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BIOENERGETICS OF GROWTH ENHANCED TRANSGENIC ATLANTIC SALMON (*SALMO SALAR*) REARED UNDER SIMULATED AQUACULTURE CONDITIONS

A Thesis

Submitted to the Graduate Faculty

in Partial Fulfilment of the Requirements

for the Degree of

Master of Science

in the Department of Health Management

Faculty of Veterinary Medicine

University of Prince Edward Island

J. Todd Cook

Charlottetown, P.E.I.

May 13, 1999

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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 more information relating to the bioenergetics of several commercially important production traits. The primary objective of the study was to compare the growth rate, protein and energy digestibility, feed conversion, and body composition of F_2 generation growth enhanced transgenic Atlantic salmon (*Salmo salar*) with that of non-genetically modified salmon, over a presmolt growth interval of 8 to 55 g. As a secondary objective, the routine oxygen consumption was measured to determine if rapid growth exhibited by transgenic salmon reared under simulated commercial culture conditions significantly altered metabolic rate. The third objective was to investigate the influence of food deprivation on metabolism in an attempt to relate oxygen consumption to the rate of mobilization of energy reserves in transgenic fish relative to their non-transgenic counterparts.

The growth enhanced transgenic fish had a 162-185% higher growth rate relative to non-transgenic salmon over the body weight interval examined. Daily feed consumption over the body weight interval examined was 114-162% higher for the transgenic fish compared to the controls. 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. Similarly, there was no significant difference in feed conversion between the two experimental groups. Body protein, dry matter, ash, lipid and energy were significantly lower in the transgenic salmon relative to controls. Over the body weight interval examined, routine oxygen consumption rates (mg O_2/h) with inclusion of the heat increment associated with feeding were 54-70% higher for transgenic fish compared to the controls. However, integrated over time, the transgenic salmon consumed 37% 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 were 58-130% higher for transgenics over controls.

Throughout most of the eight weeks of starvation, transgenic fish exhibited a greater rate of oxygen consumption compared to control salmon, but also exhibited a more rapid decline in oxygen consumption with starvation time. Consequently, depending on initial weight and length of feed deprivation, the rate of oxygen consumption of transgenic fish declined to where it equaled or was less than the oxygen consumption of control fish. Transgenic fish depleted body protein, dry matter, lipid and energy at a faster rate than did the controls. Additionally, in both groups, lipid was catabolized faster than was protein.

The transgenic experimental subjects used throughout the present study possessed the physiological plasticity necessary to accommodate an acceleration in growth well beyond the normal range for this species with few effects other than greater appetite, higher metabolic rate and leaner body. However, with their higher metabolism with respect to control fish, combined with lower energy reserves, transgenic fish may have lower survival outside intensive culture conditions.

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1.0 GENERAL INTRODUCTION

1.1 Current state of salmon aquaculture.

World aquaculture as a food production industry has increased significantly over the last two decades. The culture of salmonids (salmon and trout) has been dominated by Atlantic salmon (*Salmo salar*) with global output expanding from 27,000 metric tonnes in 1984 to 472,000 metric tonnes in 1995 (FAO, 1997). Globalization of the fisheries trade has created competitive pressures in international markets (Brown and Stechey, 1997) consequently forcing market prices down. Application of new culture techniques, improvements in nutrition, and advances in aquatic biotechnology to improve relevant economic traits have contributed to reducing production costs in an attempt to stabilize dwindling profit margins.

1.2 Salmon life history.

Atlantic salmon are anadromous; adults return to freshwater streams or rivers, from the sea, to reproduce (Saunders, 1995). Spawning in Canadian waters typically occurs in October and November. Females excavate "redds", shallow depressions in the gravel river bottom. Upon completion of the nest, the female settles into the depression, a male aligns himself beside her, and eggs and sperm are released (Scott and Scott, 1988). Fertilized eggs are covered by the female with 12-25 cm of gravel. The female does not release all her eggs at one time (Sedgwick, 1982). The same spawning pair deposits eggs in a number of different redds over a 1-2 week period.

After extrusion from the body the eggs (5-7 mm in diameter) remain buried in the

gravel over winter where development takes place very slowly due to low water temperatures (Scott and Scott, 1988). After approximately 440 degree days the sac fry, also called alevins, hatch but remain in the redd drawing nourishment from their stored yolk (Sedgwick, 1982). Upon emerging from the gravel, the underyearling salmon are called fry during their first summer in fresh water, and parr thereafter (Saunders, 1995). Parr are typically identifiable by characteristic dark vertical bands, "parr marks", composed of concentrations of melanophores along the fish's sides (Gorbman *et al.*, 1982). When parr become 12-15 cm long, they undergo a distinct transformation into smolts and subsequently are ready for seaward migration (Scott and Scott, 1988). This parr-smolt transformation is a complex series of morphological, physiological, biochemical, and behaviour changes which must be completed and synchronized temporally to ensure successful transition into the marine environment. Atlantic salmon spend 1-3 years growing rapidly at sea before returning to the home river to spawn (Scott and Scott, 1988).

Through environmental manipulation (artificial photoperiod, thermal control, and proper nutrition), fish husbandry practices have increased growth rates in the freshwater stage well above those normally encountered in nature. Current artificial production of Atlantic salmon, using non-genetically altered fish, consists of approximately 12-18 months in freshwater and another 12-24 months in seawater. Cultured salmon are manually spawned, the eggs fertilized, water hardened and disinfected, and then incubated. Smolts are usually transferred to marine sites in mid-April to mid-June, spend the next 12-24 months growing rapidly, and are subsequently are marketed at 3-5 kg in weight.

1.3 Aquatic biotechnology.

The application of aquatic biotechnology, in the form of chromosome-set manipulation, gynogenesis, androgenesis, and genome alteration via transgenes, have the potential to make significant contributions to the aquaculture industry.

Gynogenesis involves the activation of egg development by sperm but without the sperm making a genetic contribution to the resulting embryo. This is usually accomplished by irradiating the sperm with ultraviolet light which genetically deactivates the sperm but does not affect its ability to activate egg development (Chourrout, 1982). Rapid production of inbred lines ultimately leading to the elimination of recessive lethal or deleterious alleles has been met with limited success for tilapia (*Oreochromis niloticus*; *O. aureus*; *O. mossambicus*) culture (Mair, 1993). Developing all-female strains would be advantageous for those species in which the female has a higher rate of growth than the male. Similar to gynogenesis, androgenesis involves production of progeny in which the genetic material is derived exclusively from the male. The egg, as opposed to the sperm, is irradiated with gamma radiation before being fertilized with normal sperm (Parsons and Thorgaard, 1985). Recovery of strains from cryopreserved sperm, production of individuals with the same nuclear genotype, and generation of YY males are potential applications for this biotechnology (Scheerer *et al.*, 1986; Thorgaard *et al.*, 1990; 1992).

Techniques in polyploid induction have led to the development of triploid fish for aquaculture and fish management programs (Purdom, 1983). Concern over aquaculture fish escaping into the natural environment and altering "wild" fish gene pools could be resolved using sterile triploids. Thermal, pressure, and chemical treatments have been successfully

used to produce triploid fish through induced retention of the second polar body in eggs fertilized with viable spermatozoa (Chourrout, 1980; Refstie, 1981; Lou and Purdom, 1984). Similarly, tetraploid fish are produced by blocking the first mitotic division in the egg (Chourrout *et al.*, 1986). Although they show reduced viability, tetraploid fish have been raised to sexual maturity and demonstrated the ability to produce sterile triploids when crossed with normal diploids (Chourrout and Nakayama, 1987).

The use of various exogenous steroids, androgens (to masculinize) and estrogens (to feminize), is well established for producing unisex species populations (Hunter and Donaldson, 1983). While it is advantageous to use male tilapia (*Oreochromis mossambicus*) in the aquaculture industry due to their greater growth potential with respect to the females (Kirk, 1972), salmonid females typically sexually mature at an older age than their male counterparts; hence feminization is desirable to prevent deterioration in flesh quality prior to harvest (Yamazaki, 1983).

1.3.1 Genome alteration via transgenes.

Another form of biotechnology being explored for the aquaculture industry is the incorporation of transgenes to improve relevant economic traits. Animals into which new genetic material has been artificially introduced are termed "genetically-modified" or "transgenic". The potential benefits of gene transfer technology in improving fish growth rates, combatting disease, and altering other aspects of fish production are just beginning to be realized. Transgenic organisms may also act as bioreactors for producing valuable compounds and pharmaceuticals (Dziadek, 1996). The isolation and construction of genes for desirable traits, and their subsequent transfer into broodstock, could complement and/or

provide substantial advantages over conventional approaches of selective genetic breeding. Evidence from avian studies has provided support that gene transfer technology can successfully confer resistance to a retroviral pathogen (Salter and Critterden, 1988).

Commercial marine fish culture in ocean pens is presently not possible in many areas of the world due to lethal low water temperatures encountered during winter months. Although there has been preliminary success in producing transgenic Atlantic salmon containing antifreeze protein genes (Fletcher *et al.*, 1990; 1992), the levels of gene expression are still too low to confer significant freeze resistance.

1.3.1.1 Growth hormone gene.

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. One of the major factors influencing the success of any aquaculture enterprise is the growth performance of the cultured organism. While improvements in selective breeding, husbandry techniques, and environmental control has shortened the time to harvest thereby reducing expenses (capital maintenance and labour) while also lowering period of exposure to risks (predation and storm damage losses), progress has been relatively slow.

Over twenty years ago, research began to focus on the use of hormones and steroids to increase growth. The effects of bovine GH (Higgs *et al.*, 1975; Kayes, 1977; Markert *et al*, 1977; Danzmann *et al.*, 1990), thyroid and steroid hormones (Higgs *et al.*, 1979; Yu *et al.*, 1979; Higgs *et al.*, 1982), 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 concluded that solution dips, slow-release implanted pellets, and injection of these hormones and steroids will stimulate (in varying efficiencies depending on the technique used) significant increases in growth and, in some cases, feed conversion. The limited availability and high cost of commercial quantities of GH, the labour intensive nature of the treatment, and the need to periodically re-administer these hormones have not made this an economically viable strategy. Commercial production of GH and advances in GH digestibility by fish when used as a feedstuff additive has produced promising results for enhancing growth (McLean *et al.*, 1993; Tsai *et al.*, 1997). Oral administration, however, does not address potential problems of worker safety in handling such feed nor the possibility that uneaten feed passing through a sea cage could be eaten by wild organisms.

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 concluded that transgenic fish would be the most practical approach to growth enhancement. Transgenic Atlantic salmon have been successfully developed with the foreign GH gene construct being expressed and transmitted to the F_3 generation (Fletcher *et al*, 1990;1992; Du *et al.*, 1992). Devlin *et al.* (1994; 1995a,b) produced growth enhanced transgenic coho salmon (*Oncorhynchus kisutch*) that were 10- to 37-fold larger in weight (at the same age) than their non-transgenic counterparts. A significant enhancement in growth has also been observed in transgenic rainbow trout (*O. mykiss*), cutthroat trout (*O. clarki*) and chinook salmon (*O. tshawytscha*) using the same transgene construct consisting of a chinook salmon GH gene spliced to an ocean pout (*Macrozoarces americanus*) gene

promoter (Devlin *et al.*, 1994; 1995a,b).

Transgenes have been constructed from various bacterial (Rahman and Maclean, 1992), viral (Yoon *et al.*, 1990), and mammalian (Xie *et al.*, 1993) gene and promoter sources. The commercial production of transgenic fish destined for human consumption has raised social and ethical considerations which have encouraged the development of "all fish" gene constructs. The selection of the proper gene promoter/enhancer to a large extent determines the tissue in which the gene is expressed and the timing of expression. Growth hormone, normally secreted from the pituitary and under control of the hypothalamus, regulates somatic growth (Chen *et al.*, 1994; Norris, 1997). The influence of GH on growth is mediated primarily through the induction of insulin-like growth factors (IGF) produced mainly in the liver that subsequently facilitate amino acid uptake into the cells (Sakamoto and Hirano, 1993). The liver of ocean pout produce antifreeze proteins year round via antifreeze genes (Fletcher *et al.*, 1985; 1990; Gong *et al.*, 1992). Fletcher *et al.* (1990) have constructed a transgene using the promoter region from this antifreeze gene to drive the expression of a chinook salmon growth hormone gene. It is speculated that GH will therefore be produced continuously by the liver.

1.4 Bioenergetics.

Bioenergetics is the quantification of the interchanges and transformations of energy between a biological system and the surrounding environment (Lucus, 1996). Results from bioenergetic studies can provide valuable information to the aquaculture industry enabling improvements to be made in productivity, efficiency and profitability.

A constant supply of energy is necessary for all animals to sustain life. Energy drives

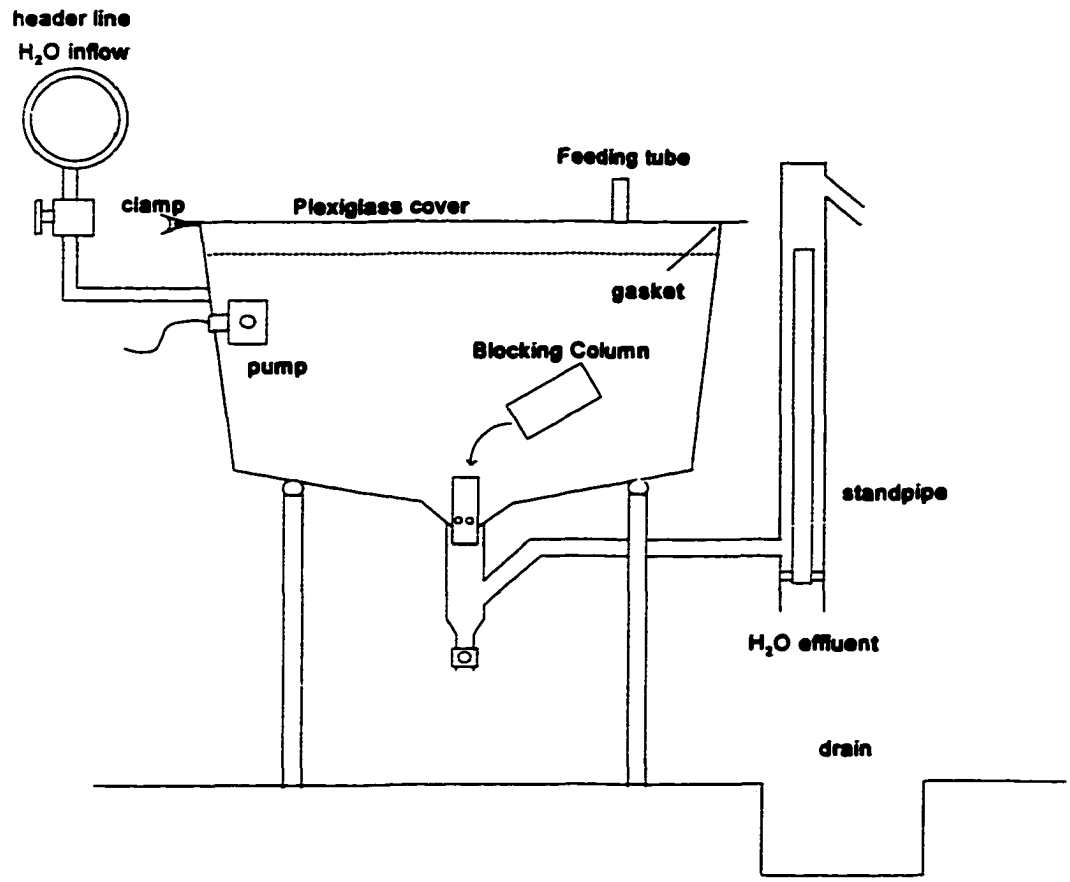
the chemical reactions required for tissue repair and regeneration, muscle activity, digestion, respiration, etc. Ingested feed or body reserves are the sources of energy to maintain life. However, the amount of ingested energy available for maintenance and growth is dependent on the ability of the fish to digest and utilize energy from the feed. Energy lost in the form of faeces, urine and gill excretions, or used in metabolism, is not available for deposition as body growth. Different species possess varying capacities to digest different sources of energy and protein. For example, some herbivorous fish species such as tilapia can use carbohydrates as a major energy source (Buddington, 1979). Salmonids, however, which are carnivorous, use carbohydrates poorly and rely more on protein and fat for energy (Hajen *et al.*, 1993b; Bureau *et al.*, 1997). Accordingly, an understanding of any effects transgenesis has on energy digestibility and conversion is a critical bioenergetic consideration for aquaculture practitioners.

1.4.1 Digestibility.

Fish, like all animals, must conform to the laws of thermodynamics. Energy cannot be created or destroyed (Kleiber, 1975). Not all energy contained within the ingested food, however, is available for metabolism. Some energy, in the form of undigested protein, carbohydrate, and lipid, is lost as components of faeces. The difference between the intake of a nutrient such as protein, and the amount recovered in the faeces is generally considered the portion of protein digested. The quantity of energy which is available to the fish is dependent not only on how much feed they eat but also to what extent they digest the energy in the feed; similarly, the proportion of each nutrient contained in the feed which is available to the fish is dependent to what degree they digest each nutrient.

The relative efficiencies of different methods of faecal collection employed in fish digestibility experiments remain controversial. Difficulties arise in that faecal excretions of fish are suspended or dissolved in large volumes of water. Direct methods of faecal collection rely upon the complete collection of faeces and an accurate measure of feed intake (Choubert *et al.*, 1982; Vens-Cappell, 1985). The indirect or indicator method, (i.e., adding an indigestible marker such as chromic oxide (Cr_2O_3) to the feed), is less technically demanding than the direct method in that only samples of faeces and feed are required for analysis. Nutrient concentrations in the feed and the faeces are measured and compared with those of an indigestible marker. The tank design used throughout the present study simulated that which is typically encountered in a commercial aquaculture facility but was modified, using a 'blocking column', to prevent faeces or feed from exiting down the tank drain (Figure 1.1). Hajen *et al.* (1993a) found no significant difference in digestibility using direct or indirect methods. Cho *et al.* (1982) and De Silva and Perera (1992) postulated that with proper faecal collection methods, leaching of nutrients does not have a significant effect on the digestibility calculations while Austreng (1978) and Lied *et al.* (1982) stated that calculations based on faeces collected from water overestimate digestibility due to loss of water-soluble nutrients. Stripping faeces from the fish directly or removal by intestinal dissection avoids the possibility of leaching but introduces the probability of removing faeces not completely digested (Austreng, 1978; Ferraris *et al.*, 1986), or contaminated with urine and body mucus (Hajen *et al.*, 1993a). Faecal material removed from the intestine before completion of natural digestion will result in lower digestibility than that obtained

Figure 1.1
Diagram of the growth tank. Water is supplied from the main header line, circulates through the tank, and exits by the drain. A 'blocking column' is used to prevent faeces and excess feed from exiting the tank on data collection days.



with faeces collected from the water. De Silva and Perera (1984) suggested that premature evacuation of faeces by excited fish may decrease estimates of digestibility compared to unexcited fish. Hajen *et al.* (1993a) reported almost 9% of the total "faecal" dry weight was fish scales. None of the aforementioned fish faecal collection techniques are without error. The goal of the present study was to measure the feed digestibility by transgenic fish and control fish, using methods which attempt to minimize the aforementioned sources of error.

Faeces are a combination of undigested food components and the remains of mucosal cells, digestive enzymes and other digestive secretions (Cho *et al.*, 1982). Unless an allowance is made for the faecal inclusion of endogenous origin, a "true" estimate of digestibility can not be made but instead an "apparent" digestibility is determined, as calculated with the equation below.

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

1.4.2 Feed conversion.

Providing nourishment for rapidly growing salmon can be expensive, with feed purchases accounting for over 40% of the total production costs (Saunders, 1995). The fish protein and oil needed to make fish feed is becoming increasingly difficult and costly to acquire as the aquaculture industry expands. Consequently it is important to maximize feed conversion efficiency. Better understanding of fish nutritional requirements has improved diet quality and consequently feed conversion (Cho *et al.*, 1976). Appetite stimulation and

improved feed conversion have been observed in fish as a result of exogenous hormone treatment (Markert *et al.*, 1977; Higgs *et al.*, 1979; Higgs *et al.*, 1982; Gill *et al.*, 1985).

