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Growth rate, body composition and feed digestibility/conversion of growth-enhanced transgenic Atlantic salmon (*Salmo salar*)

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Abstract

Although dramatic improvements in growth rates have been documented in growth-enhanced transgenic salmonid fish, prior to commercial implementation of this technology, there is a need for further information relating to the physiology of a number of commercially important production traits. Growth rate, feed digestibility, feed conversion, and body composition of F_2 generation growth-enhanced transgenic Atlantic salmon were therefore compared with that of non-genetically modified salmon, over a presmolt growth interval of 8–55 g.

The growth-enhanced transgenic fish exhibited a 2.62- to 2.85-fold greater rate of growth relative to non-transgenic salmon over the body weight interval examined. Daily feed consumption over this body weight interval was 2.14- to 2.62-fold greater for the transgenic fish compared to the control fish. Transgenesis did not affect the extent to which protein and energy were digested, with digestibility coefficients 88% and 81%, respectively for transgenic fish, and 90% and 84%, respectively for control fish, both measured over comparable body weight intervals. However, transgenic salmon relative to control fish exhibited a 10% improvement in gross feed conversion efficiency. Body protein, dry matter, ash, lipid and energy were significantly lower in the transgenic salmon relative to controls while moisture content was significantly higher.

The transgenic experimental subjects used throughout the present study possessed the physiological plasticity necessary to accommodate an acceleration in growth well beyond the normal

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range for this species with few effects other than a greater appetite and a leaner body.

Keywords: Transgenic; Body composition; Salmon; Growth rate; Feed conversion; Feed digestibility; Growth hormone

1. Introduction

The current farmed production schedule of Atlantic salmon (using genetically unaltered fish) consists of 12–18 months in freshwater and another 12–24 months in seawater. Over the past 10 years, improvements in selective breeding, husbandry techniques, and environmental control has shortened the time to harvest thereby reducing production costs.

Contributions from the field of molecular genetics are projected to play an increasing role in aquaculture through the application of transgenic technology to alter the genome of fish in order to enhance such commercially important production traits as growth rate, disease resistance and cold tolerance. Animals into which new genetic material has been artificially introduced are termed ''genetically modified'' or ''transgenic''. Advances in transgenic technology employing growth hormone (GH) transgenes are being increasingly recognized because of the extraordinary induced phenotypic effect that has clear commercial significance. Because many of the production costs in an aquaculture venture are time dependent, abbreviating the time to market size will reduce these expenditures and also lower the exposure time to a variety of risks (disease vectors, predators, and losses due to storm damage). While conventional methods of selective breeding and improved husbandry techniques have been responsible for significant improvement in the growth rates of domesticated strains of Atlantic salmon, progress has been relatively slow.

The effects of bovine GH (Higgs et al., 1975; Kayes, 1977; Markert et al., 1977; Danzmann et al., 1990; McLean et al., 1997), thyroid and steroid hormones (Higgs et al., 1979, 1982; Yu et al., 1979), genetically engineered rainbow trout GH (Danzmann et al., 1990) and recombinant salmon GH (Moriyama et al., 1993) on the growth rates and feed conversions of salmonid fish have been extensively studied. The prevailing general consensus is that immersion, injection or the use of slow-release implants containing GH will stimulate (in varying efficiencies depending on the technique used) significant increases in growth and, in some cases, feed conversion. The advent of commercial production of sufficient fish GH along with advances in GH oral delivery in feed has produced promising results for enhancing growth (McLean et al., 1993, 1997; Tsai et al., 1997); However, given the large numbers of animals generally held within an aquaculture facility, and the requirement for a time- and cost-effective GH delivery system (Dunn et al., 1990), it has been suggested that the use of transgenic fish may be the most practical approach to growth enhancement. For these reasons, transgenic Atlantic salmon containing a chinook salmon GH transgene have been developed for accelerated growth (Fletcher et al., 1992). Differences in growth enhancement between transgenic lines probably arise from a number of factors such as the chromosomal site of integration, the

number of tandem gene copies integrated at loci, and the type/effectiveness of promoter used (Moav et al., 1992).

While there have been a number of studies demonstrating the superior growth of transgenic fish (Du et al., 1992; Fletcher et al., 1992; Devlin et al., 1994, Saunders et al. 1998), few have evaluated the effect transgenesis has on nutrient utilization by fish (Fu et al., 1998). The aim of the present study was to quantify and compare growth rate, protein and energy utilization, feed conversion, and body composition of transgenic salmon under simulated aquaculture conditions with those of genetically unaltered salmon. This information is required to define some of the more important production parameters and to reveal any special husbandry requirements that will be necessary to accommodate such fish in a commercial setting.

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 partially domesticated 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 the fall of 1989, the GH transgene was micro injected (approximately 10^6 copies per egg) through the micropyle into the cytoplasm of fertilized, non-water activated salmon eggs (Shears et al., 1992). This transgene was composed of a chinook salmon GH gene attached to an antifreeze protein promoter sequence taken from the ocean pout (Hew et al., 1995). 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 families of embryos and alevins were incubated in separate trays in flow-through, stacked-tray incubator. To facilitate having transgenic and control fish of approximately the same weight at the start of the experiment, the batch 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 greater for the transgenic fry than for control fry.

In 1996, the 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 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 ‰ (Stevens et al., 1998); water temperature was maintained at 12.6°C ± 0.03 (s.e.m.) and lighting within the hatchery simulated natural photoperiod.

2.2. Diet preparation and chemical analysis

The formulation and chemical composition of the experimental diet presented in Table 1 was felt to be representative of commercial presmolt Atlantic salmon diets. Chromic oxide (Cr_2O_3) was included in the diet at 0.45% dry matter basis which was later confirmed by atomic absorption spectrophotometry (Arthur, 1970). The experimental diet was steam-pelletized using a laboratory-scale, California pellet mill equipped

Table 1
Values presented as means ± s.e.m. of 2.0- and 3.0-mm pellets

Ingredients	kg/100 kg of diet
Formulation of experimental diet	
Wheat (shorts)	13.83
Fish meal (75% protein)	59.70
Blood meal	2.49
Vitamin pre-mix	0.75
Mineral pre-mix	0.75
Protein supplement	4.98
Choline chloride	0.20
DL-Methionine	0.10
Lecithin	0.50
Carophyll pink (astaxanthin)	0.01
Potato starch	4.98
Chromium oxide	0.50
Herring oil	11.23
Chemical analysis of experimental diet on a dry matter basis	
% Dry matter (DM)	92.40 ± 0.29
Ash (%)	8.17 ± 0.07
Energy (kcal)/g DM	5.76 ± 0.02
Protein (%)	55.69 ± 0.19
Lipid (%)	18.57 ± 0.20
Cr_2O_3 (%)	0.45 ± 0.02

with a 2.0- and 3.0-mm die. Pellets were sifted to remove any fine particles, cooled to room temperature in a fan-ventilated chamber, and stored in a −20°C freezer until required for feeding.

