2 kg Atlantic salmon fed to excess in 8.5°C seawater has also been reported (Forsberg, 1997), a value lower than that calculated from our regression equations and likely attributed to differences in water temperature. Stevens et al. (1998), using transgenic and non-transgenic salmon siblings of weight similar to those used in the present study, reported oxygen consumption values 375 mg O_2 kg $fish^{-1}$ h^{-1} for transgenic fish and 220 mg O_2 kg $fish^{-1}$ h^{-1} for control fish as measured in a closed respirometer. Even with the inherent variability typically observed between metabolic experiments, especially those using different styles of respiration chambers, the oxygen consumption values reported by Stevens et al. (1998) are similar to those reported in the present study. Associated with the elevated metabolic rates, Stevens and Sutterlin (1999) observed a 24% increase in gill respiratory surface area (relative to control fish) in the same family of transgenic fish used in the current study; this was mainly due to an increase in the length of the gill filaments.

5. Conclusion

Transgenic juvenile Atlantic salmon exhibited significantly higher routine oxygen consumption rates over the entire weight range of 8–55 g, when compared to non-genetically modified fish reared under simulated aquaculture conditions. The higher rate of oxygen consumption was, in part, a function of the elevated rates of daily feed intake by the transgenic fish relative to control fish. However, the higher rate of oxygen

consumption by transgenic salmon prevailed following feed deprivation, a condition in which the metabolic effects of feed were removed. When the total quantity of oxygen consumed was integrated over the time to reach smolt size, transgenic fish (due to their higher growth rates) consumed approximately 42% less total oxygen than their non-genetically modified counterparts.

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