Accurate measure of feed intake is important when attempting to quantify how much feed is converted into fish tissue. In growth trials where fish are fed to satiation, there is usually uneaten feed left in the tank and this remaining uneaten feed must be subtracted from the amount of feed offered to determine actual feed intake. The amount of feed eaten can be determined through measurements on the gastro-intestinal tract using feed labelled with isotopes (Storebakken *et al.*, 1981) or X-ray opaque particles (Talbot and Higgins, 1983). However, these techniques stress the fish when measurements are taken thereby being disadvantageous during extended growth trials. Accurate measurement of the feed entering the fish tank and waste feed remaining after each feeding, ensuring that no particulate matter is lost due to mechanical disruption, is typically used to determine the feed consumption of fish and is the method adopted in this study.

1.4.3 Proximate analysis.

Proximate analysis is a means of evaluating diets and carcass composition in terms of the chemical content of six major components: water, crude protein, lipid, crude fibre, carbohydrate, and ash. Knowledge of a fish's nutrient intake and the corresponding changes in body composition permits determination of the efficiency of nutrient transfer from the feed to the fish. For example, Net Protein Utilization (NPU) is a measure of the protein gain during an experimental period per unit protein digested by the fish.

$$NPU = \frac{[P_{end} - P_{start}]}{[P_{input} \times P_{dg}]}$$

where P_{end} and P_{start} is the protein content (g) of the fish at the end and start of the experimental period, respectively, P_{input} is the protein fed (g) to the fish, and P_{dg} is the protein digestibility coefficient.

Utilization of body reserves during starvation can also be evaluated using proximate analysis. Depletion of carcass components does not necessarily mean the components are oxidized as an energy source. For example, body protein can be converted to carbohydrate through gluconeogenesis (Lauff and Wood, 1996).

Shearer (1994) reviewed the main factors affecting the body composition of cultured fish, particularly salmonids. Such factors may be biologically endogenous to the fish including size, sex, appetite, and life cycle stage; exogenous physical factors include temperature, water current velocity, photoperiod and feed composition/availability. Information pertaining to the effects of transgenesis on fish body composition has been limited to common carp (*Cyprinus carpio*). Chatakondi *et al.* (1995) reported that the muscle composition of F_1 generation adult transgenic carp had higher percent protein, lower percent lipid, and lower percent moisture content than controls. Fu *et al.* (1998) reported similar results in total carcass composition of F_4 generation juvenile transgenic carp.

1.4.4 Metabolism.

The first law of thermodynamics establishes that energy is neither created nor lost (Kleiber, 1975). Consequently, the sum of all forms of feed derived energy liberated or

absorbed while passing through a fish must equal dietary energy intake (E_{in} = F+U+RE+M where E_{in} is dietary energy intake, F is the energy lost in the faeces, U is total energy lost in the urine or excreted via the gills, RE is retained energy, i.e., growth, and M is the energy lost to metabolism). From the above energy budget, the term 'metabolism' is introduced. According to the second law of thermodynamics, change of energy from one form to another is not 100% efficient (Kleiber, 1975). Tissue turnover, physical activity, and the transformation of dietary nutrients into tissue components is accompanied by the liberation of heat. Total heat production, a measure of an animal's metabolism, is the noncombustible energy transferred to the environment and is useful in determining biological energy requirements. During the production of fish for food, energy released as heat is no longer available for incorporation into body tissue.

1.4.4.1 Respirometry: Oxygen as a measure of metabolism.

Methods of measuring metabolic rate fall into two basic categories: direct and indirect calorimetry. Calorimetry is the measure of heat production by an animal. The metabolic rate of an organism can be determined by measuring the amount of energy released as heat, typically called direct calorimetry. However, the accurate measurement of heat production directly in aquatic poikilotherms is not practical because of their relatively low heat production rates compared to terrestrial animals, combined with high heat capacity of the aquatic environment (Cech, 1990).

Indirect calorimetry has become the conventional method of determining metabolic rates in fish and depends on the measurement of a factor related to energy utilization other than heat production (Jobling, 1994). Respirometry is such an indirect method of

determining metabolic rate which involves measuring the rate of oxygen consumption of the organism. The amount of oxygen dissolved in water can be determined with relative ease and reliability (Cech, 1990). The energy contained in a nutrient's molecules is released when those molecules are oxidized, and during aerobic oxidation, the amount of oxygen consumed can be related to the quantity of energy utilized by the fish.

Data of oxygen consumption by fish also provides the necessary information for determining water flow requirements in a fish culture operation (Fivelstad, 1988). Knowledge of the dissolved oxygen concentration in the delivery water and the fish's rate of oxygen consumption can be used to determine the required flow rate of water. With the high cost of pumping water or generating oxygen in land-based aquaculture facilities, any major departures in water/oxygen demand by rapid growing transgenic fish will have to be included in the overall cost of production.

1.4.4.1.1 Activity level and metabolism.

In fish respirometry studies, the activity state of the animal must be considered. Fry (1971) categorized aerobic metabolism in fish as standard, routine, and active metabolism.

(i) Standard metabolism

For mammals and birds, the rate of energy metabolism in a thermoneutral environment, under quiescent conditions and in a post-absorptive state, is designated as the basal metabolic rate (Eckert *et al.*, 1988). Because the body temperature of poikilotherms depends on ambient temperature, and as metabolic rate varies with temperature (Beamish and Mookherjii, 1964), a fish's minimum energy requirement at a given temperature (while resting and in a post-absorptive state) is termed the standard metabolic rate (Brett and

Groves, 1979; Jobling, 1994).

(ii) Routine metabolism

Most metabolic values reported for fish are routine rates, which include the metabolic costs associated with daily cycles of activity in the form of spontaneous swimming, a condition most representative of circumstances encountered in finfish aquaculture (Fry, 1971; Brett and Groves, 1979; Cech, 1990; Stevens *et al.*, 1998). Under routine conditions fish should be in a post-absorptive state and swimming speed limited to a maximum of 1 body length/second (Cech, 1990). Additionally, oxygen consumption rates at routine activity levels, but inclusive of the heat increment associated with feeding (section 1.4.4.1.2), can provide fundamental information, such as water flow rates (relating to dissolved oxygen levels), necessary for culturing fish.

(iii) Swimming and active metabolism

The use of swimming or tunnel respirometers has allowed the measurement of oxygen consumption at various voluntary or forced levels of swimming. Active metabolism is the maximum rate of energy expenditure while swimming at the greatest sustainable velocity. Interpolation to zero activity is one method of calculating standard metabolic rates (Fry, 1971; Brett and Groves, 1979; Cech, 1990).

1.4.4.1.2 Factors influencing fish metabolism.

Both environmental and intrinsic factors can influence metabolic rate. Environmental variables include temperature (Caulton, 1978; Brown *et al.*, 1984; Cech *et al.*, 1985), stocking density (Christiansen *et al.*, 1991), oxygen levels (Cech *et al.*, 1985; Waller *et al.*, 1997) and salinity (Gasca-Leyva *et al.*, 1991). Care must therefore be taken to ensure that

fish are acclimated to the temperature at which measurements are to be made (Jobling, 1994). Because acute stress caused by transferring fish to a separate respiration chamber can also increase metabolic rate and oxygen consumption (Barton and Schreck, 1987), a common rearing/respiration chamber has been employed in this study.

Intrinsic factors which can influence the metabolic rate of fish include body mass (Brown *et al.*, 1984) and the mechanisms associated with digestion (activity, nutrient assimilation) (Tandler and Beamish, 1979; Dabrowski, 1986). The effect of body size on metabolic rate has received considerable attention. On a quantitative basis, larger fish generally consume more oxygen than small fish; however, on a unit-weight basis, oxygen consumption decreases as body mass increases (Brett and Groves, 1979; Eckert *et al.*, 1988; Cech, 1990; Jobling, 1994). Metabolic rate is a power function of body mass described by the equation (Brett and Groves, 1979):

$$VO_2 = a \times BW^b$$

or

$$\text{Log } VO_2 = \text{Log } a + b \text{ Log } BW$$

where 'VO_2' is oxygen consumption rate (mg O_2/h), 'BW' is body weight, 'a' is the weight coefficient, and 'b' is the weight exponent. The log-log plot of 'BW' and 'VO_2' is a linear one, but with a slope 'b' that is less than one (Cech, 1990).

The metabolic rates of fed fish are higher than those of fish in a post-absorptive feeding state. Specific dynamic action (SDA) or the heat increment of feeding is defined as the increase in heat production following consumption of feed by an animal (Jobling and Davies, 1980; Jobling, 1981). This heat is produced as a consequence of the extra work

expended due to ingestion, digestion and utilization of the feed. Both quantity of feed and balance of nutrients in the feed will affect metabolism (Beamish, 1974; Jobling and Davies, 1980; Forsberg, 1997). Tandler and Beamish (1980) reported SDA was positively related to energy ingested and the level of dietary protein.

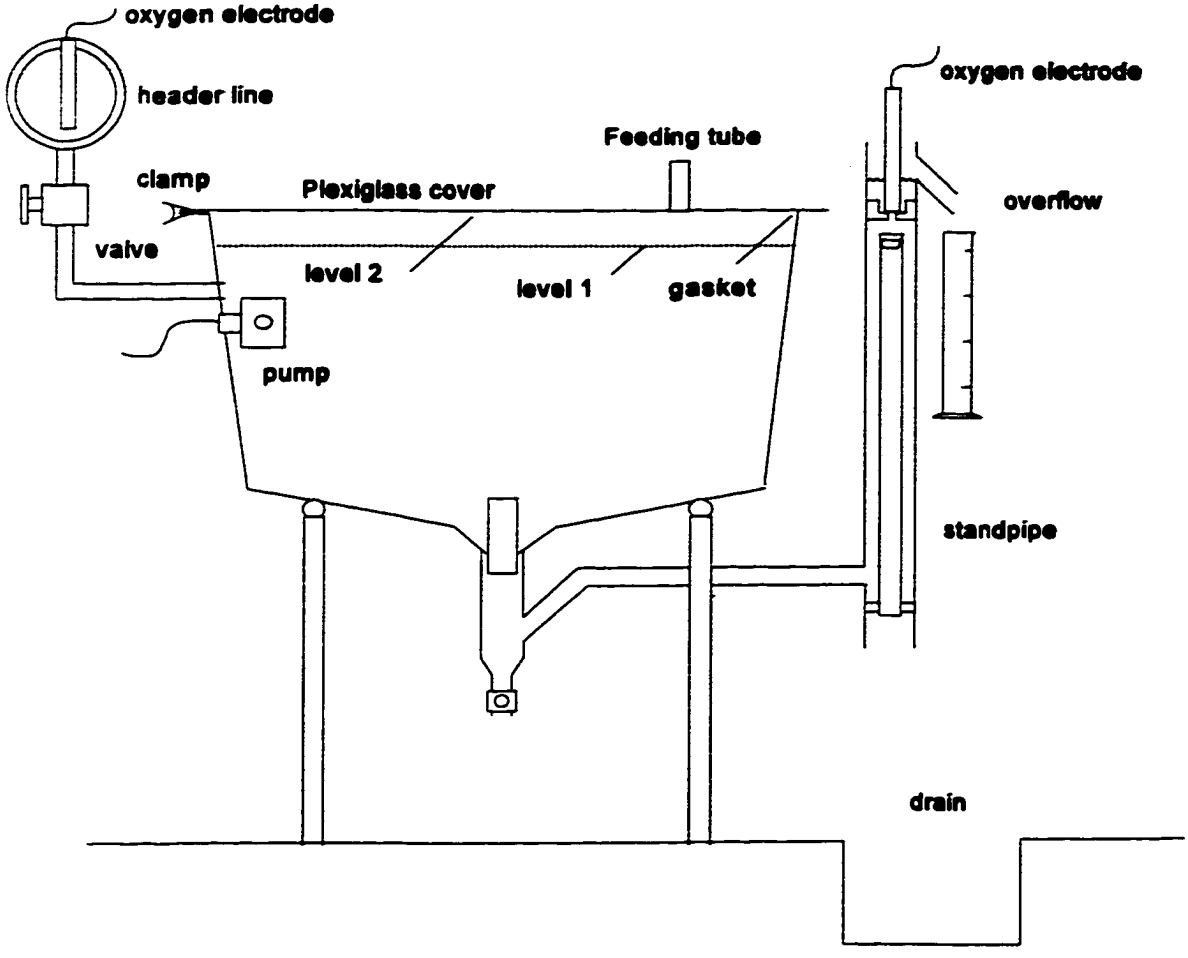
1.4.4.1.3 Respiration chambers.

While a large variety of respiration chambers have been designed to accommodate the diversity of fish sizes, shapes, and behaviours, systems generally fall into two categories: closed and flow-through respirometers.

In a closed respirometer, be it a static or swimming design, the dissolved oxygen concentration progressively decreases as the fish utilize the oxygen (Brown *et al.*, 1984; Cech, 1990; Lucas, 1996). The water in a closed, static, respirometer does not move; conversely, in closed, swimming respiration chambers (typically designed as a tunnel or tube) the water flow rate can be regulated and measured. Fish swim against a current and oxygen uptake in relation to swimming speed is determined (Brett, 1964; Cech, 1990; Lucas, 1996; Grottum and Sigholt, 1998).

Open or flow-through respirometers are designed to have a constant water flow entering and leaving the respiratory chamber, with the capability of adjusting flow rates to ensure an appreciable difference between the oxygen content measured at the entrance and exit of the chamber (Cech *et al.*, 1985; Cech, 1990; Davenport *et al.*, 1990; Lucas, 1996). To eliminate the effects of stress typically encountered when fish are transported from a given tank to the respirometer, the rearing tanks used in the present study were designed to facilitate their rapid conversion into a flow-through respiration chamber (Figure 1.2).

Figure 1.2
Diagram of the single pass, flow-through respirometer. A rubber stopper is placed in the external standpipe to raise the water within the tank from level 1 to level 2. The tank is then sealed at the top using a plexiglass cover clamped down over a water proof gasket. Water is supplied from the main header line, circulates through the tank, and exits by the drain. Dissolved oxygen concentration is measured at the inflow and outflow.



Regardless of which system is used, it is important to include a 'blank' tank (without fish) so corrections can be made for any oxygen-consuming or producing material such as bacteria or algae in the water or attached to the chamber (Cech, 1990). The oxygen electrode should also be calibrated frequently.

1.4.5 Starvation.

During spawning migrations, wintering, or when local food abundance diminishes, many species of fish, including salmonids, undergo natural periods of starvation. If the dietary energy intake during food deprivation is less than the rate of heat production, energy reserves are redirected from growth to support basal metabolism (Cho *et al.*, 1982; Sumpter *et al.*, 1991).

As growth hormone is likely to affect appetite and other aspects of fish behavior, public concern as to the possible ecological impact of transgenic technology has led to the assessment of foraging and antipredator behaviour of growth enhanced transgenic salmon (Abrahams and Sutterlin, in press). Under natural conditions where food is likely to be limited, the growth rates of transgenic salmon may be significantly reduced relative to cultured counterparts. Abrahams and Sutterlin (in press) tested the hypothesis that transgenic salmon exhibiting greater appetite would subject themselves to greater risks of predation to secure food. This study demonstrated that transgenic Atlantic salmon spent significantly longer periods than control fish feeding in the presence of a predator and consumed more feed at that location. Since transgenics have not been tested by the process of natural selection, it is likely that transgenic fish, when subjected to the rigours of nature, will have

reduced fitness with respect to non-genetically modified fish. The response by fish to extended periods of food shortage is decreased metabolism, reflected by lower oxygen consumption rates (Mehner and Wieser, 1994; Lauff and Wood, 1996). If transgenic fish maintain a higher metabolic rate than controls during feed deprivation, this may render them at a disadvantage outside of the intensive aquaculture environment as their endogenous energy reserves will be used up quicker.

1.5 Experimental fish.

Due to significant dissimilarity in growth rates between transgenic fish and non-genetically modified controls, there is a limited period when experiments can be simultaneously conducted using fish of comparable body weight. Consequently, a "transgenic line" with an intermediate growth rate was used in the present experiment to extend the period in which transgenic and control fish were of equal body weights. Differences in growth enhancement between transgenic lines probably arise from a number of factors such as the chromosomal site of integration, the number of gene copies integrated, and the type/effectiveness of promoter used (Moav *et al.*, 1992).

Experimental fish used as recipients of the growth hormone transgene as well as the non-genetically modified controls were Atlantic salmon (*Salmo salar*) from Saint John River stock, New Brunswick, Canada. All eggs and fish were produced and reared at AquaBounty Farms in Prince Edward Island, a government-inspected hatchery designed with the required security systems to prevent the escape of genetically modified organisms into the natural environment.

An "all fish" chimeric transgene was constructed of an antifreeze protein promoter

from ocean pout (*Macrozoarces americanus*) driving the expression of a chinook salmon (*Oncorhynchus tshawytscha*) growth hormone gene (Hew *et al.*, 1995). In fall 1989, this transgene was microinjected (approximately 10^6 copies per egg) through the micropyle into the cytoplasm of normal fertilized, non-activated 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. The progeny of this last pairing exhibited a bimodal size distribution at the fingerling stage in June, a phenomenon not normally seen until the first autumn of growth (Thorpe, 1977; Thorpe *et. al*, 1980). Consequently, the two modes could be separated based on fork length above and below 8.0 cm, with a ratio of 50:50 between the two modes, typical of the Mendelian segregation of a gene insert on a single chromosome. This separation was later confirmed by the exclusive presence of the transgene in the upper modal group using polymerase chain reaction (Table 1.1). The transgenic fish used in the present experiment were from the upper modal group of the 1996 spawning. Also in the fall of 1996, non-transgenic milt and eggs from the same Saint John River stock were used to generate non-transgenic fish used as controls.

1.6 Objectives.

Unlike mammalian livestock, the eggs of most farmed fish are readily available in high numbers, are fertilized externally and do not require further use of the female reproductive tract for the completion of development; such characteristics position fish as excellent candidates for genetic manipulation. In addition to Atlantic salmon, several other

Table 1.1
Detection of an "all fish" chimeric growth hormone transgene in F_2 generation transgenic Atlantic salmon (*Salmo salar*) using polymerase chain reaction (PCR). Both blood and tissue samples were used for transgene verification.

	Blood sample		Tissue sample (skin and muscle)	
Experimental group	# Positive	# Negative	# Positive	# Negative
Control	0	24	N/A	N/A
Transgenic	44	0	112	0

commercially important aquaculture species such as tilapia, *Oreochromis niloticus* (Rahman and Maclean, 1992), channel catfish, *Ictalurus punctatus* (Dunham *et al.*, 1987), common carp, *Cyprinus carpio* (Chen *et al.*, 1993; Moav *et al.*, 1995), and Arctic char, *Salvelinus alpinus*, have currently been inserted with various transgenes and the potential benefits of this technology to fish farming is being assessed.

The purpose of the present study is to compile basic bioenergetic information on presmolt growth enhanced transgenic salmon. This information is required to define some of the more important production parameters and to reveal any requirements that will be necessary to accommodate such fish in a commercial setting.

The specific objectives of this research are:

(I) To quantify and compare feed digestibility and conversion, as well as growth rate and body composition, in growth-enhanced transgenic Atlantic salmon reared under simulated aquaculture conditions to those of genetically unaltered salmon over a weight range representative of the fresh water smolt production phase;

(ii) To quantify and compare the routine metabolism, assessed as rate of oxygen consumption, of transgenic salmon relative to that of control fish under simulated aquaculture conditions; and

(iii) To investigate the effects of transgenesis on metabolism and body composition during food deprivation.

2.0 GROWTH RATE, PROTEIN AND ENERGY DIGESTIBILITY, FEED CONVERSION, AND BODY COMPOSITION OF GROWTH ENHANCED TRANSGENIC ATLANTIC SALMON (*SALMO SALAR*)

2.1 Introduction.

The success of any aquaculture venture depends upon the growth performance of cultured animals. The current farmed production schedule of Atlantic salmon (using genetically unaltered fish) usually 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 expenses (capital maintenance and labour) while also lowering period of exposure to risks (predation and storm damage losses).

Molecular geneticists are now playing an increasing role in aquaculture through the use of transgenic technology by altering a fish's genome to enhance commercially important production traits such as growth rate and freeze resistance. While there have been numerous studies demonstrating the superior growth of transgenic fish (Du *et al.*, 1992; Fletcher *et al.*, 1992; Devlin *et al.*, 1994), few have evaluated the effect transgenesis has on fish bioenergetics (Fu *et al.*, 1998). The aim of the present study was to quantify and compare growth rate, protein and energy digestibility, 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.