Feed samples were ground to 1 mm and analysed for dry matter, protein and ash using standard methods (AOAC, 1990) and gross energy using an isoperibolic calorimeter (No. 1261, Parr Instruments, Moline, IL). Lipid extraction and quantification was carried out using methodologies of Bligh and Dyer (1959) and Kates (1972).

2.3. Protocol

Six hundred and sixty transgenic salmon, average weight 9.42 ± 0.09 g, were randomly distributed in 12 tanks with a total of 55 fish per tank. Six hundred and sixty control salmon, average weight 6.62 ± 0.05 g, were randomly assigned to 12 additional tanks with a total of 55 fish per tank. The fish were allowed an acclimation period of 3 weeks to the experimental tanks and diet.

The number and total weight of fish in a minimum of three and a maximum of 12 replicate tanks containing each of the two experimental groups of fish were used to calculate growth rates; each tank contained at least 30 transgenic or control fish, the difference resulting from periodic sampling for body composition. Sets of triplicate tanks of both groups of fish were periodically diverted into the food deprivation study (Cook et al., 2000b) Energy and protein digestibility as well as feed conversion data were measured on three replicate tanks of fish per experimental group. The rates of water flow to individual 92-l fibreglass, flow-through experimental tanks were periodically adjusted (taking into account fish size and number) to maintain water oxygen levels above 6 mg/l, which was well above the critical level for both groups of fish (Stevens et al., 1998). Oxygen levels were measured using an Oxyguard Handy Mark 4 oxygen sensor (Point Four Systems, Port Moody, British Columbia, Canada).

At the start of the experiment, all the fish were anaesthetized using tertiary-amyl-alcohol (1.0 ml/l), weighed, and the mean wet weights were 13.72 ± 0.21 and 6.98 ± 0.07 g for transgenic and control fish, respectively. Fish were fed to satiation three times per day with at least 10% excess feed delivered to each tank. Every 2 weeks cumulative wet fish weights per tank were measured and divided by the number of fish in the tank to calculate the average weight per fish. At the start of the experiment and at approximately 10 g wet weight intervals, subsamples of five fish per tank from three replicate tanks per experimental group (using tanks not previously sampled) were euthanised with an anaesthetic overdose, wrapped in cellophane and stored at −20°C for whole body composition analysis. The experiment was terminated when the fish reached a wet weight of approximately 55 g. Specific growth rate (SGR) was calculated according to the following equation:

$$\mathrm{SGR}\,(\%\,\text{body wt. gain/day}) = \left[\frac{(\mathrm{Log}_n\,\text{Final fish wt.} - \mathrm{Log}_n\,\text{Initial fish wt.})}{\text{Time interval}}\right]100$$

Protocol for collection of uneaten feed consisted of turning off the in-tank, water circulation pump just prior to feeding and placing a water diversion standpipe over the

centre drain to prevent uneaten feed from exiting the tank, yet still allowing water to leave at the top of the standpipe. Approximately 20 min after feeding, uneaten feed was siphoned out of the tank and filtered through a 10-μm pre-weighed filter, oven-dried for approximately 36 h at 60°C and then weighed. The in-tank, water circulation pump was then turned back on.

For determination of digestibility, voided faeces were collected at three day intervals and averaged between each measurement date. The water diversion standpipe was left over the centre drain to prevent voided faeces from exiting the tank. Just prior to the second and third daily feed, (i.e., 4 h after the previous feeding), the well-defined faecal casts were syphoned out of the tank, filtered and stored at −20°C for composite analysis at a later date. Because the weight of dry matter of the faeces collected over a 2-week period was insufficient to perform chemical analysis, faeces collected from each tank throughout the entire experimental period for each experimental group were pooled for analysis.

2.4. Fish and faecal sample preparation and chemical analysis

Frozen whole fish were autoclaved for 20 min at 120°C, homogenized with a known volume of distilled water, lyophilized, then equilibrated to room humidity, weighed, and further homogenized to a fine powder. Samples were analysed for dry matter, protein and ash using standard methods (AOAC, 1990) and for gross energy using an isoperibolic calorimeter (No. 1261, Parr Instruments, Moline, IL). Lipid extraction and quantification were carried out using the methodologies of Bligh and Dyer (1959) and Kates (1972). All chemical analyses were done in duplicate and averaged.

Frozen faecal samples were lyophilized, ground to an even powder consistency, and analysed for protein and energy as described above. Chromic oxide in the feed and faeces as well as the total carcass minerals was determined by atomic absorption spectrophotometry (Arthur, 1970). The AD of a given nutrient was calculated using the following equation:

$$\text{Apparent digestibility}\,(\%) = 100 - 100\left[\frac{\%\text{nutrient faeces}}{\%\text{nutrient feed}} \times \frac{\%Cr_2O_3\ \text{feed}}{\%Cr_2O_3\ \text{faeces}}\right]$$

A Student's *t*-test was performed to demonstrate any significance between the transgenic and control replicate means using 95% as the critical level of significance.

Fig. 1. (a) Growth in relation to time for growth enhanced transgenic Atlantic salmon (open triangles) and controls (solid circles) at 12.6°C and fed to satiation three times/day on a commercial diet. Each data point represents one tank of fish. Data is presented with fitted regression lines (solid lines) with 95% confidence intervals (dashed lines). (b) Specific growth rates for growth enhanced transgenic Atlantic salmon and controls.