2.2 Methods.

2.2.1 Experimental fish.

Atlantic salmon from Saint John River stock, New Brunswick, Canada, were used as recipients of the growth hormone transgene and genetically unaltered controls. All eggs and fish were produced and reared at AquaBounty Farms, Prince Edward Island.

Transgenic and control embryos were incubated separately in flow-through Heath incubators. To facilitate having both experimental groups, transgenic and controls, at approximately the same weight at the start of the experiment, the transgenic eyed eggs were incubated at a lower water temperature (4°C) relative to control eggs (7°C). Consequently, time to first feeding was approximately 17 days longer for the transgenics relative to controls (Table 2.1). Lighting within the hatchery mimicked the natural photoperiod. Fresh 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 ppt (Stevens *et al.*, 1998).

2.2.2 Diet preparation and chemical analysis.

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 commercial diet was steam-pelleted using a laboratory-scale, California pellet mill equipped with a 2.0 mm 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.

For chemical analysis, feed samples were ground to 1 mm and analysed for dry matter, protein and ash using standard AOAC 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

Table 2.1

History of the Atlantic salmon (*Salmo salar*) used in the present study. Transgenic eggs were incubated at a lower water temperature to delay development. This was to facilitate having both experimental groups at approximately the same wet body weight at the commencement of the study.

Date	Julian day		Control Wt. (g)	Temp (°C)	Days Fed	Transgenic Wt. (g)	Temp (°C)	Days Fed
01Nov96	305	spawn						
		egg incuba-tion		7			4	
19Feb97	50		0.12		1		5	
08Mar97	67		nd	13	18	0.12[a]	13	1
28Apr97	118		0.78	14	69	0.50 [a]	14	52
26May97	146		2.04	15	97	1.56[a]	15	80
12Jun97	163		3.27	15	114	3.06[a]	15	97
25Jun97	176	graded	nd	14	127	5.84[b]	14	110
08Jul97	189		6.62	16	140	9.42[b]	16	123
20Jul97	201		6.98	12.6	152	13.72[b]	12.6	135
03Aug97	215		8.87	12.6	166	24.92[b]	12.6	149
17Aug97	229		11.45	12.6	180	38.93[b]	12.6	163
31Aug97	243		13.67	12.6	194	55.76[b]	12.6	194
14Sep97	257		18.13	12.6	208			
29Sep97	272		22.53	12.6	223			
12Oct97	285		28.09	12.6	236			
26Oct97	299		31.90	12.6	250			
9Nov97	313		37.49	12.6	264			
23Nov97	326		43.13	12.6	277			
14Dec97	348		52.92	12.6	299			

nd indicates no data; weight was not measured on that day

[a] Represents total population (i.e., both upper and lower modal group [UMG and LMG])

[b] Represents UMG of the transgenic fish. Control fish were also graded and larger individuals were used; however, the frequency distribution was unimodal rather than bimodal.

(1972). The formulation and chemical composition of the commercial Atlantic salmon diet used are presented in Tables 2.2 and 2.3.

2.2.3 Protocol.

Inclusive of the experiment of comparative food deprivation (Chapter 4), a minimum of three and a maximum of twelve replicate tanks containing each of the two experimental groups of fish were used in determining fish weights for growth rate calculations, with each tank containing at least 30 transgenic or control fish. Energy and protein digestibility as well as feed conversion data were measured on three replicates per experimental group. The mean water temperature entering the 92 L fibreglass, flow-through experimental tanks (Figure 1.1) was 12.6°C ± 0.03 (s.e.m.). The rates of water flow to individual tanks were periodically adjusted (taking into account fish size and number) to maintain water oxygen levels above 6 ppm (Stevens *et al.*,1998). Oxygen levels were measured using an Oxyguard Handy Mark 4 oxygen sensor (Point Four Systems Inc., Port Moody, British Columbia, Canada).

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

At the start of the experiment, all the fish were anaesthetized using tertiary-amyl-alcohol (1.0 ml/L) and individual fork lengths and wet weights were measured. Mean wet weight was 13.72 ± 0.21 g and 6.98 ± 0.07 g for transgenic and control fish respectively. Subsamples of 5 fish per tank from three tanks in each of the two experimental groups were

Table 2.2
Formulation of experimental diet

Ingredients	Kg/100 Kg of Diet
Wheat (shorts)	13.83
Fishmeal (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

Table 2.3
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

Values presented as mean ± s.e.m. of 2.0 mm and 3.0 mm pellets

euthanised with an anaesthetic overdose, wrapped in cellophane and stored at -20°C for body composition analysis.

Fish were fed to satiation three times per day, and every two weeks cumulative wet fish weights per tank were measured. Excess feed remaining in the tank was flushed out 20 minutes post feeding. At approximately 10 g wet weight intervals, subsamples of 5 fish per tank from three tanks per experimental group, using tanks not previously sampled, were euthanised for whole body composition analysis. The experiment was terminated when the fish reached a wet weight of approximately 55 g. Weight specific growth rate (SGR) was calculated with the equation below.

$$SGR\ (\%\ body\ wt.\ gain/day) = \frac{[Log_n\ Final\ fish\ Wt. - Log_n\ Initial\ fish\ Wt.]}{Time\ interval} \times 100$$

For calculation of total feed consumption and digestibility, excess feed and voided faeces were collected at three day intervals and averaged between each measurement date. The weight of dry matter for the collected faeces (collected over a two week period) was too small to perform chemical analysis for samples; therefore, faeces collected from each tank for the whole experimental period for each experimental group were pooled for analysis. Protocol on collection days consisted of turning off the in-tank, water circulation pumps just prior to feeding and placing a poly-vinyl chloride 'blocking column' over the center drain stand to prevent uneaten feed from exiting the tank yet still allowing water to exit at a higher point in the center column (Figure 1.2). Approximately 20 minutes after the fish were fed, excess uneaten feed was siphoned out of the tank and filtered through two coffee

filters of known dry weight. The in-tank, water circulation pump was then turned back on. Excess feed was dried in a toaster oven at approximately 60°C for 36 h and then weighed. Feed conversion was calculated with the equation below.

$$\textit{Feed conversion} = \frac{\textit{Dry weight of feed fed - uneaten feed}}{\textit{Wet weight gain of fish}}$$

The blocking column 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, faeces were syphoned out of the tank and stored at -20°C for composite analysis at a later date.

2.2.4 Fish and faecal sample preparation and chemical analysis.

Frozen whole fish were autoclaved for 20 min at 120°C, homogenized in a blender with a known volume of distilled water, placed in an aluminum pan and lyophilized. Upon removal from the freeze drier, samples were equilibrated to room humidity, weighed, and further homogenized in a coffee grinder. Samples were analysed for dry matter, protein and ash using standard AOAC 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 using a mortar and pestle, and analysed for protein and energy as described above. Chromic oxide was determined by atomic absorption spectrophotometry (Arthur, 1970). The apparent digestibility (AD) of a given nutrient was calculated using the equation described in 1.4.1.

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. The relationships between wet body weight and time, and body composition and wet body weight, were tested by regression analysis. A test for common slope was used to compare regression coefficients in regression equations for transgenic fish and control fish.

2.3 Results.

2.3.1 Growth.

The growth data for transgenic and control fish fed to satiation three times per day at a water temperature of 12.6°C is shown in Figure 2.1. Data (Appendix A; Table 1) was subjected to nonlinear regression using a second degree polynomial resulting in the following relationship:

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

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

where time is julian day[1].

Transgenic fish grew at a significantly greater rate than did the control group. The mean start and end fish weights are presented in Table 2.4. Transgenic mean wet body weight was 4.08 times larger than the controls when the transgenics reached the predetermined experimental weight endpoint of approximately 55 g. However, due to the large dissimilarity in growth rates between the two experimental groups during the acclimation period, control fish weighed significantly less at the start of the experiment.

---

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

Figure 2.1
Growth in relation to time for transgenic Atlantic salmon (*Salmo salar*) and controls fed to satiation three times/day on a commercial diet. Data is presented with fitted regression lines (solid lines) with 95% confidence intervals (dashed lines).

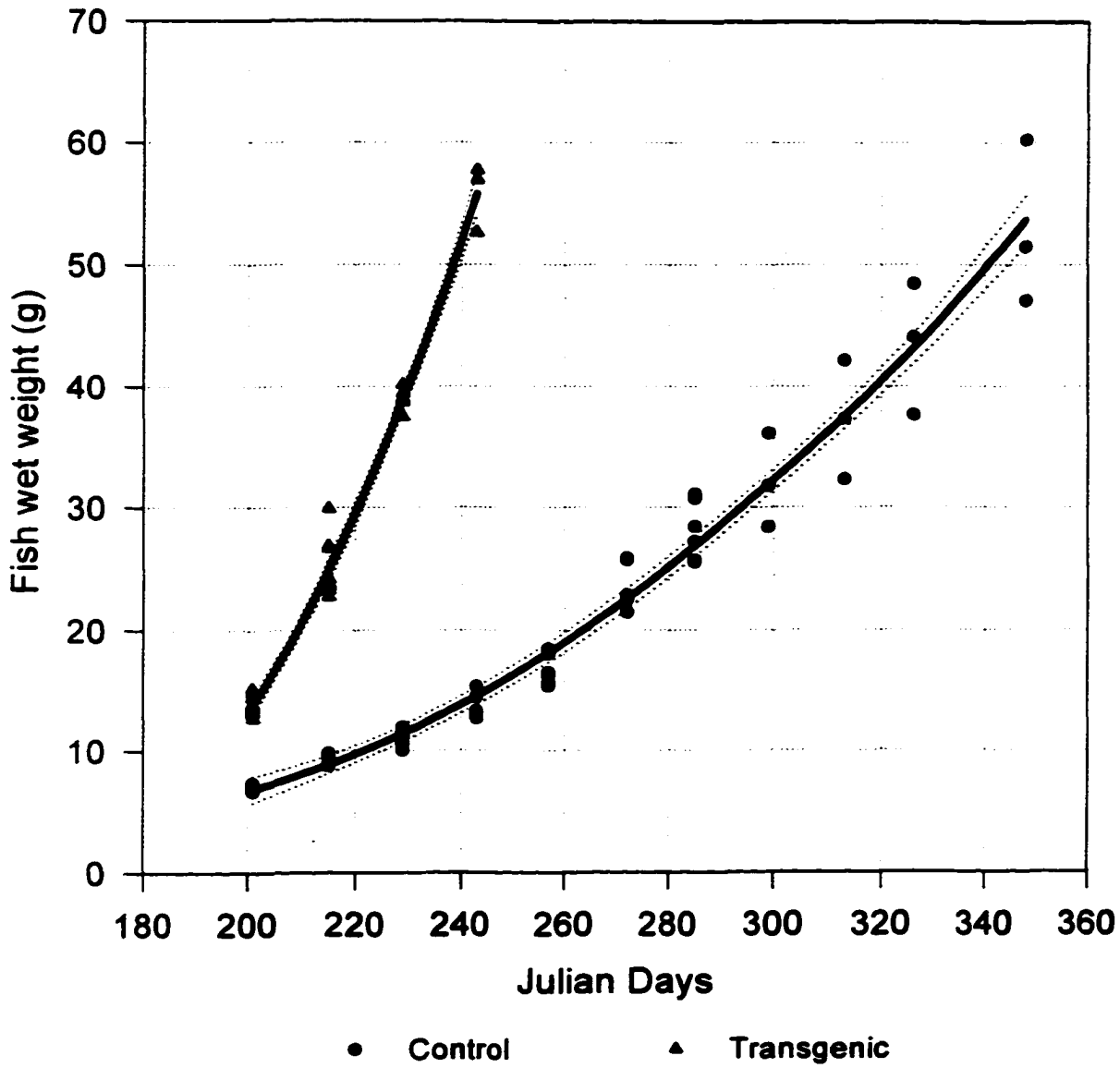


Table 2.4
Initial and final mean experimental weights of transgenic Atlantic salmon (*Salmo salar*) and controls fed to satiation three times/ day on a commercial diet.

Experimental Group	Initial wt. (g) on 201 Julian Day	Wt. (g) on 243 Julian Day (transgenic final wt.)	Wt. (g) on 285 Julian Day	Wt. (g) on 348 Julian Day (control final wt.)
Control	6.98 ± 0.07^{a}	13.67 ± 0.41^{a}	28.09 ± 0.99	52.92 ± 3.88
Transgenic	13.72 ± 0.21^{b}	55.76 ± 1.58^{b}	N/A	N/A

Fish/tank = 30-50
Number of replicates: min =3 max =12
[a,b] Means (in the same column) with different superscripts are significantly different ($P<0.05$)

There was a limited period when the two groups of fish were approximately the same body mass. Even when the time to first feeding for the transgenic fish was delayed by incubating their eggs at a lower temperature, experimentation on the control group had to be extended an additional four months for these fish to obtain a final weight equal to the terminal end weight of the transgenics.

To facilitate direct comparison between transgenic and control fish, weight specific growth rates (SGR) were calculated between each weight collection date (Figure 2.2). Rate of growth tended to vary inversely proportional to weight in both groups of fish. Data (Appendix A; Table 1) was subjected to linear regression analysis resulting in the following relationship:

$$\text{Transgenic SGR (\% body weight gain/day)} = 4.77 - 0.0414 \times \text{Weight} \quad (r^2 = 0.46)$$

$$\text{Control SGR (\% body weight gain/day)} = 1.85 - 0.0179 \times \text{Weight} \quad (r^2 = 0.21)$$

At 14 g wet weight, transgenic and control weight SGRs were 4.19% and 1.60% body weight/day, respectively; a 162% difference. The magnitude of difference increased up to 185% at 52 g, with transgenic and control weight specific growth rates 2.62% and 0.92% body weight/day, respectively. A test for common slope revealed a significant difference ($P<0.05$) indicating the rate of SGR decrease was not the same between the two groups and that the transgenics exhibited a larger decrease in growth rate than the controls as body weight increased.

2.3.2 Digestibility and feed conversion.

Mean apparent digestibility coefficients for dry matter, crude protein and energy are presented in Table 2.5. For all three parameters, there was no significant difference between

Figure 2.2
Weight specific growth rates for transgenic Atlantic salmon (*Salmo salar*) and controls fed to satiation three times/day on a commercial diet. Data is presented with fitted regression lines (solid lines) surrounded by 95% confidence intervals (dashed lines).

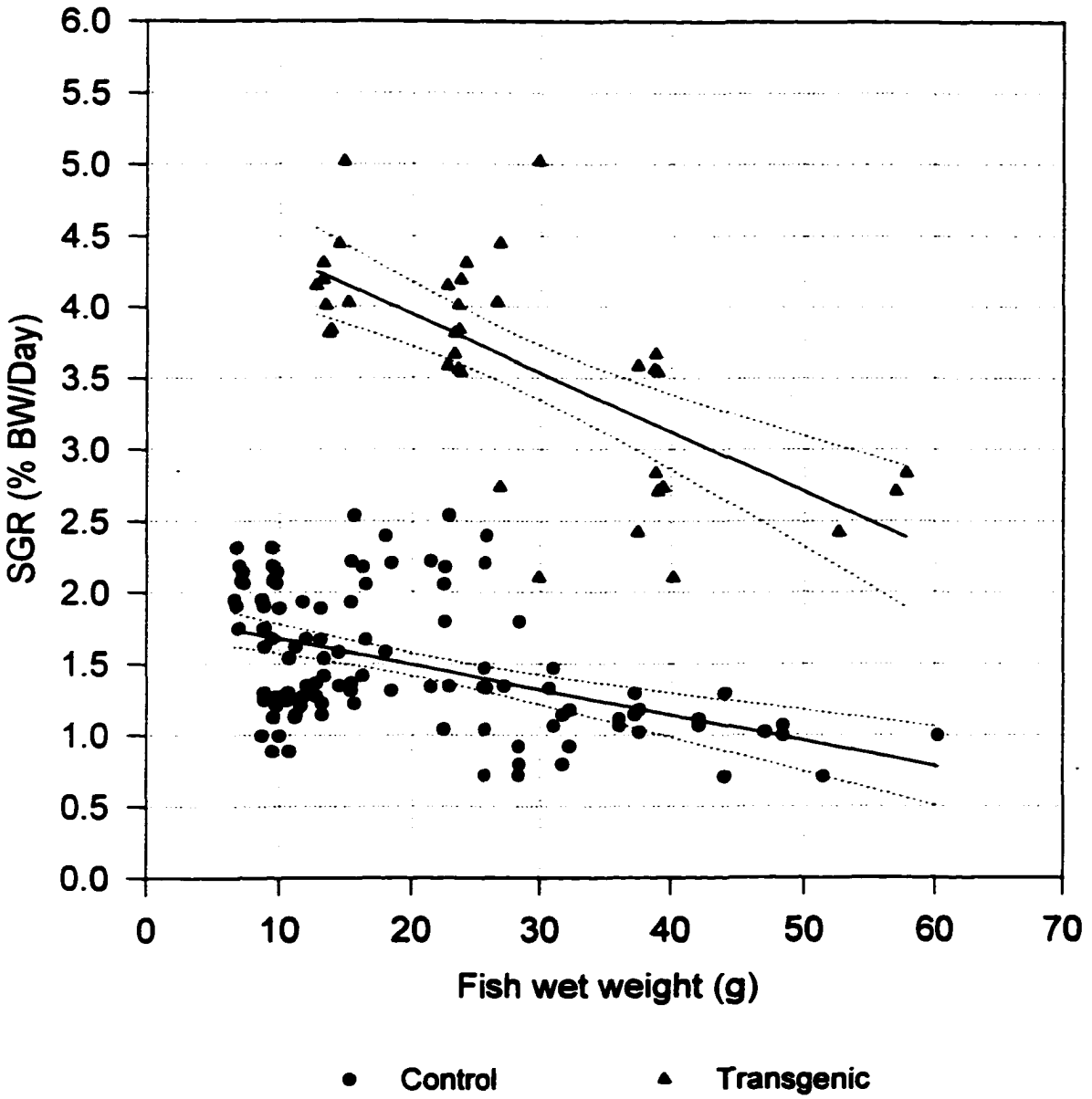


Table 2.5
Apparent digestibility (AD) of dry matter, energy and crude protein of diet fed to transgenic Atlantic salmon (*Salmo salar*) (13-55 g) and controls (7-53 g) fed to satiation three times/day.

	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

transgenic salmon and controls ($P > 0.05$). Faeces remained relatively cohesive in the water, with clearly defined faecal casts.

Protein, energy, feed conversion, gain:feed, and utilization values are presented in Table 2.6. There was no significant difference ($P > 0.05$) between transgenic and control values. Daily feed consumption (% body weight/day), however, was significantly higher in the transgenic group. At 14 g, transgenic and control daily feed consumption was 3.53% and 1.35% of body weight per day, respectively; a 162% difference. This magnitude of difference decreased to 114% at approximately 55 g, with transgenic and control daily feed intake 1.67% and 0.78% of body weight per day, respectively.

Small amounts of faecal material were observed in the collection of waste feed and included in the total weighed sample. Although this will give an underestimation of the amount of feed eaten, it was assumed to be negligible (proportionally small amount).

2.3.3 Fish body composition.

Transgenesis significantly affected body composition. The experimental results concerning absolute values of dry matter, protein, lipid, energy, and ash content per fish are shown in Appendix A (Tables 2 and 3). The carcass of transgenic fish contained significantly lower ($P<0.05$) levels of all body parameters than control fish except moisture content which was greater in the transgenic fish. From the overall relations shown in Figures 2.3-2.7, the increase in each component with body weight exhibited by the controls exceeded the transgenics. Regression coefficients are displayed in Table 2.7.

Body composition as a percentage basis of the fish's wet weight is shown in Figure 2.8. Data (Appendix A; Tables 4 and 5) was subjected to nonlinear regression using a second degree polynomial. The regression coefficients are displayed in Table 2.8. Protein,

Table 2.6
Protein, energy and feed conversion in transgenic Atlantic salmon (*Salmo salar*) (13-55 g) and controls (7-53 g) fed to satiation three times/day on a commercial diet.

	Control	Transgenic
Protein conversion[1]	2.60 ± 0.16	2.45 ± 0.19
Protein:gain[2]	0.41 ± 0.02	0.42 ± 0.03
Net protein utilization[3]	0.46 ± 0.03	0.47 ± 0.03
Energy conversion[1]	2.51 ± 0.16	2.71 ± 0.17
Energy:gain[2]	0.44 ± 0.03	0.38 ± 0.02
Net energy utilization[3]	0.52 ± 0.03	0.45 ± 0.03
Feed conversion[4]	0.83 ± 0.05	0.72 ± 0.05
Feed:gain[5]	1.30 ± 0.08	1.42 ± 0.09

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

Note[1] nutrient fed/nutrient gain

[2] nutrient gain/nutrient fed

[3] nutrient gain/(nutrient fed×digestibility coefficient)

[4] dry feed/wet weight gain

[5] wet weight gain/dry feed

Figure 2.3
Protein content (absolute weight) in relation to wet body weight of transgenic Atlantic salmon (*Salmo salar*) and controls fed to satiation three times/day on a commercial diet. Data is presented with fitted regression lines (solid lines) surrounded by 95% confidence intervals (dashed lines).

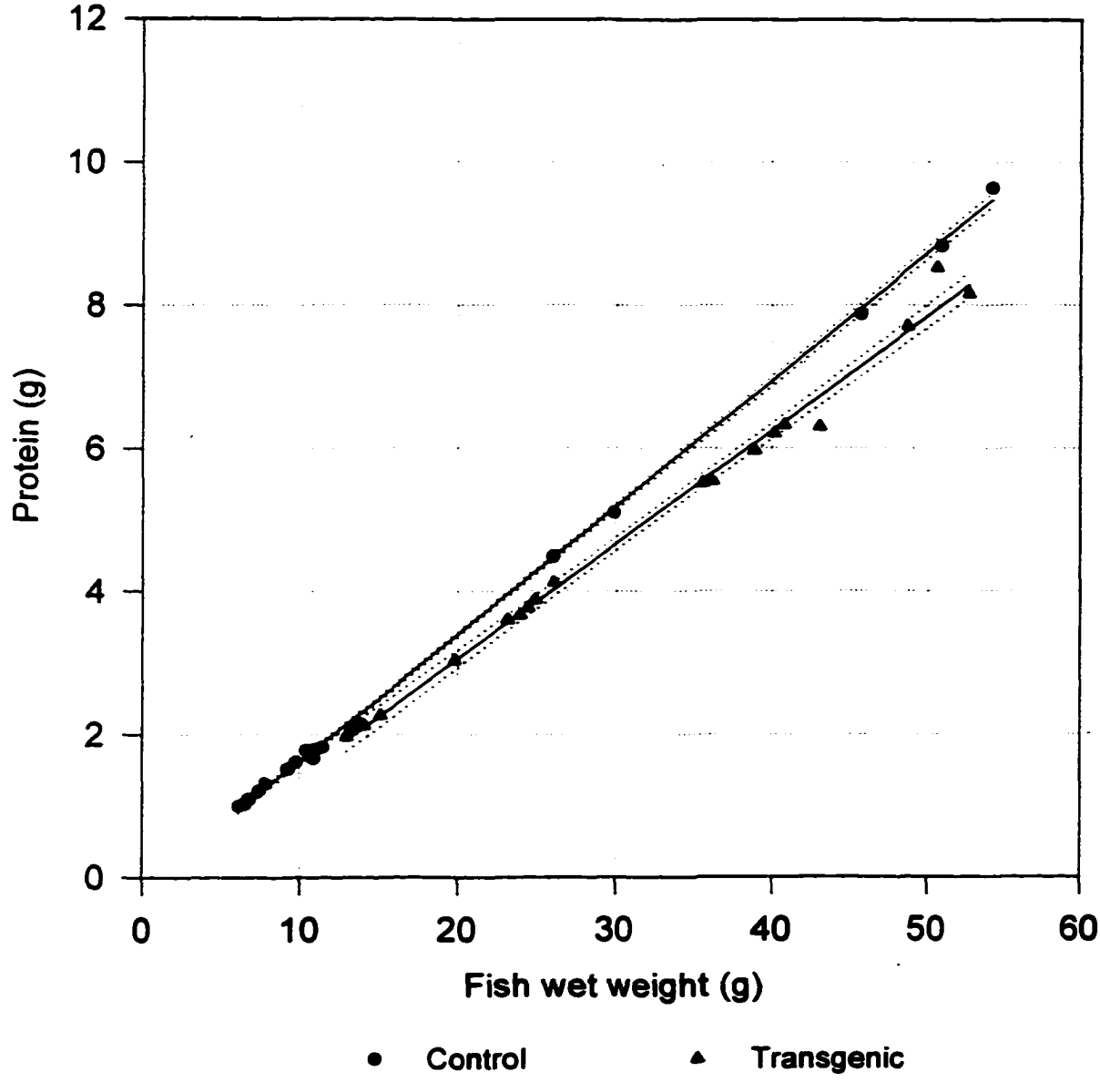


Figure 2.4
Dry matter content (absolute weight) in relation to wet body weight of transgenic Atlantic salmon (*Salmo salar*) and controls fed to satiation three times/day on a commercial diet. Data is presented with fitted regression lines (solid lines) surrounded by 95% confidence intervals (dashed lines).

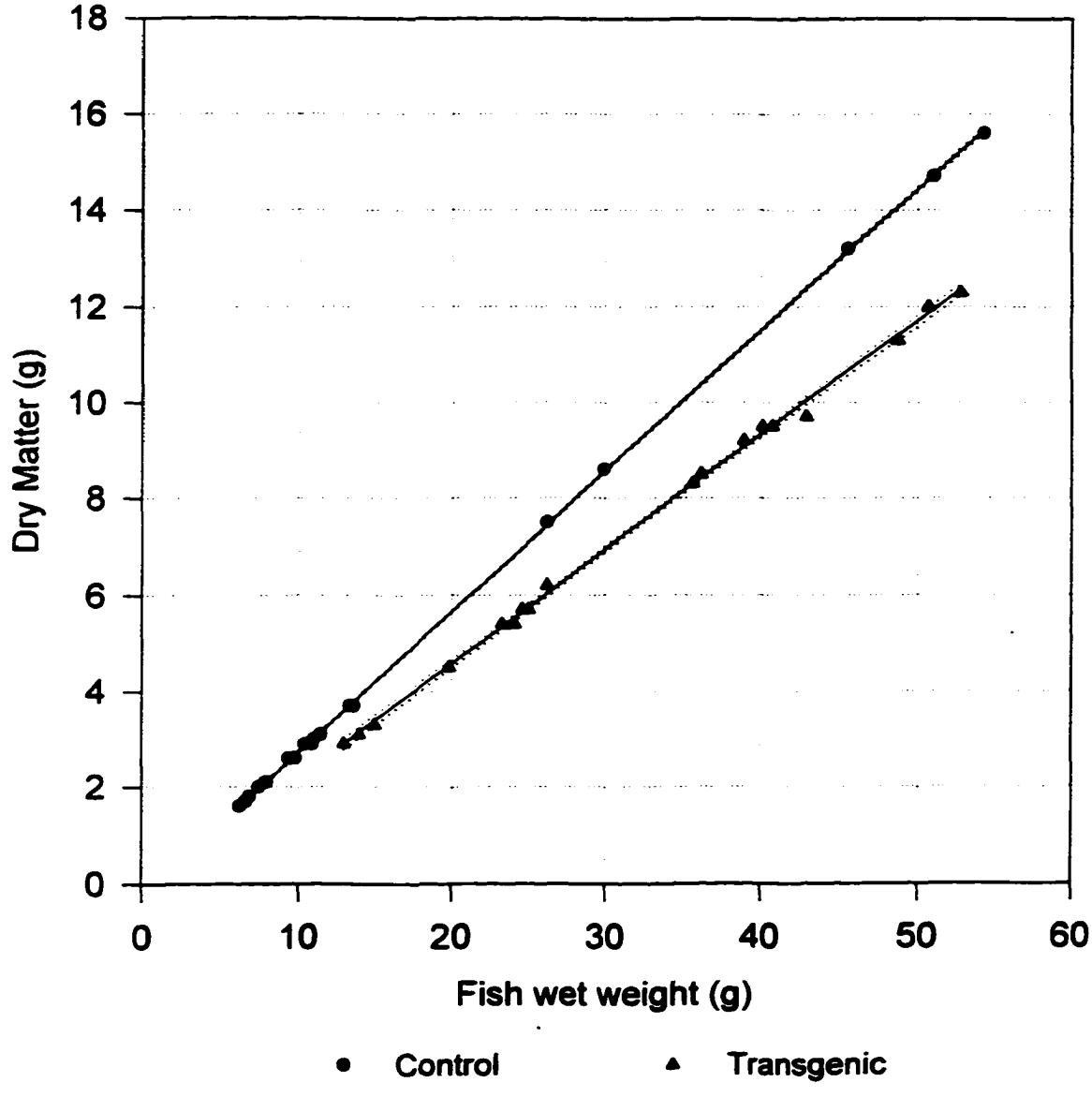


Figure 2.5
Ash content (absolute weight) in relation to wet body weight of transgenic Atlantic salmon (*Salmo salar*) and controls fed to satiation three times/day on a commercial diet. Data is presented with fitted regression lines (solid lines) surrounded by 95% confidence intervals (dashed lines).

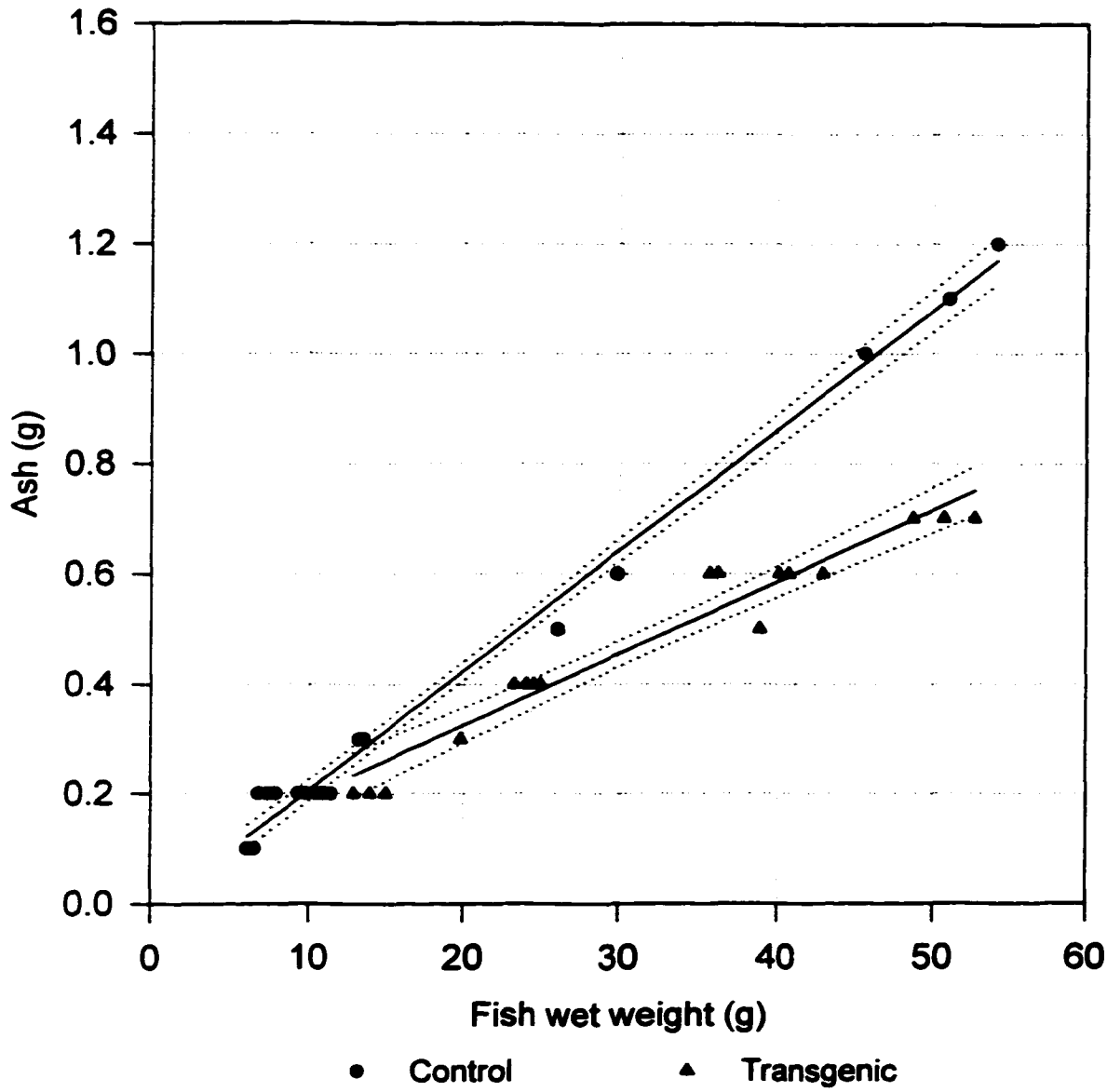


Figure 2.6
Lipid content (absolute weight) in relation to wet body weight of transgenic Atlantic salmon (*Salmo salar*) and controls fed to satiation three times/day on a commercial diet. Data is presented with fitted regression lines (solid lines) surrounded by 95% confidence intervals (dashed lines).

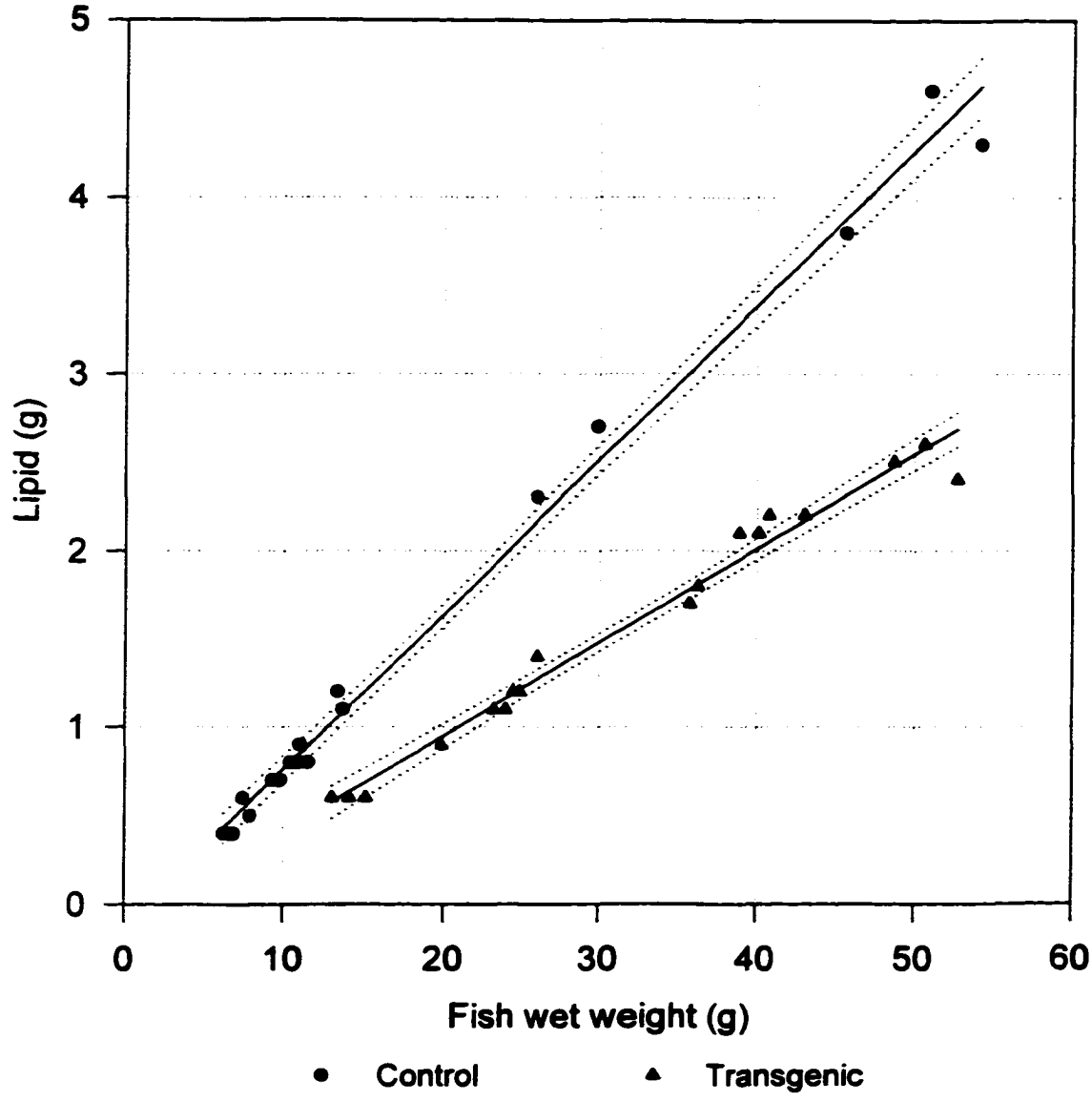


Figure 2.7
Energy content in relation to wet body weight of transgenic Atlantic salmon (*Salmo salar*) and controls fed to satiation three times/day on a commercial diet. Data is presented with fitted regression lines (solid lines) surrounded by 95% confidence intervals (dashed lines).

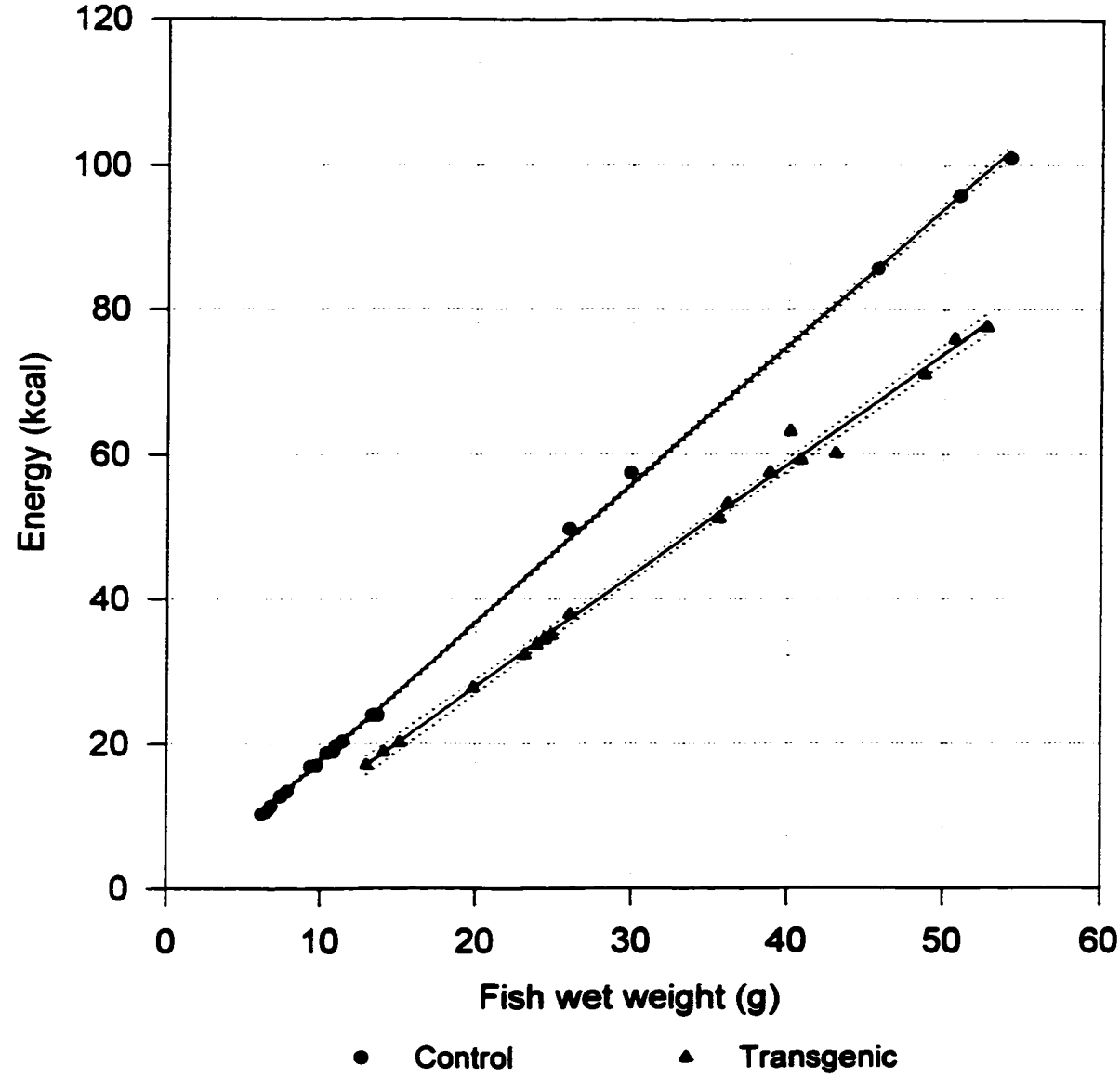


Table 2.7

Regression coefficients for the relation between body composition and energy content per fish wet weight of transgenic Atlantic salmon (*Salmo salar*) and controls fed to satiation three times/day on a commercial diet: $Y = b_o + b_1 \times BW$ where 'Y' is absolute nutrient or energy content, 'b_o' and 'b_1' are regression coefficients, and 'BW' is wet body weight.