The relationship between wet body weight and time, as well as body composition and wet body weight, was demonstrated by regression analysis. A test for common slope

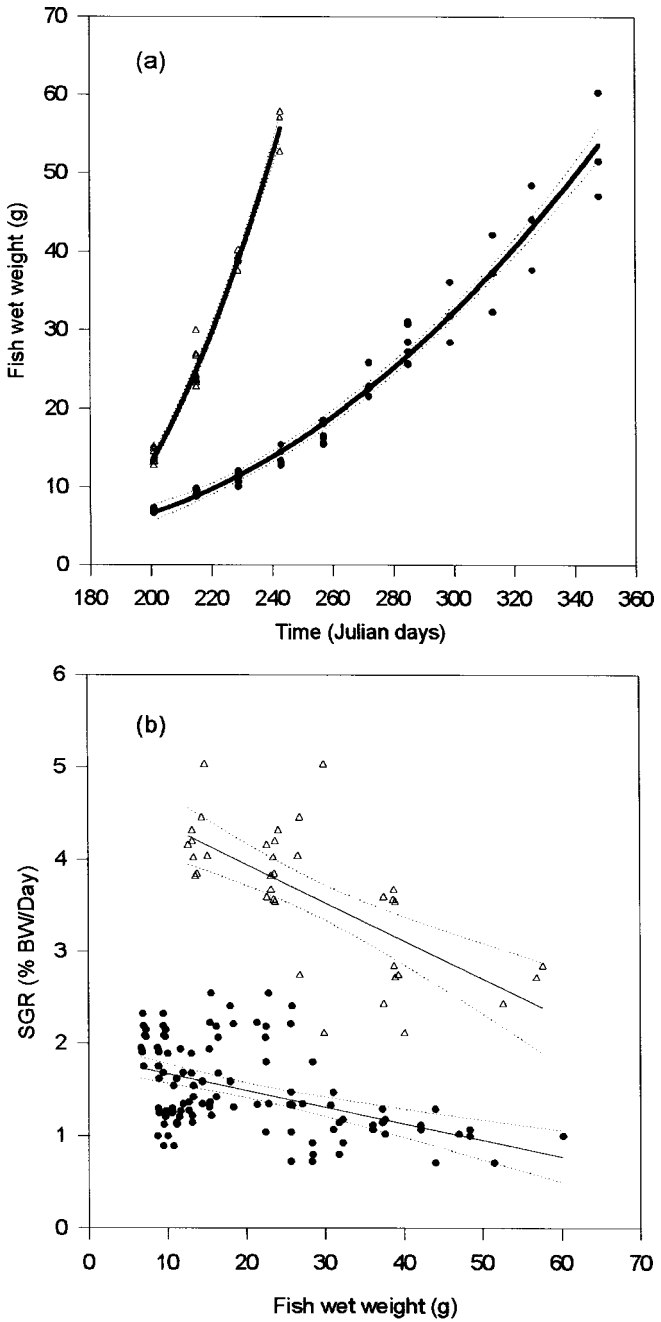

Table 2
Apparent digestibility (AD) of dry matter, energy and crude protein of diet fed to growth enhanced transgenic Atlantic salmon (13–55 g) and controls (7–53 g) fed to satiation three times/day

	Control	Transgenic
Dry matter AD (%)	75.55 ± 0.22	70.62 ± 3.9
Energy AD (%)	83.86 ± 0.53	80.74 ± 2.76
Protein AD(%)	89.96 ± 0.28	87.98 ± 1.67

No significant difference ($P > 0.05$) between digestibility parameters for transgenic and control fish.

was used to compare coefficients in regression equations for transgenic fish and control fish.

3. Results

3.1. Growth

The growth data for transgenic and control fish fed to satiation three times per day at a water temperature of 12.6°C (Fig. 1a) was subjected to nonlinear regression using a second degree polynomial resulting in the following relationship:

$$\text{Transgenic Weight (g)} = 163 - 2.18 \times \text{Time} + 0.00717 \times \text{Time}^2 \qquad (r^2 = 0.99)$$

$$\text{Control Weight (g)} = 31.6 - 0.380 \times \text{Time} + 0.00127 \times \text{Time}^2 \qquad (r^2 = 0.97)$$

where Time is Julian day.[1]

Transgenic fish grew at a significantly greater rate ($P < 0.05$) than did the control fish. Although the transgenic fish weighed nearly twice as much as the control fish at the beginning of the experiment, the mean wet body weight of transgenic fish was 4.08 times larger than the control fish when the transgenics reached the predetermined final weight of approximately 55 g. Even when the time to first feeding for the transgenic fish was significantly delayed by incubating their eggs at a lower temperature, the control fish had to be reared an additional 4 months for these fish to obtain a weight equal to the terminal weight of the transgenic fish. To facilitate direct comparison between transgenic and control fish, SGRs were calculated between each weight collection date (Fig. 1b), and as expected growth was inversely related to body weight in both groups of fish. Data was subjected to linear regression analysis resulting in the following relationship:

$$\text{Transgenic SGR} = 4.77 - 0.0414 \times \text{Weight} \qquad (r^2 = 0.46)$$

$$\text{Control SGR} = 1.85 - 0.0179 \times \text{Weight} \qquad (r^2 = 0.21)$$

At a common wet weight of 14 g, transgenic and control SGRs were 4.19%/day and 1.60%/day, respectively — a 2.62-fold difference. The magnitude of difference increased to 2.85-fold at 52 g, with transgenic and control SGRs 2.62%/day and

[1] Julian day — a serial number equal to the number of days elapsed since January 1st.

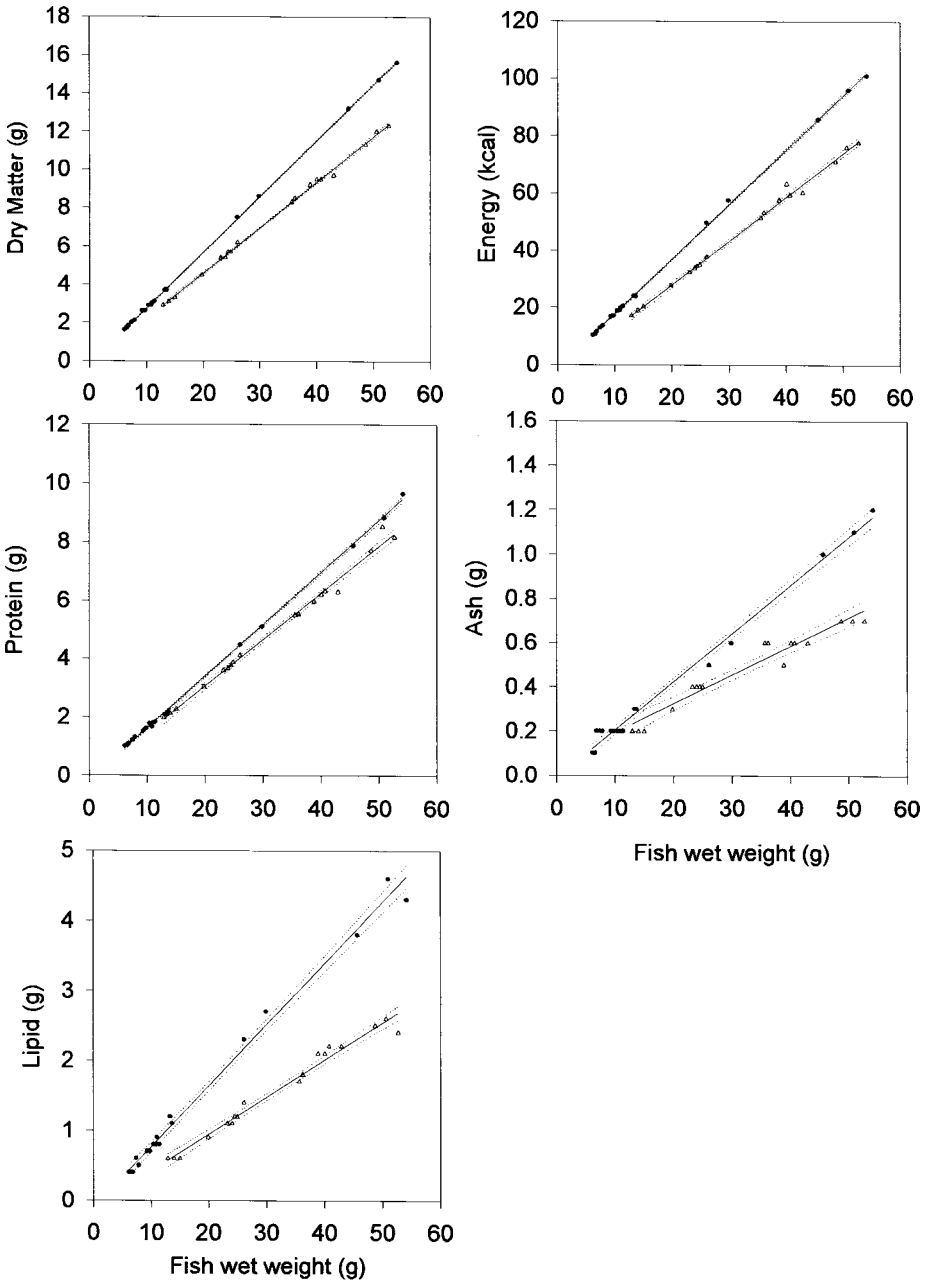

Fig. 2. Body composition and energy content in relation to wet body weight of growth enhanced transgenic Atlantic salmon (open triangles) and controls (solid circles) fed to satiation three times/day on a commercial diet. Each data point represents a subsample of five fish. Data is presented with fitted regression lines (solid lines) surrounded by 95% confidence intervals (dashed lines).

0.92%/day, respectively. A test for common slope revealed that the SGR regression line for the transgenic fish was significantly ($P < 0.05$) steeper than the SGR regression slope for the control fish.

3.2. Digestibility and feed conversion

Transgenic fish consumed more feed (% body weight/day) than control fish of comparable size. At 14 g, daily feed consumption by transgenic and control fish was 2.8% and 1.0% of body weight per day, respectively — a 2.8-fold difference; the magnitude of which decreased to 2.2-fold at approximately 55 g, with transgenic and control daily feed intake 1.7% and 0.77% of body weight per day, respectively. Mean AD coefficients for dry matter, crude protein and energy (Table 2) were not significantly different ($P > 0.05$) between transgenic and control salmon. Gross feed conversion efficiency (wet weight gain by fish/dry weight of feed consumed) over the entire growth period averaged 1.17 ± 0.05 for control fish and 1.52 ± 0.05 for transgenic salmon. Adjusting this efficiency for the higher moisture level in the transgenic fish results in a value of 1.29 for transgenic fish, which represents 10% improvement in feed utilization by the growth-enhanced fish.

3.3. Fish body composition

With the exception of moisture content, which was 5–6% greater ($P < 0.05$) in transgenic fish, the carcass of transgenic fish contained significantly lower absolute levels of all body constituents than control fish at all measured body weights (Fig. 2; Table 3). When body composition data (Fig. 3), expressed as a percentage basis of the fish wet weight, was subjected to nonlinear regression using a second degree polynomial (Table 4), percent protein, dry matter, ash, and lipid varied little (< 1–2%) within

Table 3
Regression coefficients for the relation between body composition and energy content per fish wet weight of growth enhanced transgenic Atlantic salmon and controls fed to satiation three times/day on a commercial diet: $Y = b_0 + b_1 \times \mathrm{BW}$ where 'Y' is absolute nutrient or energy content, 'b_0' and 'b_1' are regression coefficients, and 'BW' is wet body weight

Y (g or kcal)/fish	Fish strain	b_0	b_1	r^2
Protein	Control	−0.158	0.178	0.99
	Transgenic	−0.137	0.160	0.99
Dry matter	Control	−0.207	0.293	0.99
	Transgenic	−0.185	0.238	0.99
Ash	Control	−0.013	0.022	0.99
	Transgenic	0.065	0.013	0.93
Lipid	Control	−0.110	0.087	0.99
	Transgenic	−0.110	0.053	0.98
Energy (kcal)	Control	−1.411	1.909	0.99
	Transgenic	−2.794	1.533	0.99

Comparable regression coefficients between the two experimental groups are all significantly different ($P < 0.05$).

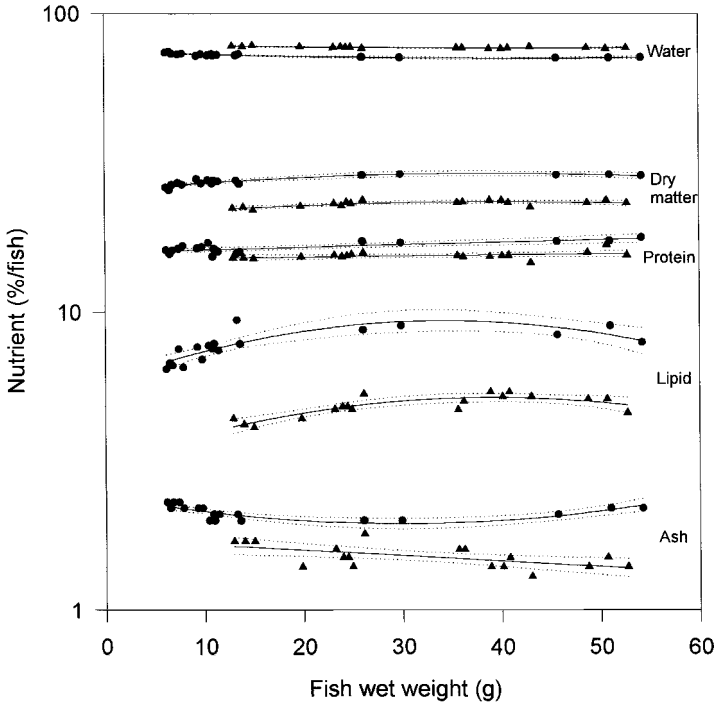

Fig. 3. Nutrient content as percent wet body weight of growth enhanced transgenic Atlantic salmon (open triangles) and controls (solid circles) fed to satiation three times/day on a commercial diet. Each data point represents a subsample of five fish. Data is presented with fitted regression lines (solid lines) surrounded by 95% confidence intervals (dashed lines).