Y (g or kcal)/fish	Fish strain	b_o	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 ($P < 0.05$)

Figure 2.8
Nutrient content (%) in relation to wet body weight of transgenic Atlantic salmon (*Salmo salar*) and controls fed to satiation three times/day on a commercial diet. Data is presented with fitted regression lines (solid lines) surrounded by 95% confidence intervals (dashed lines).

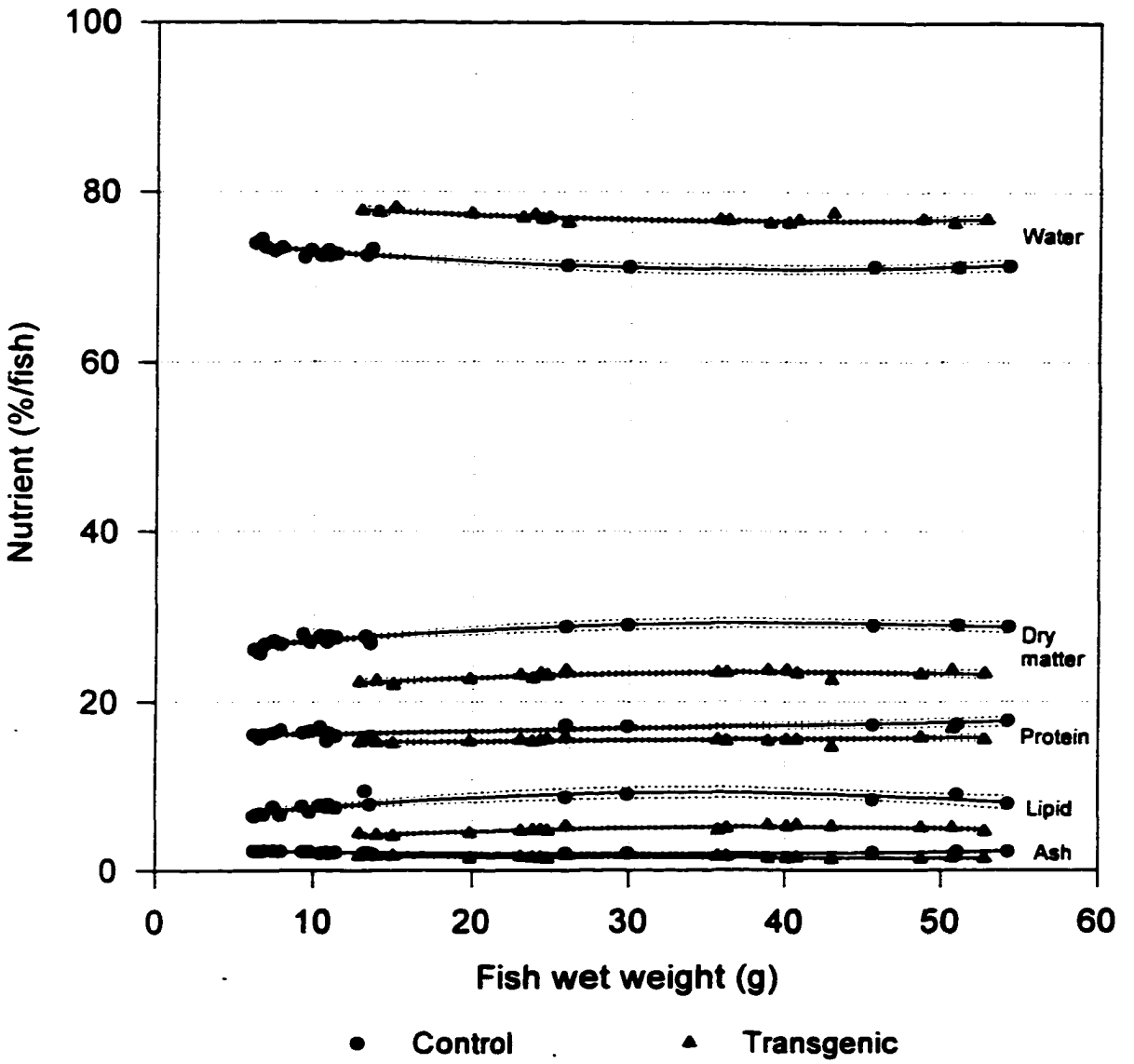


Table 2.8

Regression coefficients for the relation between body composition and energy content per unit wet weight of transgenic Atlantic salmon (*Salmo salar*) and controls fed to satiation three times/day on a commercial diet: $Y = b_0 + b_1 \times BW + b_2 \times BW^2$ 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 wt. 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	0.75
	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 ($P < 0.05$)

dry matter, ash, and lipid percentages varied little (<1-2%) with change in wet body weight.

2.4 Discussion.

2.4.1 Growth.

The present study confirmed that growth rates of transgenic Atlantic salmon containing a growth hormone gene were significantly greater than control salmon. Weight specific growth rates were nearly 3-fold larger than for non-genetically altered controls. Variable results have been observed for growth enhanced transgenic Atlantic salmon between different transgenic lines. Du *et al.* (1992) and Fletcher *et al.* (1992) reported transgenic Atlantic salmon 2- to 6- fold 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. Unfortunately, these authors did not report specific growth rates; direct comparison to growth growth rates in the present study is difficult. The magnitude of growth enhancement in this study was also lower than those reported by Devlin *et al.* (1995b) who found the average weight of transgenic coho salmon (*Oncorhynchus kisutch*) 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 gene copy number and the type of promoter used.

Exogenous hormone treatment, while showing relatively positive results, has not yielded rates of growth greater than the 185% increase reported in the present study. Cavari *et al.* (1993) observed that fingerling gilthead seabream (*Sparus aurata*), injected with bovine or human growth hormone, were 15% larger than control fish of the same age. Juvenile coho salmon injected with bovine growth hormone exhibited 118% weight gain over non-injected controls (Higgs *et al.*, 1977). Gill *et al.* (1985) found that injection of

recombinant chicken or bovine growth hormone into juvenile Pacific salmon increased weight gain by approximately 100% and lowered feed conversion ratios. Supplementation of feed with recombinant porcine or fish growth hormone has resulted in a 50-60% 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). Danzmann *et al.* (1990) reviewed 37 cases where salmonid growth rates were enhanced through exogenous hormone treatment, all of which still exhibited smaller magnitudes of growth acceleration quantified in the present experiment.

Although the transgenic fish displayed a greater absolute drop in SGR over a set weight range in respect to the control fish, they had a proportionally smaller (with respect to their SGR at 14 g) decrease in SGR as body weight increased (Figure 2.2). The magnitude of difference in daily feed consumption between the two experimental groups also changed with body weight. Transgenic fish of 14 g consumed 161% more feed than control fish; this declined to 114% for 55 g fish. The difference in SGR was attributed to disproportionate changes in daily feed intake between transgenic and control salmon and a faster decrease in growth rate by the controls. As salmonids continue to grow throughout their life cycle, even small differences in specific growth rate between strains of fish quickly compound into large differences in final body weight.

2.4.2 Digestibility and feed conversion.

Transgenesis does not appear to affect the extent to which protein, dry matter, and energy are digested (Table 2.5). Transgenic and control protein digestibility coefficients were 88% and 90% respectively, which correspond to the value of 87% reported by Shearer *et al.* (1992) for juvenile Atlantic salmon. As diet composition such as protein content and

source can significantly affect digestibility (Jobling, 1983; Cho and Bureau, 1995), comparison of results with literature values was only done in cases where diets of similar composition to that used in the present study were used. Most current commercial salmonid diets contain approximately 50-55% protein and 15-25% lipid. Hajen *et al.* (1993a) observed protein digestibility in chinook salmon of 76-87%. Apparent energy digestibility values of 80-86% for rainbow trout (Cho *et al.*, 1976) and 73-80% in chinook salmon (Hajen *et al.* 1993a) are similar to the values (81-84%) obtained in the present study. The dry matter digestibility values reported here (71-76%) are lower than the 87% observed by Shearer *et al.* (1992) in juvenile Atlantic salmon.

Researchers often use dissimilar methods for measuring digestibility, and the published literature consequently contains several apparently conflicting values. Methods of faecal collection in fish digestibility experiments still remain controversial after decades of study and often account for the large degree of variability reported between experiments. The goal in the present study was not to perfect a digestibility technique but to quantify feed digestibility by transgenic salmon in relation to non-genetically manipulated controls using a methodology which minimized known sources of error typically encountered in digestibility studies. For example, apparent digestibility estimates were made using faecal material collected over the period between the last feeding and just prior to the next 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 4 h hence minimizing nutrient leaching. 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) stated that calculations based on faeces

collected from water will give an overestimation of digestibility of water soluble nutrients. Differences in water temperature and fish size will also affect digestibility measurements (Jobling, 1983; Cho and Bureau, 1995). Consequently, water temperature was kept constant throughout the experiment and digestibility values were only compared between the two experimental groups for fish of similar size.

Aquaculture production of salmonids is based on using manufactured feed and it is important to maximize feed conversion efficiency. Appetite stimulation and improved feed conversion have been observed through exogenous hormone treatment (Higgs *et al.*, 1979; Higgs *et al.*, 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 growth hormone. Garber *et al.* (1995) injected two-year-old rainbow trout (300-700 g) with recombinant bovine growth hormone, with improved feed efficiency as the end result. Although daily feed consumption was higher for the transgenics in the present study, the total weight of feed consumed to grow to a given weight was similar to that consumed by the controls. Dry matter, protein, and energy conversions were slightly lower, but not significantly, for transgenics with respect to the control group. Feed conversion values observed here (0.72 and 0.83 for transgenics and controls, respectively) were slightly lower than those (0.91-3.02) reported by Storebakken and Austreng (1987) for fingerling Atlantic salmon. Protein utilization values of 21-29% and feed conversion of 1.35-1.64, obtained by Bromley and Smart (1981) on rainbow trout (70-250 g), were lower than the values exhibited in the present experiment (41-42 % and 0.72-0.83 for protein conversion and feed conversion, respectively). Cho *et al.* (1976) obtained feed:gain ratios of 1.05-1.45 for fingerling rainbow trout which were comparable to those observed for each

of the two experimental groups in the current study.

Growth hormone (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, transgenic fish using an antifreeze gene promoter from ocean pout to drive the expression of a growth hormone transgene, may be able to secrete growth hormone continuously year round (Fletcher *et al.*, 1985; 1990; Gong *et al.*, 1992). It is hypothesized that growth hormone secretion (in this particular line of transgenic fish) is not under control of neuroendocrine factors but occurs in the liver. Providing there is adequate nutrition, continuous growth hormone secretion would presumably mediate faster growth through continuous induction of IGF production. Transgenic salmon exhibit comparable feed digestibility and conversion but have greater daily feed consumption in relation to non-genetically altered salmon. Together with a larger digestive surface area (Stevens *et al.*, unpublished data), the transgenic salmon were able to convert feed into weight gain at a faster rate.

2.4.3 Body composition.

Previously reported effects of growth hormone 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 growth hormone. Treated fish had significantly enhanced growth rates and protein conversion, yet possessed significantly lower percentage of muscle protein per unit of wet fish weight and a greater percentage of muscle water than untreated fish. Rainbow trout, injected with recombinant fish growth hormone 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 parameters of body composition measured. Absolute weight per fish for each composition parameter was used for direct comparison between transgenic and control fish. Caution must be taken when comparing body composition between experimental groups (Shearer, 1994). The comparison of percentages of nutrients on a dry weight basis can be misleading as a change in one component will affect the proportion of other components. Also, exogenous factors such as previous nutritional history (feeding rate) can affect body components, particularly lipid and moisture contents (Reinitz, 1983); the amount of whole body lipid is dependent on dietary energy input. When energy requirements are exceeded, energy is stored as body lipid. The transgenic salmon in the present study had less body fat than control fish which was a function of their higher energy demand, i.e., higher metabolic rate (Chapter 3). Chatakondi *et al.* (1995) reported that the muscle of F_1 generation transgenic carp (1.60 kg) had a slight, although significantly, lower percent of lipid, a higher percent protein, 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 reported in the present study. It is unknown whether there is a genetic disposition, relating to the degree of growth acceleration, which may have played a role in the transgenic salmon displaying slightly lower protein and ash contents than controls. Feeding regime and/or diet composition could also have resulted in the transgenic

fish having altered body compositions in comparison to the control fish. Due to the physical logistics of manually feeding a large number of tanks of fish, a feeding schedule of three times per day was adopted and this may not have been sufficient to meet the transgenic's protein, lipid, energy, vitamin and mineral requirements. A diet with energy levels higher than what are typically present in commercial salmon feed is also recommended to meet the transgenic's higher metabolic rate (Stevens *et al.*, 1998) in the hopes of sparing dietary protein from being catabolized for energy and instead converted into body protein. Similarly, current dietary nutrient requirements should be reevaluated for such growth-accelerated fish.

2.5 Conclusion.

The present study represents a systematic evaluation of the performance of F_2 generation transgenic fish cultured under simulated aquaculture conditions. Atlantic salmon transgenic for the ocean pout promoter/chinook salmon growth hormone gene grew considerably faster than non-transgenic Atlantic salmon of the same stock during their first year of life. Daily feed intake was significantly higher for transgenic fish throughout this study. However, total feed consumption, digestibility, and conversions were unaffected by transgenesis. Body dry matter, protein, lipid, ash, and energy contents were significantly lower in transgenic fish with respect to controls. Increases in growth performance of the magnitude observed in this study holds significant potential for increasing the efficiency of aquaculture production by reducing production times.

3.0 METABOLIC RATE OF PRESMOLT GROWTH ENHANCED TRANSGENIC ATLANTIC SALMON (*SALMO SALAR*) REARED UNDER SIMULATED AQUACULTURE CONDITIONS

3.1 Introduction.

Transgenic Atlantic salmon (*Salmo salar*) containing a chinook salmon (*Oncorhynchus tshawytscha*) growth hormone transgene have been developed for accelerated growth (Chapter 2). Hogendoorn (1983) revealed that there is a direct linear relationship between the quantity of oxygen consumed and the dry weight gain for some fish on different feed intake levels. Consequently, the rapid growth exhibited by growth enhanced transgenic fish might come with some metabolic cost. Exogenous growth hormone treatment has been reported to significantly increase metabolism in parr and presmolt Atlantic salmon in relation to non-treated controls (Seddiki *et al.*, 1996). Transgenic fish have greater daily feed consumption in comparison to control fish (Chapter 2); Forsberg (1997) determined that the oxygen consumption of post-smolt Atlantic salmon increased proportionally with increased feed intake. Transgenic salmon also have higher activity levels than non-transgenic fish (Abrahams and Sutterlin, in press) which will significantly affect rate of oxygen consumption (Fry, 1971).

An animal's oxygen consumption rate provides a basis for understanding of its energy requirements. With the relatively high cost of pumping water (or generating oxygen) in land-based hatchery facilities, an understanding of culture oxygen requirements for fast growing transgenic salmon is needed. 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.

3.2 Methods.

3.2.1 Protocol.

Six hundred and sixty transgenic salmon (see Chapters 1 and 2 for experimental fish history), average weight 9.42 ± 0.09 g, were randomly distributed to twelve tanks for a total of 55 fish per tank. Six hundred and sixty control salmon, average weight 6.62 ± 0.05 g, were randomly assigned to twelve additional tanks for a total of 55 fish per tank. The fish were acclimated for three weeks in the experimental tanks. Fish were fed to satiation three times per day. Mean weights at the start of the experiment were 13.72 ± 0.21 g and 6.98 ± 0.07 g for transgenic and control fish, respectively. As part of ongoing growth and food deprivation experiments (Chapters 2 and 4), the fish in a minimum of three, and up to a maximum of twelve, replicate tanks for each of the two experimental groups were used in measuring routine oxygen consumption. A non-invasive protocol was developed whereby the aforementioned experimental growth tanks (Chapter 2) were temporarily converted to metabolic respiration chambers.

3.2.2 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. The mean water temperature entering the single pass, 92 L fibreglass flow-through experimental tanks (Figure 1.2) was 12.6°C ± 0.03. Water entered the tank at the periphery and circulated towards the center drain. The rates of water flow to individual tanks were periodically adjusted (taking into account fish size and number)

to maintain water oxygen levels above 6 ppm (Stevens *et al.*, 1998). Oxygen levels were measured using an Oxyguard Handy Mark 4 oxygen sensor (Point Four Systems Inc., Port Moody, British Columbia, Canada).

At the start of the experiment and every two weeks thereafter, the experimental growth tanks were converted to flow-through respiration chambers and the oxygen consumption of the fish (within) measured. For 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 gasket on the rim of the tank allowed for the plexiglass cover to be sealed airtight. Clamps were quickly placed around the tank edge and any air remaining inside the respirometer was bled out. Water exited at a higher point on the external standpipe resulting in a slightly positive pressure in the sealed tank. Flow rates were adjusted for each individual tank to facilitate an oxygen concentration drop, resulting from fish respiration, to approximately 60% of saturation. 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 slow current velocity. After tank sealing was accomplished, the fish were given an acclimation period of approximately 24 h before the first oxygen consumption readings commenced (Brett and Zala, 1975). Fish were continually fed to satiation three times per day on each of 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. Water flow rates and dissolved oxygen concentrations (DO) in the inflow and outflow water were measured every 4 hours over a 24 h period; the means were used to calculate oxygen consumption in fish reared under intensive culture conditions (Jarboe,

1995). The respiration tanks were unsealed after the last measurements were taken and the fish biomass was measured. Dissolved oxygen readings, measured to $\pm$ 0.1 mg O_2/L, flow rate, and tank biomass of fish were used to calculate the fish's oxygen consumption rate, according to the following equation (Cech, 1990):

$$VO_2 = (\Delta[O_2] \times 60\ \text{min/h} \times Vw)_{fishtank} - (BOD \times 60\ \text{min/h} \times Vw)_{blanktank}$$

where VO_2 is the routine oxygen consumption rate (mg O_2/h), $\Delta[O_2]$ is the DO concentration change from the inflowing water supply (mg O_2/L), $[O_2]_{inflow}$ and the outflowing drain water, $[O_2]_{outflow}$, Vw is the flow rate (L/min) and BOD is the biological oxygen demand (mg O_2/L).

The rate of oxygen consumption (mg O_2/h) was related to fish body weight according to the power relationship in the following equation (Cech, 1990).

$$VO_2 = a \times BW^b$$

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

To standardize for changes in body weight, VO_2 was divided by the fish biomass in the respirometer.

$$MO_2 = \frac{(\Delta[O_2] \times 60\ \text{min/h} \times Vw)_{fishtank} - (BOD \times 60\ \text{min/h} \times Vw)_{blanktank}}{W}$$

where MO_2 is the routine weight specific oxygen consumption rate (mg O_2/kg of fish/h) and

W equals the total fish biomass (kg) in the chamber. To check for microbial oxygen consumption, oxygen measurements were made concurrently on 'blank' tanks. These tanks normally had fish in them and therefore were representative as containing the same film of microbial flora that would result from nutrient enrichment associated with daily fish feeding and fish defecation. The 'blank' tank oxygen consumption rate was subtracted from the calculated fish oxygen consumption to obtain a true representation of the fish's metabolism.

At approximately 10 g intervals in fish wet weight, oxygen consumption rates of fish in a post-absorptive state were measured. Subsequent to the metabolic measurements inclusive of the heat increment associated with feeding, the respirometers were kept sealed and the fish fasted for 24 h (Brett and Zala, 1975). Inflow and outflow oxygen concentrations and flow rates were measured every 4 hours over the next 24 h period. Each tank was then unsealed, fish biomass measured, and the fish's mean oxygen consumption rate calculated according to the previous equations.

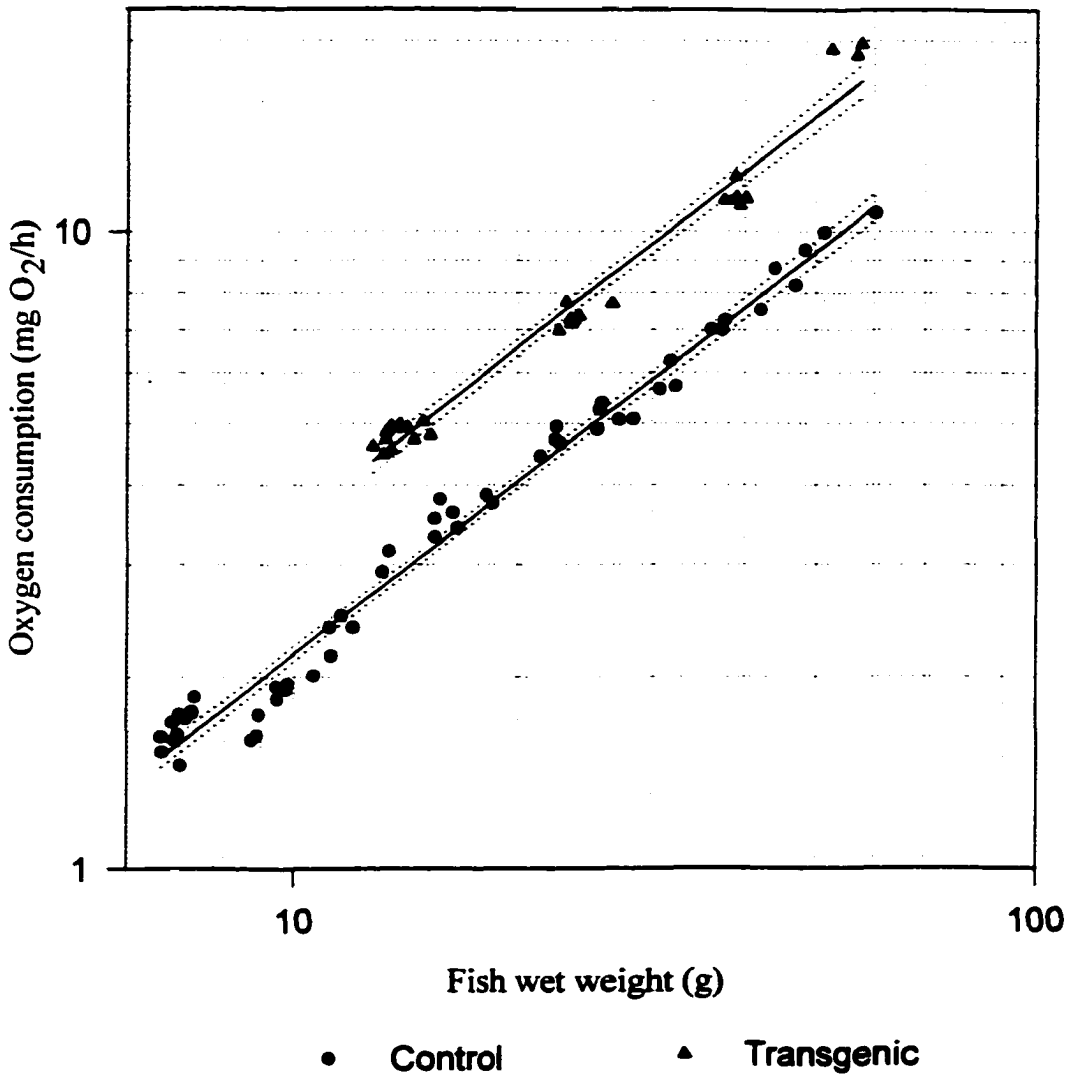
A Student's t-test was used to test the significance of any difference in oxygen consumption between transgenic and control fish at measured weights. The relationship between oxygen consumption and weight was tested by regression analysis, using 95% as the critical level for confidence intervals. A test for common slope was used to compare regression coefficients in regression equations for transgenic fish and control fish.

3.3 Results.

3.3.1 Routine oxygen consumption and weight exponent.

Routine oxygen consumption rates inclusive of the heat increment associated with being fed to satiation three times per day are shown in Figure 3.1. After logarithmic

Figure 3.1.
Routine oxygen consumption in relation to body weight of transgenic Atlantic salmon (*Salmo salar*) and controls, with inclusion of the heat increment associated with feeding. Data is presented with fitted regression lines (solid lines) surrounded by 95% confidence intervals (dashed lines).



transformation of the transgenic and control oxygen consumption data (Appendix B;Table 1), the regression equations shown in Table 3.1 accounted for 97-98% of the data's variance. There was a significant difference ($P<0.05$) between the rates of oxygen consumption of transgenic and control fish (inclusion of the heat increment associated with being fed) at all measured body weights. The slope of the regression line was significantly ($P>0.05$) steeper for the transgenic fish compared to the control fish, indicating 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 g to 52 g revealed that at 14 g, the rates of oxygen consumption of transgenic and control fish were 4.86 mg O_2/h and 2.90 mg O_2/h, respectively, representing a 1.68-fold difference. This difference in oxygen consumption marginally, although significantly ($P<0.05$), increased up to 1.69-fold at 52 g, where the oxygen consumption rates were 16.03 mg O_2/h and 9.46 mg O_2/h for transgenic and control fish, respectively. However, the total quantity of oxygen consumed by transgenic fish, when integrated over the time to grow from 14 g to 52 g, was 10:18 g, approximately 37% less (due to their higher growth rates; Chapter 2) than the 16.06 g of oxygen consumed by their non-transgenic counterparts.

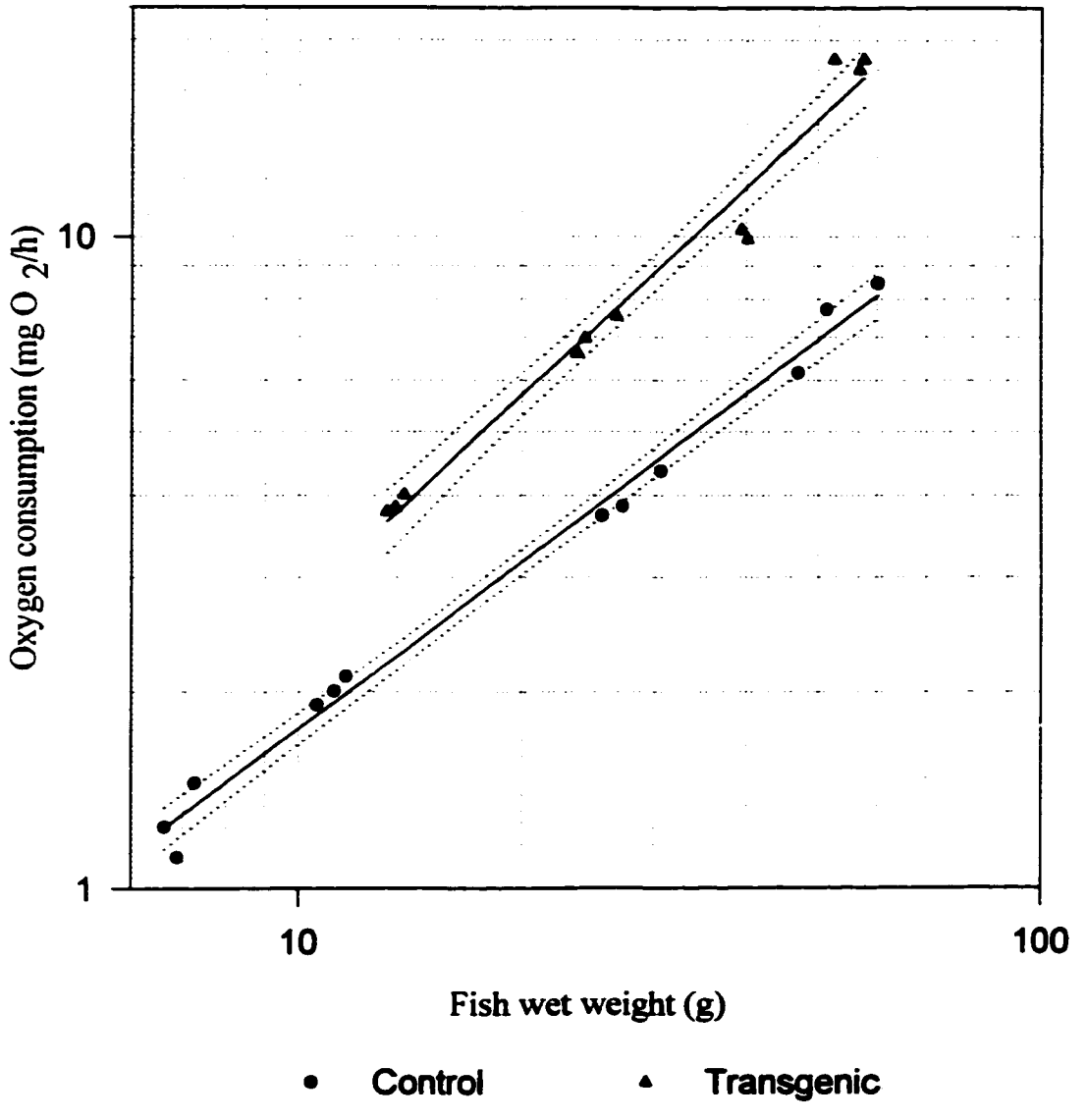
At 14 g, transgenic and control daily feed intakes as a percentage of body weight were 3.53% and 1.35%, respectively, representing a 2.61-fold difference. Upon reaching a wet body mass of 52 g, transgenic and control daily feed intakes were 1.67% and 0.78%, respectively, representing a 2.14-fold difference.

Routine oxygen consumption rates in a post-absorptive state are shown in Figure 3.2. After logarithmic transformation of the transgenic and control oxygen consumption data (Appendix B; Table 2), the regression equations shown in Table 3.1 accounted for 98-98%

Table 3.1.
Regression coefficients for the relation between routine oxygen consumption 'VO_2' (mg O_2/h) and body weight 'BW' (g) of transgenic Atlantic salmon (*Salmo salar*) and controls: $VO_2 = a \times BW^b$ where 'a' is the regression coefficient and 'b' is the weight exponent

Feeding state	Experimental group	a	b	r^2
Fed	control	0.27	0.9	0.98
	transgenic	0.44	0.91	0.97
Fasted	control	0.25	0.85	0.99
	transgenic	0.24	1.06	0.98

Figure 3.2.
Routine oxygen consumption, in relation to body weight, of transgenic Atlantic salmon (*Salmo salar*) and controls fasted for 24 h. Data is presented with fitted regression lines (solid lines) surrounded by 95% confidence intervals (dashed lines).



of the data's variance. There was 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. The slope of the regression line was significantly ($P>0.05$) steeper for the transgenic fish compared to the control fish, indicating the transgenics had a more rapid increase in oxygen consumption with increase in body weight. 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 mg O_2/h 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 mg O_2/h and 7.19 mg O_2/h, respectively.

The double logarithmic plots of metabolic rate in relation to body weight revealed weight exponents of 0.91 and 0.90 for transgenic and control fish respectively, while fed (Figure 3.1). Consequently, every time the transgenic fish doubled in body weight, their oxygen consumption increased by 91%; control oxygen consumption increased by 90% for every doubling of their body weight. The weight exponents in a post-absorptive state were 1.06 and 0.85 for transgenics and controls, respectively (Figure 3.2). In both the fed and post-absorptive state, the weight exponents were significantly different ($P<0.05$) between the two experimental groups.

In both the fed and fasting measurements, there was a small amount of 'tank' oxygen consumption, likely attributed to microbial film on the inner surface of the respiration chamber. The mean 'blank' tank oxygen consumption rate throughout the experiment was 5.31 ± 0.51 mg O_2/h.

Control salmon exhibited, with respect to the transgenic group, a significantly greater decrease in oxygen consumption when measured from a fed to a fasting state. The drop in

oxygen consumption from the fed to the fasted state was 22% at 14 g and declined to 1% at 52 g for the transgenic group; values for controls at comparable body weights were 23% and increasing to 32%, respectively.

3.3.2 Routine weight specific oxygen consumption.

On a quantitative basis, the larger the fish, be it transgenic or control, the more oxygen it consumed. However, on a unit-weight basis, oxygen consumption decreased as body mass increased. Weight specific routine oxygen consumption rates with inclusion of the heat increment associated with being fed to satiation three times per day are shown in Figure 3.3. The transgenic data (Appendix B; Table 3) was subjected to nonlinear regression using a second degree polynomial. Data for the control fish (Appendix B; Table 3) was subjected to linear regression as a second degree polynomial was found to produce no better fit. Regression equations for both experimental groups are shown in Table 3.2.

There was a significant difference ($P<0.05$) between transgenic and control rates of fed oxygen consumption at all weights measured during the present experiment. At a body weight of 14 g, transgenic and control oxygen consumption rates were 348.74 mg O_2/kg/h and 214.57 mg O_2/kg/h respectively, representing a 1.63-fold higher weight specific oxygen consumption rate for the transgenics over the controls. Transgenic fish exhibited a parabolic oxygen response in relation to body size (Figure 3.3), reaching a minimum metabolic rate at approximately 35 g. The magnitude of difference between the two experimental groups decreased to 1.48-fold at approximately 30 g where oxygen consumption rates were 295.30 mg O_2/kg/h and 199.23 mg O_2/kg/h for transgenic and control fish, respectively. A subsequent increase of oxygen consumption by the transgenic salmon to 326.32 mg O_2/kg/h resulted in an 1.83-fold difference over the controls (178.32 mg O_2/kg/h) at the final body

Figure 3.3.
Weight specific routine oxygen consumption in relation to body weight of transgenic Atlantic salmon (*Salmo salar*) and controls, with inclusion of the heat increment associated with feeding. Data is presented with fitted regression lines (solid lines) surrounded by 95% confidence intervals (dashed lines).

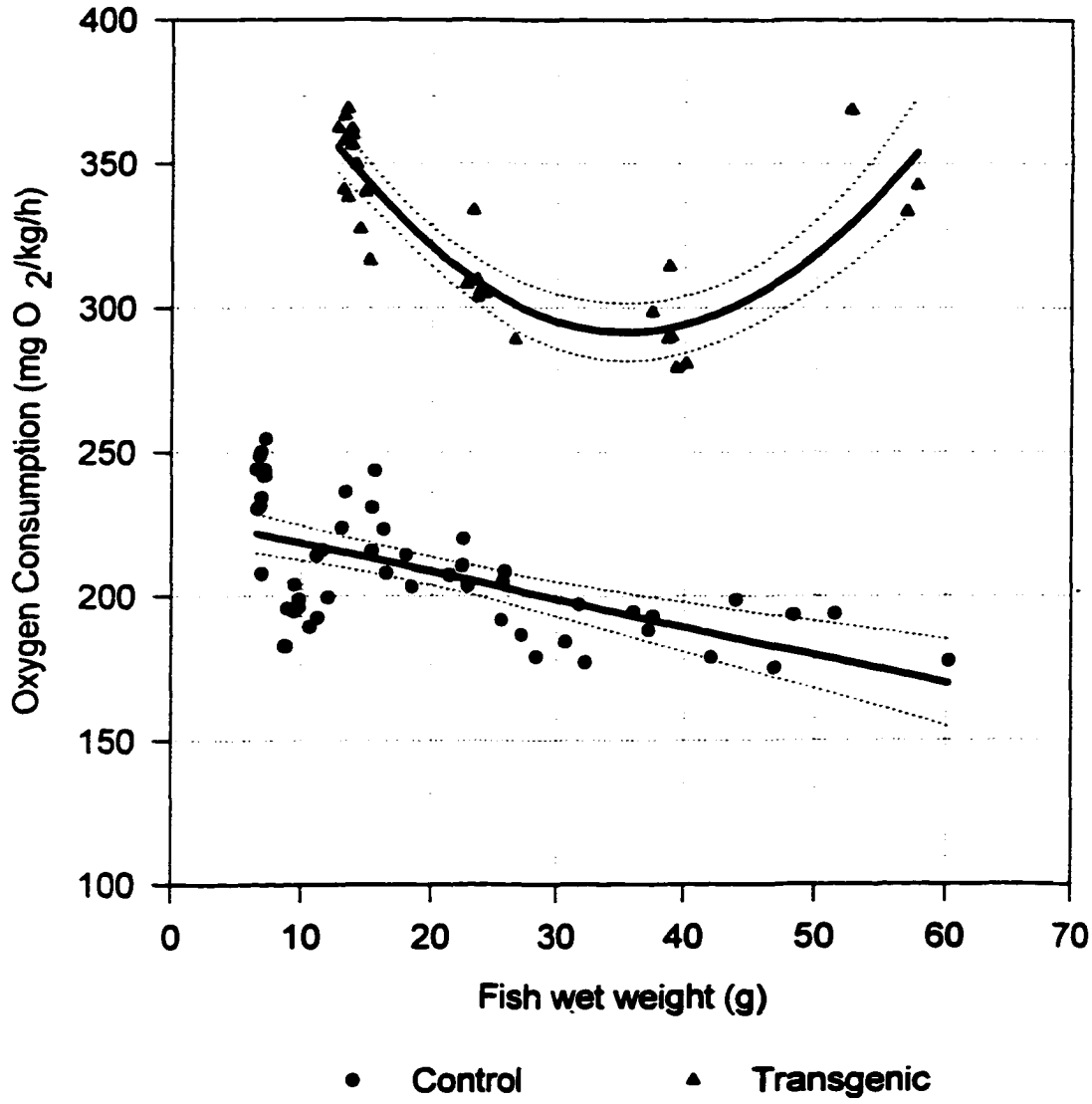


Table 3.2.
Regression coefficients for the relation between weight specific routine oxygen consumption 'MO_2' (mg O_2/kg of fish/h) and body weight 'BW' (g) of transgenic Atlantic salmon (*Salmo salar*) and controls: $MO_2 = b_o + b_1 \times BW + b_2 \times BW^2$ where 'b_o', 'b_1', and 'b_2' are regression coefficients

Feeding state	Experimental group	b_o	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	345	-5.01	0.08	0.56

weight of approximately 52 g.

Weight specific oxygen consumption rates for transgenic and control fish in a post-absorptive state are shown in Figure 3.4. The data for both transgenics and controls (Appendix B; Table 4) was subjected to nonlinear regression using second degree polynomials. Regression equations for both experimental groups are shown in Table 3.2.