Table 4
Regression coefficients for the relation between body composition and energy content per unit wet weight of growth-enhanced transgenic Atlantic salmon and controls fed to satiation three times/day on a commercial diet: $Y = b_0 + b_1 \times \mathrm{BW} + b_2 \times \mathrm{BW}^2$ where 'Y' is percent constituent, 'b_0', 'b_1' and 'b_2' are regression coefficients, and 'BW' is wet body weight

Y (% constituent)/g wet weight fish	Fish strain	b_0	b_1	b_2	r^2
Protein	Control	15.8416	0.0332	n/a	0.61
	Transgenic	15.0225	0.0137	n/a	0.17
Dry matter	Control	25.3839	0.192	−0.0024	0.82
	Transgenic	20.7596	0.1336	−0.0017	0.58
Ash	Control	2.4031	−0.0305	0.0005	
	Transgenic	1.8401	−0.0147	0.0001	0.37
Lipid	Control	5.5731	0.2148	−0.0031	0.69
	Transgenic	2.8451	0.1177	−0.0015	0.71
Moisture	Control	74.6161	−0.192	0.0024	0.82
	Transgenic	79.2404	−0.1336	0.0017	0.58

Comparable regression coefficients between the two experimental groups are all significantly different ($P < 0.05$).

transgenic and control groups throughout the range of wet body weights. Fifty-gram control salmon had 1.33 times more ash per unit dry body weight and 1.51 times more ash per unit wet body weight than did transgenic salmon of comparable size (Fig. 2). This is largely accounted for by the fact that the dried carcass of transgenic fish has only 36% of the body calcium and 75% of the body phosphorus as does that of a control salmon (Sutterlin, unpublished data), which suggest incomplete bone mineralization in these presmolt transgenic salmon.

4. Discussion

4.1. Growth

The present study confirmed that growth rates of transgenic Atlantic salmon containing a GH transgene were significantly greater than control salmon, and is supportive of the findings of Saunders et al. (1998), that transgenic smolts can be produced eight months after egg fertilization. SGRs were nearly threefold greater than transgenic salmon compared to non-genetically altered controls. These results are consistent with those of Du et al. (1992) and Fletcher et al. (1992) who reported transgenic Atlantic salmon two- to sixfold larger in weight at a specific age than control salmon, with the largest transgenic fish 13-fold larger than the mean weight of control fish. Because, these authors did not report SGRs, direct comparisons to growth rates in the present study is not possible. Nevertheless, the magnitude of growth enhancement in these studies appear to be lower than those reported by Devlin et al. (1995) who found the average weight of transgenic coho salmon was 10-fold larger than control fish of the same age. Zhu (1992) speculated that differences in growth enhancement between transgenic lines probably arise from the chromosomal site of integration, the number of tandem gene inserts at a loci as well as the type and effectiveness of the promoter used.

Exogenous hormone treatment, while showing positive results, has generally not yielded growth rates of a magnitude comparable to the 2.85-fold enhancement reported in the present study. Cavari et al. (1993) observed that fingerling gilthead seabream, injected with bovine or human GH, were 1.15-fold larger than control fish of the same age. Juvenile coho salmon injected with bovine GH exhibited 2.18-fold weight gain over non-injected controls (Higgs et al., 1977). Gill et al. (1985) found that injection of recombinant chicken or bovine GH into juvenile Pacific salmon increased weight gain by approximately twofold and lowered feed conversion ratios. McLean et al. (1997) observed a very impressive fourfold elevation in growth rate in chinook salmon using a single injection of a slow releasing formulation of bovine GH. Supplementation of feed with recombinant porcine or fish GH has resulted in a 1.5- to 1.6-fold increase in weight for coho salmon and juvenile black seabream (*Acanthopagrus schlegeli*) over control fish fed a non-supplemented diet (McLean et al., 1993; Tsai et al., 1997), and Danzmann et al. (1990) reviewed 37 cases where salmonid growth rates were enhanced through exogenous hormone treatment, all of which exhibited lesser magnitudes of growth acceleration than quantified in the present experiment. As many of these authors did not report SGR adjusted for temperature or body weight, direct comparison to growth rates in the present study is not possible.

Transgenic salmon exhibited a larger absolute decrease in SGR than control fish as body weight increased (Fig. 1b), initially suggesting that the effect of transgenesis is transient. This is largely due to sampling being restricted to a limited size range of fish, as growth rates of transgenic Atlantic salmon and rainbow trout observed by Sutterlin (unpublished data) indicate that these fish continue to exhibit enhanced growth well into the post smolt phase.

GH, normally secreted in the pituitary and under control of the hypothalamus, is involved in the regulation of somatic growth primarily through the induction of insulin-like growth factors (IGF) (Chen et al., 1994; Norris, 1997). Secretion of GH typically occurs in bursts and varies seasonally in fish. However, the transgenic fish used in this study employ an antifreeze gene promoter from ocean pout to drive the expression of a GH transgene, and it is hypothesized that GH secretion (in this particular line of transgenic fish) is not under control of neuroendocrine factors, but occurs predominately in the liver and perhaps other tissues (Fletcher et al., 1985, 1990; Gong et al., 1992). Providing there is adequate nutrition, continuous GH secretion would presumably mediate faster growth through continuous induction of IGF.