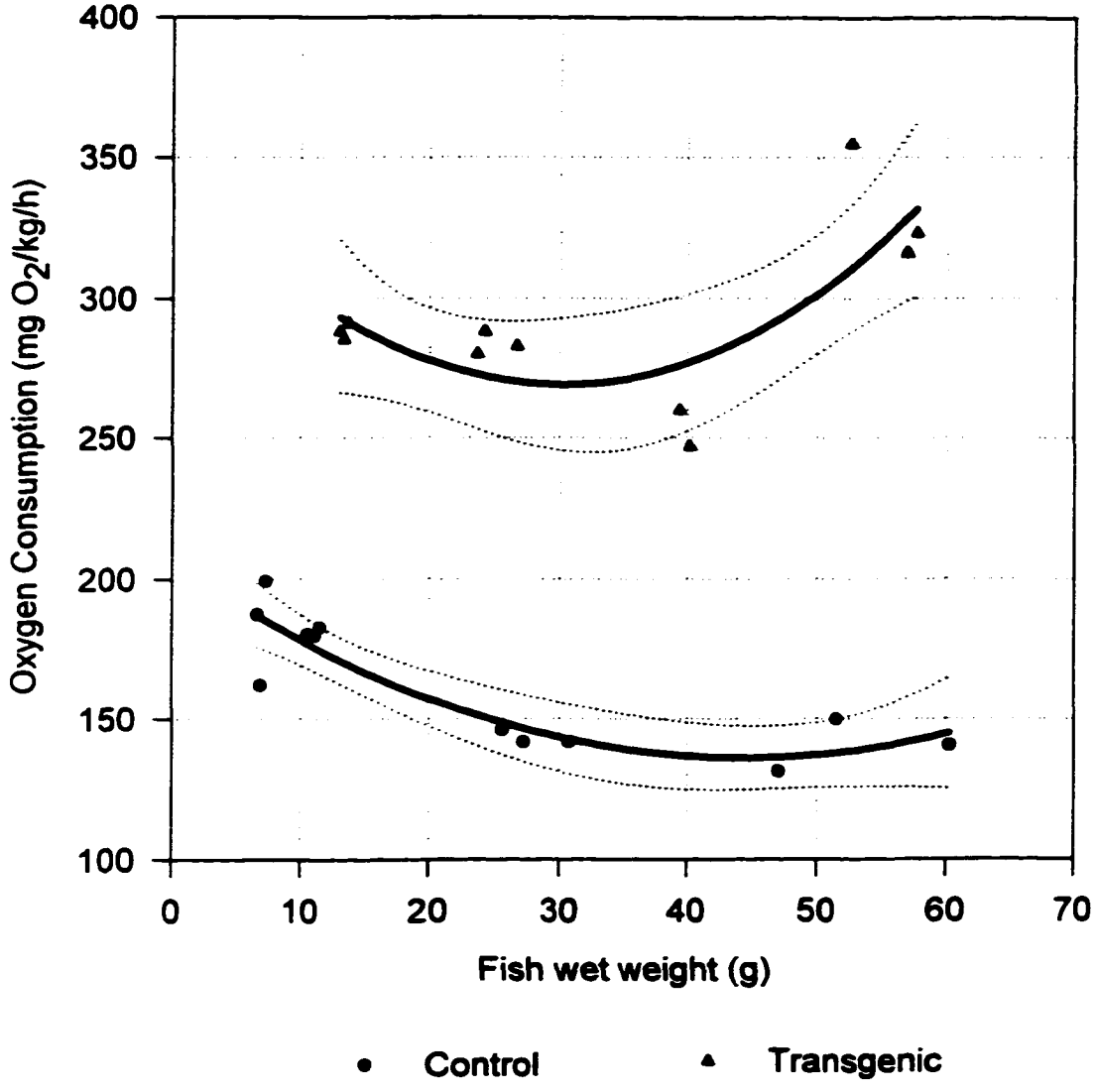
At a body weight of 14 g, the transgenic and control oxygen consumption rates were 291.11 mg O_2/kg/h and 169.50 mg O_2/kg/h, respectively, a 1.72-fold difference. The divergence in metabolism between the two experimental groups increased up to 2.23-fold at 52 g, where the oxygen consumption rates were 308.64 mg O_2/kg/h and 138.44 mg O_2/kg/h for transgenics and controls, respectively. There was a significant difference ($P<0.05$) between transgenic and control rate of fasting oxygen consumption at all weights measured during the present experiment.

3.4 Discussion.

3.4.1 Routine oxygen consumption and weight exponent.

Routine metabolism for F_2 generation growth enhanced transgenic Atlantic salmon was significantly higher than for non-transgenic controls over a weight range representative of smolt production. The heat release associated with metabolism generally represents a loss of energy to fish. The transgenic's higher oxygen consumption was largely a function of their larger daily feed intake compared to that of control fish. Forsberg (1997) established that oxygen consumption increased proportionally with increased feed intake in post-smolt Atlantic salmon reared in commercial scale fish tanks. Hogendoorn (1983) noted a direct linear relationship between the amount of oxygen consumed and dry weight gain for fish on different ration levels. More oxygen is consumed as the feed intake increases. However, the

Figure 3.4.
Weight specific routine oxygen consumption, in relation to body weight, of transgenic Atlantic salmon (*Salmo salar*) and controls fasted for 24 h. Data is presented with fitted regression lines (solid lines) surrounded by 95% confidence intervals (dashed lines).



drop 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. A 24 h starvation may not have been sufficient for the fish, especially the transgenics, to be in a true post-absorptive state. 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. However, transgenic fish maintained a significantly higher oxygen consumption than control fish after 24 h feed deprivation. Even if 24 h feed deprivation was not sufficient to put the fish in a post-absorptive state, extended periods of feed deprivation (Chapter 4) have shown that transgenic fish maintain a significantly higher oxygen consumption rate with respect to control fish.

The relationship of body weight to oxygen consumption is often expressed as $VO_2 = a \times BW^b$ where "b", the weight exponent (slope on a double log plot), has an approximate 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, respectively, and 1.06 and 0.85 after 24 h feed deprivation, respectively. 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 by 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 control weight exponent decreased when measured from a feeding to a fasting state; consequently, measurements over a larger weight interval may provide a better assessment of the relationship of the changes in oxygen consumption with respect to changes in body weight. Beamish (1964) evaluated brown trout (*Salmo trutta*), brook trout (*Salvelinus fontinalis*), common white sucker (*Catostomus commersonii*), brown bullhead (*Ictalurus nebulosus*) and carp (*Cyprinus carpio*) and found weight exponents of 0.877, 1.052, 0.864, 0.925, and 0.894 for fasted standard metabolism, respectively. Even 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).

3.4.2 Weight specific routine oxygen consumption.

Direct comparison between oxygen consumption studies is frequently difficult due to the variability which is inherent within a given species and the error imposed by insufficient attention to variables such as fish weight and water temperature (Brown *et al.*, 1984; Cech *et al.*, 1985; Cai and Summerfelt, 1992; Cech *et al.*, 1994), 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 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 (smoltification) typically occurs in the spring of the second year 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, Steven *et al.*, (1998) reported that the transgenic fish took on a silver coloration and lost the dark vertical bars 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 presmolts cultured under hatchery conditions. Similar results with growth enhanced transgenic fish have been reported by Saunders *et al.* (1998). Seddiki *et al.* (1996), while describing the positive effect of ovine growth hormone treatment on the seawater adaptability of salmon presmolts, also noted that the treatment caused a 30% 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 grams but then gradually increased until the termination of the experiment at approximately 52 grams. Although Wiggs *et al.* (1989) found an increase in the oxygen consumption for smolt over parr, this normally occurs in the spring of the year with non-genetically altered salmon. This size-dependent early smoltification appears to be an obligatory consequence of using fast growing transgenic salmonids. Hence, studies using juvenile transgenic salmonids may be faced with the unavoidable confounding physiological factor of smoltification.

Bergheim *et al.* (1991) illustrated the relationship between oxygen consumption and temperature in fed 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 of fish/h for 20-75 g non-transgenic fish were determined. 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 fish's oxygen consumption (Bergheim *et al.*, 1991). A weight specific oxygen consumption rate of 120 mg O_2/kg/h for 2 kg Atlantic salmon fed to excess in 8.5°C seawater has also been reported (Forsberg, 1997), a value lower than those reported here and is likely attributed to differences in fish size and water temperature. Stevens *et al.* (1998), using transgenic and non-transgenic salmon siblings of similar weight to those used in the present study, reported oxygen consumption values 375 mg O_2/kg of fish/h for transgenic fish and 220 mg O_2/kg of fish/h 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.

3.5 Conclusion.

Transgenic juvenile Atlantic salmon exhibited significantly higher routine oxygen consumption rates, over the entire weight range examined, when compared to non-genetically modified fish reared under simulated aquaculture conditions. While a higher rate of oxygen consumption was partially a function of a larger daily feed intake by the transgenic fish with respect to control fish, a significantly higher oxygen consumption was apparent when 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 37% less total oxygen than their non-genetically modified counterparts.

4.0 EFFECT OF FOOD DEPRIVATION ON METABOLISM AND BODY COMPOSITION OF GROWTH ENHANCED TRANSGENIC ATLANTIC SALMON (*SALMO SALAR*)

4.1 Introduction.

The enhanced growth rates (under hatchery conditions) exhibited by growth enhanced transgenic Atlantic salmon relative to non-genetically modified salmon are largely a function of their higher feed intake (Chapter 2). The following experiment in long term food deprivation was conducted to determine if the higher metabolic rates in feeding and growing transgenic fish (Chapter 3), relative to control fish, would persist in the absence of feed and result in a more rapid depletion of body energy reserves, tissue breakdown, etc., relative to control fish. Although commercial implementation of this technology will involve the use of reproductively incapable fish (female triploids), there remains some unresolved concern relating to the capability of these growth-enhanced fish to survive and compete with wild populations should they escape fish culture confinement. Therefore, public concerns on the possible environmental impact of transgenic technology has prompted the assessment of the bioenergetics relating to life history-related events such as food deprivation. Fish undergo natural periods of food deprivation throughout a normal life cycle and have consequently evolved the capability to endure prolonged food shortages. Energy expenditure can be reduced during such periods possibly by reducing activity, reflected by lower oxygen consumption (Beamish, 1964). However, extensive body energy reserves may be lost as the fish metabolize their own tissues to meet critical energy requirements. The probability and extent of ecological effects is dependent upon the fitness of transgenic individuals to cope

with changes in food abundance. Transgenic growth rates may be lowered significantly under more natural conditions such as during spawning migrations, wintering, or where food is not available in unlimited quantities.

4.2 Methods.

The bioenergetics of food-deprived juvenile transgenic and control salmon, at weight increments over a weight range of approximately 8 g to 55 g, was evaluated using the following methods. Six hundred and sixty transgenic salmon (see Chapter 1 for experimental fish history), average weight 9.42 $\pm$ 0.09 g, were randomly distributed to twelve tanks for a total of 55 fish per tank. Six hundred and sixty control salmon, average weight 6.62 $\pm$ 0.05 g, were randomly assigned to twelve additional tanks for a total of 55 fish per tank. The fish were allowed an acclimation period of three weeks in the experimental tanks. Fish were maintained at a temperature of 12.6°C $\pm$ 0.03. Feeding was conducted to satiation three times per day. Mean weights at the start of the experiment were 13.72 $\pm$ 0.21 g and 6.98 $\pm$ 0.07 g for transgenic and control fish, respectively. Fish in three transgenic and control tanks each were fasted and their oxygen consumption rates measured in a post-absorptive state (after 24 h starvation) using methodology described in Chapter 3. Fasting was continued and oxygen consumption rates were measured every two weeks thereafter until the fish lost approximately 15 percent of their initial wet body weight. Subsamples of 5 fish per tank were euthanized, subsequent to each oxygen measurement, for analysis of body composition and gross energy (Chapter 2).

The fish remaining in the nine tanks from each of the transgenic and control groups continued to be fed to satiation three times per day. At approximately 10 g wet weight intervals, fish in three tanks from each of the transgenic and control groups were fasted and

oxygen consumption rates and body composition measured according to the above protocol. As the transgenic fish had a significantly higher growth rate (Chapter 2), the time at which control fish of comparable size to transgenic fish were deprived of food was considerably delayed. This procedure continued until the fish in all twelve tanks of transgenics and controls were being monitored for oxygen consumption and body composition under conditions of food deprivation.

A Student's t-test was used to test the significance of any difference in oxygen consumption rate and body composition between transgenic and control fish over a range of body weights. The relationship between wet body weight, duration of food deprivation, and the rate of oxygen consumption or changes in body composition (dry matter, protein, lipid, ash, and energy) was tested by multiple regression analysis, using 95% as the critical level for significance. A test for common slope was used to compare regression coefficients in regression equations for transgenic fish and control fish.

4.3 Results.

4.3.1 Oxygen consumption.

Starvation resulted in cessation of weight increase and ultimately led to weight loss as the period of food deprivation progressed. The relationship between wet body weight and time of starvation for transgenic and control fish is shown in Table 4.1. Transgenic and control oxygen consumption rates (mg O_2/weight of fish) in relation to initial wet body weight and duration of starvation are shown in Figure 4.1. Initial weight represents the weight of the fish at zero time of starvation. The oxygen consumption data (Appendix C; Table 1) for each experimental group was subjected to multiple regression with initial wet body weight and starvation time as independent variables (Table 4.2). The rates of oxygen

Table 4.1
Final wet body weight 'FBW' (g) in relation to initial wet body weight 'IBW' (g) and duration of starvation 'Time' (days) of transgenic and non-genetically modified Atlantic salmon (*Salmo salar*) where: $FBW=b_0+b_1\times IBW+b_2\times Time$ and 'b_0', 'b_1', and 'b_2' are regression coefficients

Experimental group	b_0	b_1	b_2	r^2
Control	1.519[a]	0.945	-0.077[a]	0.99
Transgenic	1.817[b]	0.945	-0.096[b]	0.99

[a,b] Coefficients (in the same column) with different superscripts are significantly different ($P<0.05$)

Figure 4.1

Oxygen consumption rate (mg O_2/h) in relation to initial wet body weight (g)[day = 0] and duration of starvation (days) of transgenic and non-genetically modified Atlantic salmon (*Salmo salar*). Data is presented with fitted multiple regression (mesh plane) and the symbol '*' depicting axis interception by regression plane.

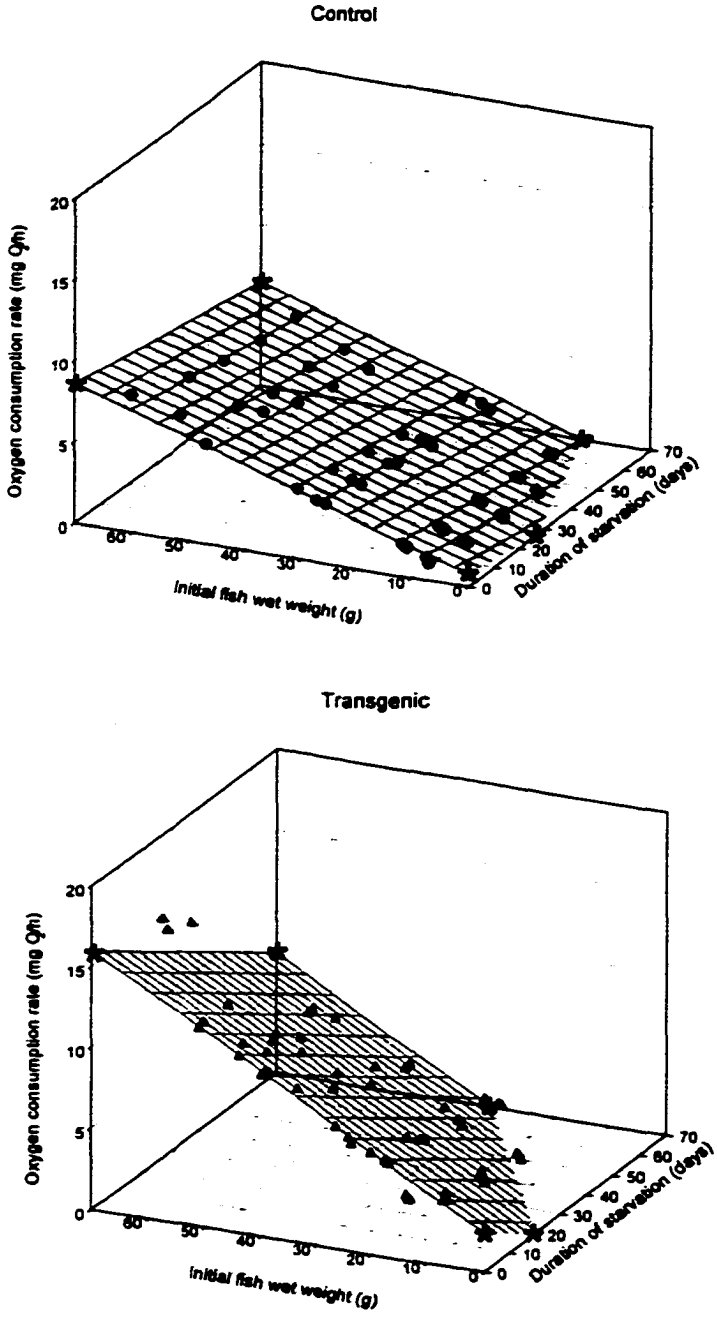


Table 4.2
Oxygen consumption rate 'Y' (mg/h) in relation to initial wet body weight 'BW' (g) and duration of starvation 'Time' (days) of transgenic and non-genetically modified Atlantic salmon (*Salmo salar*) where: $Y=b_o+b_1\times BW+b_2\times Time$ and 'b_o', 'b_1', and 'b_2' are regression coefficients

Experimental group	b_o	b_1	b_2	r^2
Control	0.814	0.111	-0.031	0.97
Transgenic	2.423	0.193	-0.122	0.82

Regression coefficients (in the same column) between the two experimental groups are significantly different ($P < 0.05$)

consumption of both transgenic and control fish decreased with increased time of starvation, to levels significantly ($P<0.05$) lower than those of fish of comparable size that had not been deprived of food (i.e. zero time). As expected from Chapter 3, transgenic fish had significantly higher rates of oxygen consumption than control fish (of comparable body weights) initially (zero time starvation) and throughout most of the period of food deprivation. However, the slopes of oxygen consumption in relation to initial body weight and time of starvation (Figure 4.1) were significantly steeper for the transgenic fish relative to control fish indicating that their rate of energy expenditure was declining more rapidly and eventually was reduced to a level where it was equal or less than that of the oxygen consumption of control fish.

4.3.2 Body composition.

The body composition and energy content of transgenic and control fish, with respect to initial wet body weight and duration of starvation, are shown in Figures 4.2-4.6. The composition data (Appendix C; Tables 2 and 3) for both experimental groups was subjected to multiple regression with initial wet body weight and starvation time as independent variables (Table 4.3). Dry matter, protein, lipid, ash, and energy contents at all measured weights were significantly ($P<0.05$) lower in the transgenic fish than in the controls (Figures 4.2-4.6). With the exception of ash content, all other fish body components in the absence of feeding decreased to significantly ($P<0.05$) lower levels than could be expected in fed fish (zero time food deprivation) of the same size. Ash content increased significantly ($P<0.05$) in the transgenic group with food deprivation time. Starvation had no significant ($P>0.05$) effect on the ash content of control fish from that expected of growing fish in a post-absorptive state (fasted for 24 h) of the same body weight. The rate of loss, or gain in

Figure 4.2
Dry matter content (absolute weight in g) in relation to initial wet body weight (g)[day = 0] and duration of starvation (days) of transgenic and non-genetically modified Atlantic salmon (*Salmo salar*). Data is presented with fitted multiple regression (mesh plane) and the symbol '*' depicting axis interception by regression plane.