4.2. Digestibility and feed conversion

Despite the elevated rate of food processing, transgenesis does not appear to affect the extent to which protein, dry matter, and energy are digested (Table 2). This may in part be due to the larger digestive surface area in these fish (Stevens et al., 1999). Protein digestibility coefficients of transgenic and control fish were 88% and 90%, respectively, which correspond to the value of 87% reported by Shearer et al. (1992) for juvenile Atlantic salmon. Because dietary protein content and source can significantly affect digestibility (Jobling, 1983; Cho and Bureau, 1995), current results were compared with literature values only in cases where diets employed were of composition similar to that used in the present study. Most current commercial salmon diets contain approximately 50–55% protein and 15–25% lipid. Hajen et al. (1993) observed protein digestibility coefficients in chinook salmon of 76–87%, respectively. Apparent energy digestibility values of 80–86% for rainbow trout (Cho et al., 1976) and 73–80% in chinook salmon (Hajen et al., 1993) are similar to the values (81–84%) obtained in the present study. The dry matter digestibility values reported here (71–76%) are slightly lower than the 87% observed by Shearer et al. (1992) in juvenile Atlantic salmon.

The validity of different methods used for faecal collection in fish digestibility experiments remain controversial after decades of study and often account for the large degree to variability reported in the literature. Also, differences in fish species, fish size, water temperature and feeding regime all likely affect digestibility measurements (Jobling, 1983; Cho and Bureau, 1995). In the present study, AD estimates were made using faecal material collected over the 3.5 h prior to the second and third daily feeding. As the fish were fed to satiation three times per day at 4-h intervals, the maximum period in which voided faecal material was exposed to the water was less than 3.5 h. While Cho et al. (1982) and De Silva and Perera (1984) postulated that leaching of nutrients does not have a significant effect on the digestibility calculation, Austreng (1978) and Lied et al. (1982) state that calculations based on faeces collected from water

will give an overestimation of digestibility of water soluble nutrients. The objective of the present study was not to perfect a digestibility technique but to quantify and compare feed digestibility by transgenic salmon to that of non-genetically manipulated control fish using a methodology which attempted to minimize known sources of error typically encountered in digestibility studies.

Appetite stimulation and improved feed conversion have been observed through exogenous hormone treatment (Higgs et al., 1979, 1982; Gill et al., 1985). Markert et al. (1977) found significantly enhanced growth rates and improved dry matter and protein conversion in yearling coho salmon injected with bovine GH. Garber et al. (1995) injected 2-year-old rainbow trout (300–700 g) with recombinant bovine GH and observed improved feed efficiency. The gross feed conversion efficiency of 1.17 for control salmon observed in current study are within the range reported by Storebakken and Austreng (1987) for fingerling Atlantic salmon.

In terrestrial livestock, the genetic gains in growth attained using traditional methods of selective breeding often are accompanied by simultaneous improvements in feed conversion efficiency. The theoretical basis for a similar expectation in fish has been presented by Gjedrem (1997). This model assumes that fish selected over generations for rapid growth will have the same energetic maintenance requirements as non-selected fish at any given weight, but that rapid growth by selected lines will accrue this energy cost over a shorter period of time, thus, accounting for improved feed conversion. The same explanation would also appear to apply to the growth-enhanced fish in the current study in that Brett and Groves (1979) estimate the maintenance ration of juvenile sockeye salmon at 15°C represents about 25% of the maximum ration. Therefore, fish growing three times more rapid and having the same net feed conversion efficiency will only require 1/3 of this food for maintenance.

4.3. Body composition

Previously reported effects of GH treatment, either exogenous (administered) or endogenous (transgenic), on body composition have not been entirely consistent. Higgs et al. (1975) and Markert et al. (1977) injected yearling coho salmon with bovine GH, and although significantly enhanced growth rates and protein conversion efficiencies were observed, treated fish had significantly lower percentage of muscle protein per unit of wet fish weight due to retention of a greater percentage of muscle water than untreated fish. Higher total carcass moisture content in transgenic salmon was also observed in the present study. Rainbow trout, injected with recombinant fish GH showed no significant difference in body composition (Agellon et al., 1988). However, in the latter study, tissue samples were collected 4 weeks after the last hormone treatment and consequently differences between treated and controls may have subsided.

In the present study, there were significant differences between transgenics and controls for all constituents of body composition measured, when each component was compared either as an absolute or percent value against wet body weight. Caution must be taken when comparing body composition between experimental groups because the comparison of percentages of nutrients on a dry weight basis can be misleading as a change in one component will affect the proportion representation of other components

(Shearer, 1994). Also, exogenous factors such as previous nutritional history (feed quality and feeding rate) can affect body components, particularly lipid and moisture contents (Reinitz, 1983). When energy requirements are exceeded, energy is stored as body lipid, and this component has been found to be inversely related to the moisture content which will decrease or increase as lipid is stored or utilized (Brett et al., 1969). The transgenic salmon in the present study had less body fat than control fish which was a function of their greater energy demand and elevated metabolic rate (Stevens et al., 1998; Cook, 1999; Cook et al., 2000a); consequently, a higher tissue moisture content was not unexpected. Chatakondi et al. (1995) reported that the muscle of F_1 generation transgenic carp (1.60 kg) had a higher percent of protein, lower percent lipid, and a lower percent moisture than did the controls; Fu et al. (1998) reported similar results in total carcass composition of F_4 generation (< 10 g) transgenic carp. The magnitude of growth acceleration of these F_4 generation transgenic carp, however, was much lower than the 2.85-fold increase reported in the present study. Feeding regime, diet composition, as well as genetic predisposition all could have resulted in the transgenic fish having altered body compositions in comparison to the control fish. The altered chemical composition observed in the presmolt transgenic salmon in this study appears to be only transient because no such differences in protein, lipid, moisture or mineral content were observed in larger 3-kg growth-enhanced Atlantic salmon and rainbow trout containing the same gene construct (Sutterlin et al., unpublished data).

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References

Agellon, L.B., Emery, C.J., Jones, J.M., Davies, S.L., Dingle, A.D., Chen, T.T., 1988. Promotion of rapid growth of rainbow trout (*Salmo gairdneri*) by a recombinant fish growth hormone. Can. J. Fish. Aquat. Sci. 45, 146–151.

AOAC (Association of Official Analytical Chemists), 1990. Official Methods of Analysis. 15th edn. AOAC, Arlington, VA.

Arthur, D., 1970. The determination of chromium in animal feed and excreta by atomic absorption spectrophotometry. Can. Spectrosc. 15, 134.

Austreng, E., 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. Aquaculture 13, 265–272.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Brett, J.R., Groves, T.D.D., 1979. Physiological energetics. In: Fish Physiology. Hoar, W.S., Randall, D.J., Brett, J.R. (Eds.), Bioenergetics and Growth vol. 8 Academic Press, NY, pp. 279–352.

Brett, J.R., Shelbourn, J.E., Shoop, C.T., 1969. Growth rate and body composition of fingerling sockeye salmon (*Oncorhynchus nerka*) in relation to temperature and ration size. J. Fish. Res. Board Can. 26, 2363–2394.

Cavari, B., Funkenstein, B., Chen, T.T., Gonzalez-Villasenor, L.I., Schartl, M., 1993. Effect of growth hormone on the growth rate of the gilthead seabream (*Sparus aurata*), and use of different constructs for the production of transgenic fish. Aquaculture 111, 189–197.

Chatakondi, N., Lovell, R.T., Duncan, P.L., Hayat, M., Chen, T.T., Powers, D.A., Weete, J.D., Cummins, K., Dunham, R.A., 1995. Body composition of transgenic common carp, *Cyprinus carpio*, containing rainbow trout growth hormone gene. Aquaculture 138, 99–109.

Chen, T.T., Marsh, A., Shamblott, M., Chan, K.-M., Tang, Y.-L., Cheng, C.M., Yang, B.-Y., 1994. Structure and evolution of fish growth hormone and insulinlike growth factor genes. In: Farrell, A.P., Randall, D.J. (Eds.), Fish Physiology vol. 13 Academic Press, San Diego, pp. 179–212.

Cho, C.Y., Bureau, D.P., 1995. Determination of the energy requirements of fish with particular reference to salmonids. J. Appl. Ichthyol. 11, 141–163.

Cho, C.Y., Slinger, S.J., Bayley, H.S., 1976. Influence of level and type of dietary protein, and of level of feeding on feed utilization by rainbow trout. J. Nutr. 106, 1547–1556.

Cho, C.Y., Slinger, S.J., Bayley, H.S., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comp. Biochem. Physiol. 73B, 25–41.

Cook, J.T., 1999. Bioenergetics of growth hormone transgenic Atlantic salmon (*Salmo salar*) reared under simulated aquaculture conditions. MSc Thesis, Atlantic Veterinary College. University of Prince Edward Island, Canada, 138 pp.

Cook, J.T., McNiven, M.A., Sutterlin, A.M., 2000a. Metabolic rate of presmolt growth enhanced transgenic (*Salmo salar*). Aquaculture.

Cook, J.T., Sutterlin, A.M., McNiven, M.A., 2000b. Effect of food deprivation on oxygen consumption and body composition of growth enhanced transgenic Atlantic salmon (*Salmo salar*). Aquaculture.

Danzmann, R.G., Van Der Kraak, G.J., Chen, T.T., Powers, D.A., 1990. Metabolic effects of bovine growth hormone and genetically engineered rainbow trout growth hormone in rainbow trout (*Oncorhynchus mykiss*) reared at a high temperature. Can. J. Fish. Aquat. Sci. 47, 1292–1301.

De Silva, S.S., Perera, M.K., 1984. Digestibility in *Sarotherodon niloticus* fry: effect of dietary protein level and salinity with further observations on variability in daily digestibility. Aquaculture 38, 293–306.

Devlin, R.H., Yesaki, T.Y., Blabi, C.A., Donaldson, E.M., Swanson, P., Chan, W.K., 1994. Extraordinary salmon growth. Nature 371, 209–210.

Devlin, R.H., Yesaki, T.Y., Donaldson, E.M., Du, S.J., Hew, C.L., 1995. Production of germline transgenic Pacific salmonids with dramatically increased growth performance. Can. J. Fish. Aquat. Sci. 52, 1376–1384.

Du, S.J., Gong, Z., Fletcher, G.L., Shears, M.A., King, M.J., Idler, D.R., Hew, C.L., 1992. Growth enhancement in transgenic Atlantic salmon by the use of an ''all fish'' chimeric growth hormone gene construct. Bio/Technology 10, 176–181.

Dunn, E.J., Polk, A., Scarrett, D.J., Olivier, G., Lall, S., Goosen, M.F.A., 1990. Vaccines in aquaculture: the search for an efficient delivery system. Aquacult. Eng. 9, 23–32.

Fletcher, G.L., Du, S.J., Shears, M.A., Hew, C.L., Davies, P.L., 1990. Antifreeze and growth gene transfer in Atlantic salmon. Bull. Aquacult. Assoc. Can. 90/4, 70–71.

Fletcher, G.L., Hew, C.L., Li, X., Haya, K., Kao, M.H., 1985. Year-round presence of high levels of plasma antifreeze peptides in a temperate fish, ocean pout (*Macrozoarces americanus*). Can. J. Zool. 63, 488–493.

Fletcher, G.L., Shears, M.A., King, M.J., Goddard, S.V., Kao, M.H., Du, S.J., Davies, P.L., Hew, C.L., 1992. Biotechnology for aquaculture: transgenic salmon with enhanced growth and freeze-resistance. Bull. Aquacult. Assoc. Can. 92/3, 31–33.

Fu, C., Cui, Y., Hung, S.S.O., Zhu, Z., 1998. Growth and feed utilization by F_4 human growth hormone transgenic carp fed diets with different protein levels. J. Fish Biol. 53, 115–129.

Garber, M.J., DeYonge, K.G., Byatt, J.C., Lellis, W.A., Honeyfield, D.C., Bull, R.C., Schelling, G.T., Roeder, R.A., 1995. Dose–response effects of recombinant bovine somatotropin (Posilac™) on growth performance and body composition of two-year-old rainbow trout (*Oncorhynchus mykiss*). J. Anim. Sci. 73, 3216–3222.

Gill, J.A., Sumpter, J.P., Donaldson, E.M., Dye, H.M., Souza, L., Berg, T., Wypych, J., Langley, K., 1985.

Recombinant chicken and bovine growth hormones accelerate growth in aquacultured juvenile Pacific salmon *Oncorhynchus kisutch*. Bio/Technology 3, 643–646.

Gjedrem, T., 1997. Selective breeding. World Aquacult. 28 (1), 33–45.

Gong, Z., Fletcher, G.L., Hew, C.L., 1992. Tissue distribution of fish antifreeze protein mRNAs. Can. J. Zool. 70, 810–814.

Hajen, W.E., Beames, R.M., Higgs, D.A., Dosanjh, B.S., 1993. Digestibility of various feedstuffs by post-juvenile chinook salmon (*Oncorhychus tshawytscha*) in sea water: 1. Validation of technique. Aquaculture 112, 321–332.

Hew, C.L., Fletcher, G.L., Davies, P.L., 1995. Transgenic salmon: tailoring the genome for food production. J. Fish Biol. 47 (A), 1–19.

Higgs, D.A., Donaldson, E.M., Dye, H.M., McBride, J.R., 1975. A preliminary investigation of the effect of bovine growth hormone on growth and muscle composition of coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 27, 240–253.

Higgs, D.A., Fagerlund, U.H.M., Eales, J.G., McBride, J.R., 1982. Application of thyroid and steroid hormones as anabolic agents in fish culture. Comp. Biochem. Physiol., Part B 73, 143–176.

Higgs, D.A., Fagerlund, U.H.M., McBride, J.R., Dye, H.M., Donaldson, E.M., 1977. Influence of combinations of bovine growth hormone, 17 alpha-methyltestosterone, and L-thyroxine on growth of yearling coho salmon (*Oncorhynchus kisutch*). Can. J. Zool. 55, 1048–1056.

Higgs, D.A., Fagerlund, U.H.M., McBride, J.R., Eales, J.G., 1979. Influences of orally administered L-thyroxine or 3,5,3′-triiodo-L-thyronine on growth, food consumption, and food conversion of underyearling coho salmon (*Oncorhynchus kisutch*). Can. J. Zool. 57, 1974–1979.

Jobling, M., 1983. A short review and critique of methodology used in fish growth and nutrition studies. J. Fish Biol. 23, 685–703.

Kates, M., 1972. Lipid extraction procedures. In: Work, T.S., Work, E. (Eds.), Techniques of Lipidology. North-Holland, Amsterdam, pp. 350–351.

Kayes, T., 1977. Effects of hypophysectomy, beef growth hormone replacement therapy, pituitary autotransplantation, and environmental salinity on growth in the black bullhead (*Ictalurus melas*). Gen. Comp. Endocrinol. 33, 371–381.

Lied, E., Julshamn, K., Braekkan, O.R., 1982. Determination of protein digestibility in Atlantic cod (*Gadus morhua*) with internal and external indicators. Can. J. Fish. Aquat. Sci. 39, 854–861.

Markert, J.R., Higgs, D.A., Dye, H.M., MacQuarrie, D.W., 1977. Influence of bovine growth hormone on growth rate, appetite, and food conversion of yearling coho salmon (*Oncorhynchus kisutch*) fed two diets of different composition. Can. J. Zool. 55, 74–83.

McLean, E., Devlin, R.H., Byant, J.C., Clark, W.C., Donaldson, E.M., 1997. Impact of a controlled release formulation of bovine growth hormone upon growth and seawater adaptation in coho (*Oncorhyncus kisutch*) and chinook (*Oncorhynchus tshawytscha*) salmon. Aquaculture 156, 113–128.

McLean, E., Donaldson, E.M., Teskeredzic, E., Souza, L., 1993. Growth enhancement following dietary delivery of recombinant porcine somatotropin to diploid and triploid coho salmon (*Oncorhynchus kisutch*). Fish Physiol. Biochem. 11, 363–369.

Moav, B., Liu, Z., Moav, N.L., Gross, M.L., Kapuscinski, A.R., Faras, A.J., Guise, K., Hackett, P.B., 1992. Expression of heterologous genes in transgenic fish. In: Hew, C.L., Fletcher, G.L. (Eds.), Transgenic Fish. World Scientific, Singapore, pp. 120–141.

Moriyama, S., Yamamoto, H., Sugimoto, S., Abe, T., Hirano, T., Kawauchi, H., 1993. Oral administration of recombinant salmon growth hormone to rainbow trout, *Oncorhynchus mykiss*. Aquaculture 112, 99–106.

Norris, D.O., 1997. Vertebrate Endocrinology. Academic Press, San Diego, 634 pp.

Reinitz, G., 1983. Relative effect of age, diet, and feeding rate on the body composition of young rainbow trout (*Salmo gairdneri*). Aquaculture 35, 19–27.

Saunders, R.L., Fletcher, G.L., Hew, C.L., 1998. Smolt development in growth hormone transgenic Atlantic salmon. Aquaculture 168, 177–193.

Shearer, K.D., 1994. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. Aquaculture 119, 63–88.

Shearer, K.D., Maage, A., Opstvedt, J., Mundheim, H., 1992. Effects of high-ash diets on growth, feed efficiency, and zinc status of juvenile Atlantic salmon (*Salmo salar*). Aquaculture 106, 345–355.

Shears, M.A., King, M.J., Goddard, S.V., Fletcher, G.L., 1992. Gene transfer in salmonids by injection

through the micropyle. In: Hew, C.L., Fletcher, G.L. (Eds.), Transgenic Fish. World Scientific, Singapore, pp. 44–60.

Stevens, E.D., Sutterlin, A., Cook, T., 1998. Respiratory metabolism and swimming performance in growth hormone transgenic Atlantic salmon. Can. J. Fish. Aquat. Sci. 55, 2028–2035.

Stevens, E.D., Wagner, G.N., Sutterlin, A.M., 1999. Gut morphology of GH transgenic salmon. J. Fish Biol. 55, 517–526.

Storebakken, T., Austreng, E., 1987. Ration level for salmonids: I. Growth, survival, body composition, and feed conversion in Atlantic salmon fry and fingerlings. Aquaculture 60, 189–206.

Thorpe, J.E., 1977. Bimodal distribution of length of juvenile Atlantic salmon (*Salmo salar* L.) under artificial rearing conditions. J. Fish Biol. 11, 175–184.

Thorpe, J.E., Morgan, R.I.G., Ottaway, E.M., Miles, M.S., 1980. Time of divergence of growth groups between potential 1+ and 2+ smolts among sibling Atlantic salmon. J. Fish Biol. 17, 13–21.

Tsai, H.-J., Hsih, M.-H., Kuo, J.-C., 1997. *Escherichia coli*-produced fish growth hormone as a feed additive to enhance the growth of juvenile black seabream (*Acanthopagrus schlegeli*). J. Appl. Ichthyol. 13, 79–82.

Yu, T.C., Sinnhuber, R.O., Hendricks, J.D., 1979. Effect of steroid hormones on the growth of coho salmon (*Oncorhynchus kisutch*). Aquaculture 16, 351–359.

Zhu, Z., 1992. Generation of fast growing transgenic fish: methods and mechanisms. In: Hew, C.L., Fletcher, G.L. (Eds.), Transgenic Fish. World Scientific, Singapore, pp. 92–119.