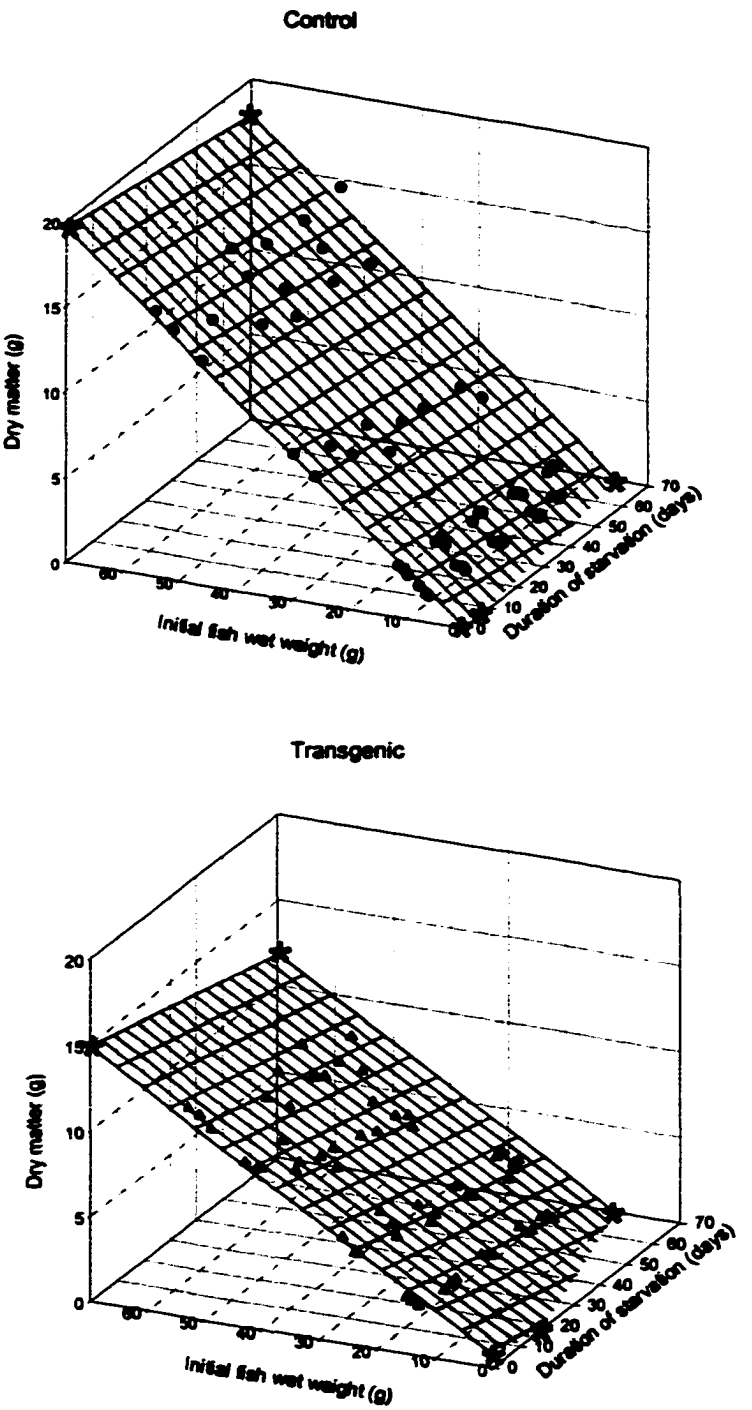


Figure 4.3
Protein content (absolute weight in g) in relation to initial wet body weight (g)[day = 0] and duration of starvation (days) of transgenic and non-genetically modified Atlantic salmon (*Salmo salar*). Data is presented with fitted multiple regression (mesh plane) and the symbol '*' depicting axis interception by regression plane.

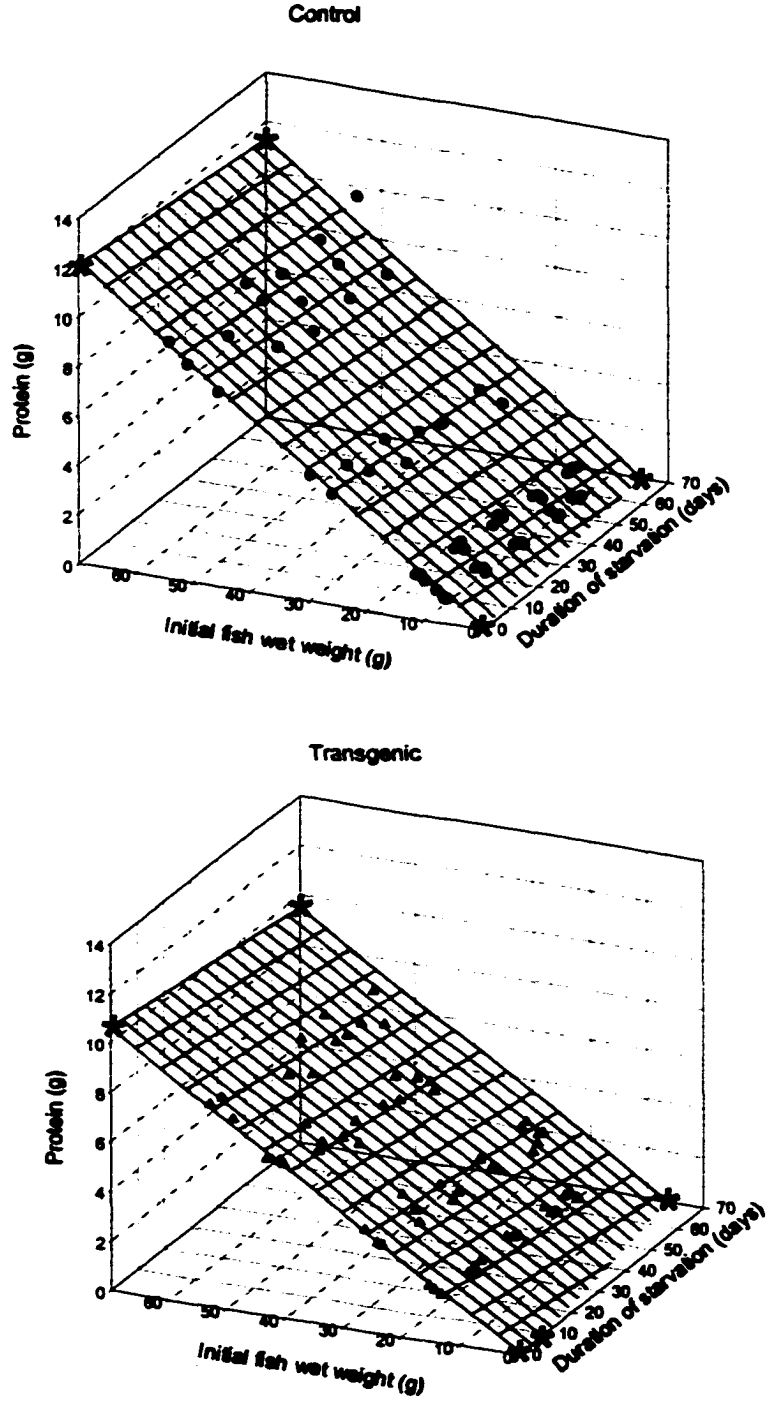


Figure 4.4
Lipid content (absolute weight in g) in relation to initial wet body weight (g)[day = 0] and duration of starvation (days) of transgenic and non-genetically modified Atlantic salmon (*Salmo salar*). Data is presented with fitted multiple regression (mesh plane) and the symbol '*' depicting axis interception by regression plane.

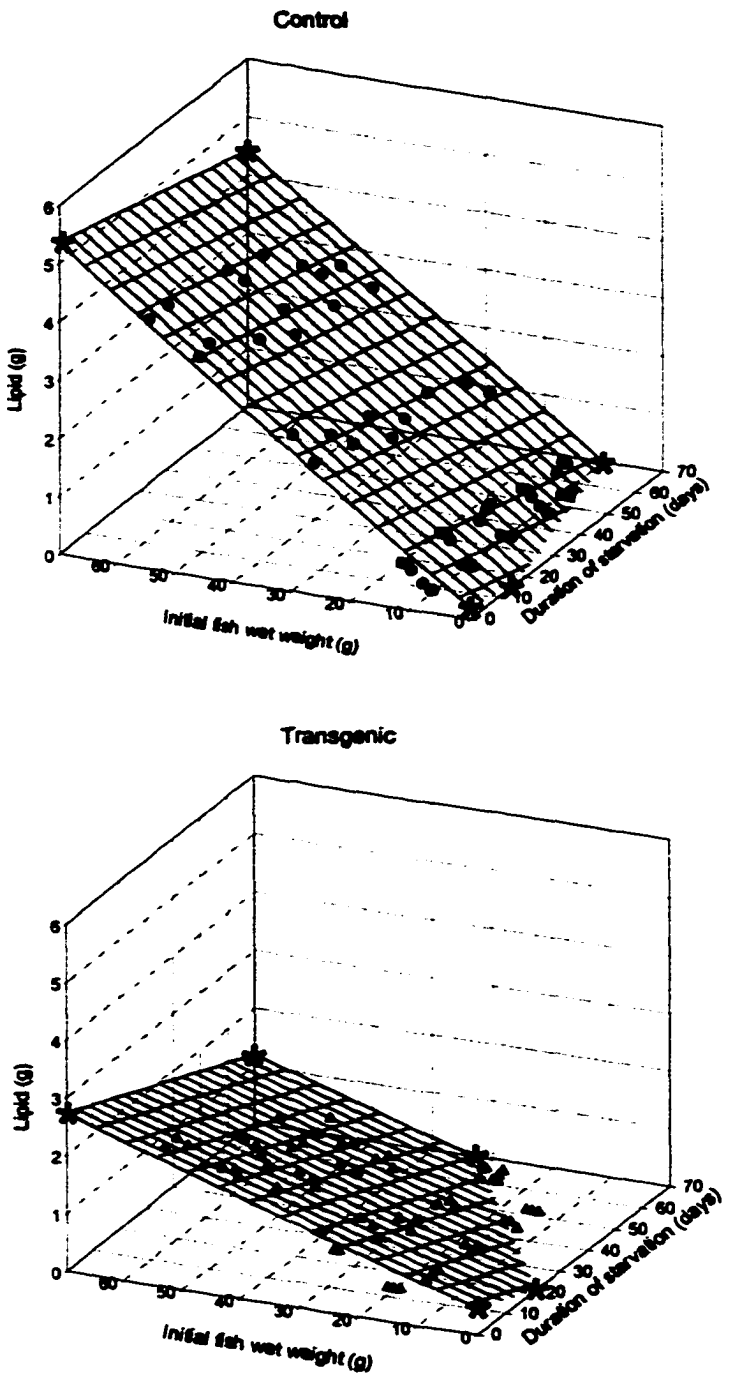


Figure 4.5
Energy content (kcal) in relation to initial wet body weight (g)[day = 0] and duration of starvation (days) of transgenic and non-genetically modified Atlantic salmon (*Salmo salar*). Data is presented with fitted multiple regression (mesh plane) and the symbol '*' depicting axis interception by regression plane.

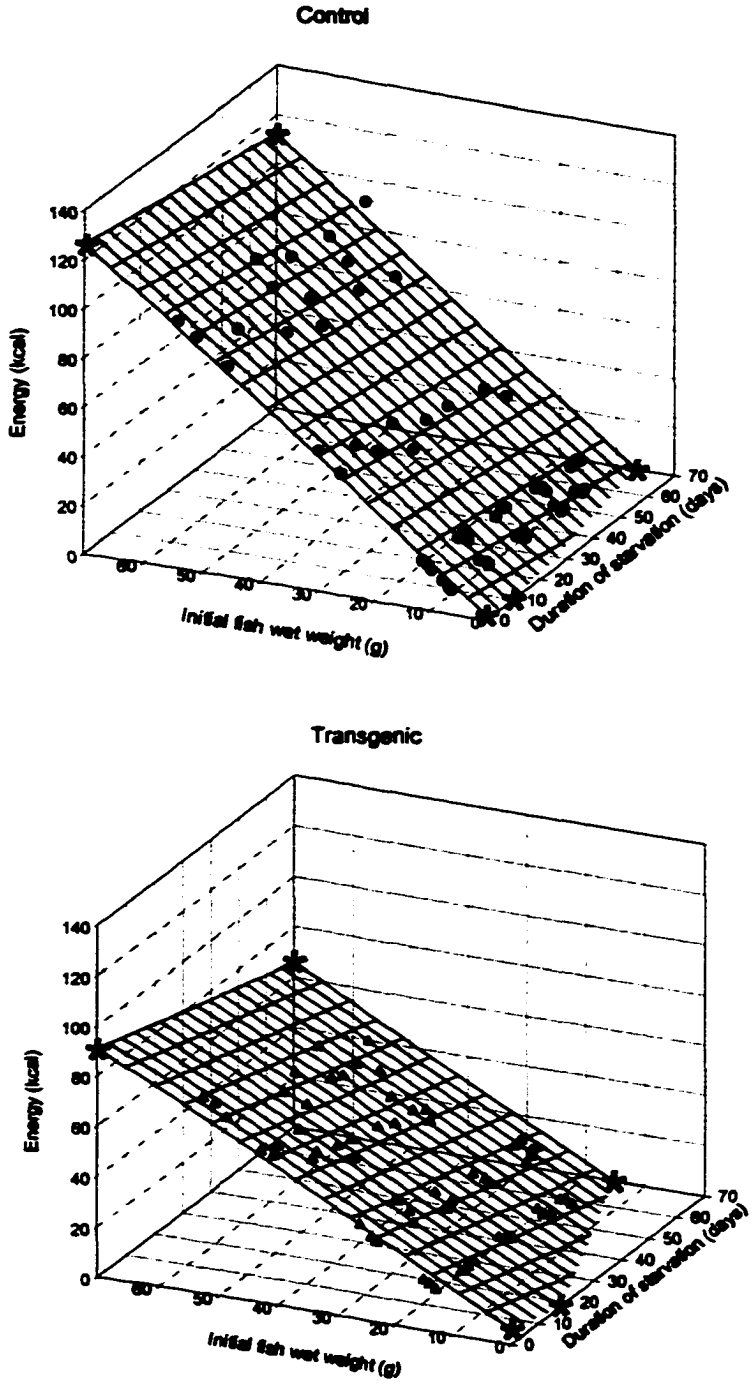


Figure 4.6
Ash content (absolute weight in g) in relation to initial wet body weight (g)[day = 0] and duration of starvation (days) of transgenic and non-genetically modified Atlantic salmon (*Salmo salar*). Data is presented with fitted multiple regression (mesh plane) and the symbol '*' depicting axis interception by regression plane.

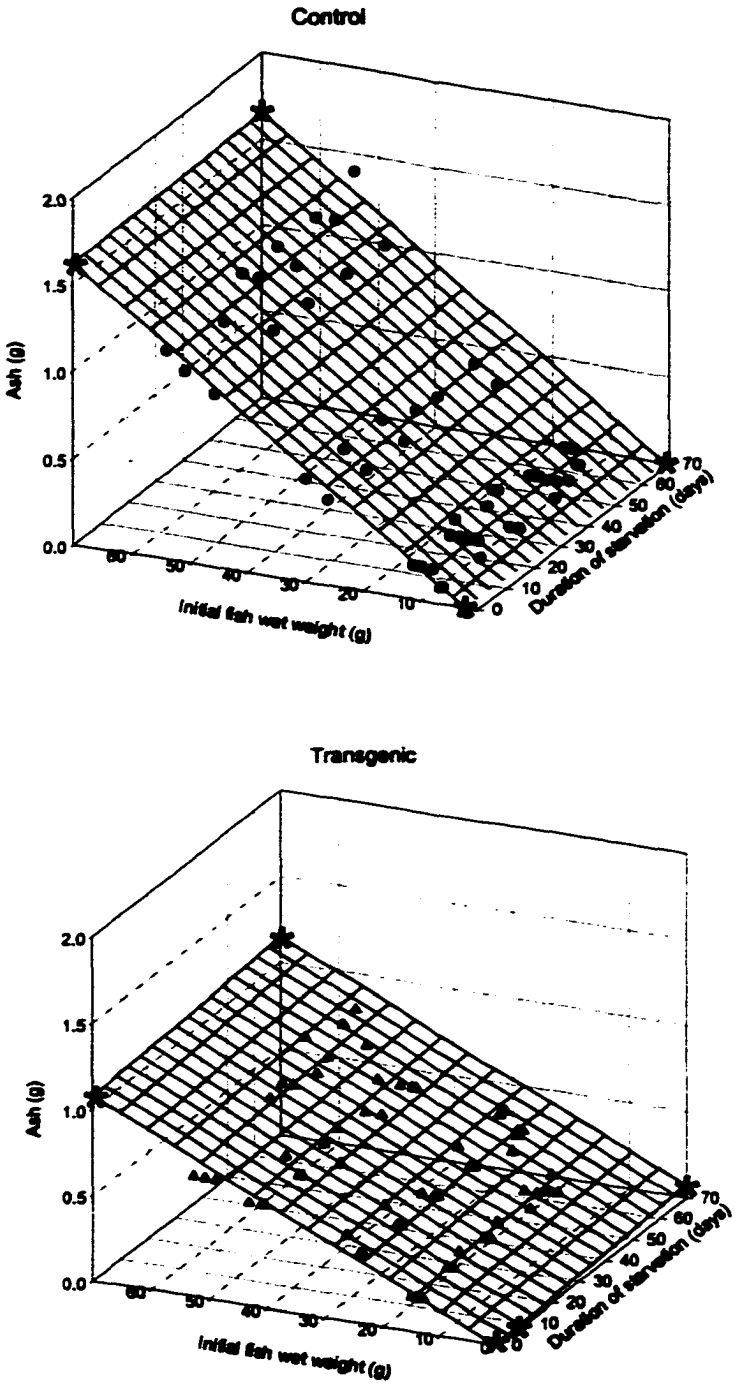


Table 4.3
Body composition 'Y' (g or kcal) in relation to initial wet body weight 'BW' (g) and duration of starvation 'Time' (days) of transgenic and non-genetically modified Atlantic salmon (*Salmo salar*) where: $Y=b_0+b_1\times BW+b_2\times Time$ and 'b_0', 'b_1', and 'b_2' are regression coefficients

Y (g or kcal)/fish	Experimental group	b_0	b_1	b_2	r^2
Dry Matter	Control	0.164	0.278	-0.025	0.96
	Transgenic	0.77	0.202	-0.044	0.94
Protein	Control	-0.044	0.173	-0.012	0.96
	Transgenic	0.132	0.15	-0.016	0.95
Lipid	Control	0.197	0.074	-0.013	0.95
	Transgenic	0.465	0.032	-0.022	0.87
Energy	Control	2.043	1.771	-0.195	0.95
(kcal)/fish	Transgenic	6.305	1.206	-0.355	0.93
Ash	Control	-0.055	0.024	n/a	0.95
	Transgenic	-0.007	0.015	0.001	0.91

Regression coefficients (in the same column) for each body constituent parameter between the two experimental groups are all significantly different ($P < 0.05$)

the case of ash, for each body component with starvation time was significantly ($P<0.05$) higher in the transgenics over the controls. Lipid reserves decreased at a faster rate than protein in both experimental groups (Table 4.3).

4.4 Discussion.

4.4.1 Oxygen consumption.

In the present study, both transgenic and non-genetically modified Atlantic salmon exhibited depression in average oxygen consumption rate when subjected to starvation conditions. As reported in Chapter 3, oxygen consumption is dependent on body weight hence it is not surprising that a decrease in body weight resulting from starvation would reduce the oxygen consumption rate. However, in both experimental groups, the subsequent decrease in oxygen consumption was greater than could be accounted for by a change in body weight. The transgenic fish, although maintaining higher oxygen consumption than control fish at most measured body weights, displayed a more rapid decline in oxygen consumption over the period when food was withheld.

An adaptation for the survival of food shortage periods is to decrease metabolism, as reflected by lower oxygen consumption. Mehner and Wieser (1994) reported that the average rate of oxygen consumption in small perch (*Perca fluviatilis*) (3-4 g) decreased when subjected to 14 days without food. Similar declines in oxygen consumption have been observed in juvenile plaice (*Pleuronectes platessa* L.) (30-60 g) (Jobling, 1980) and African catfish (*Clarias lazera*) (1-97 g) (Hogendoorn, 1983). A decline in oxygen consumption may be a consequence of lower activity by the fish in an attempt to conserve body energy reserves during periods of food shortage. However, a minimum of locomotor activity (foraging behavior) must be maintained as a trade off to ensure location and capture of prey

should it become available. Hogendoorn (1983) noted that the metabolic expenditure of African catfish (corrected for body weight) decreased with length of food deprivation, probably reflecting a decrease in fish activity. There may be an energy "set point" that reflects stored energy reserves, the likely "historic duration" of food unavailability, and determines what energy might be allocated to activity. The transgenic fish had an initial lower body energy content than control fish and a higher oxygen consumption rate. Therefore, this energy "set point" would have been reached first by the transgenic fish and may explain why their rate of oxygen consumption declined more rapidly than control fish during the period of food deprivation.

4.4.2 Body composition.

There are differential rates of mobilization of the two major body constituents, lipid and protein. Stored lipid, when metabolized, constitutes the main energy source. Because the transgenic fish had higher metabolism than controls, represented by higher oxygen consumption, their fat and protein was mobilized at a faster rate in response to food deprivation. For both transgenic and control fish, the depletion of body lipid occurred more rapidly than protein depletion; a 55 g transgenic fish, starved for 60 d, lost 38% more lipid (on a weight basis) than protein; a similar size control fish lost 16% more lipid than protein. Consequently, when the weight of depleted lipid and protein for each experimental group was multiplied by their respective caloric values (9.5 kcal/g lipid and 4.5 kcal/g protein), transgenic and control fish potentially obtained 2.9-fold and 2.4-fold more energy from lipids than protein, respectively. While protein was depleted, it is theoretically possible that it was not used for energy. For example, the protein could have been converted to carbohydrate through gluconeogenesis (Lauff and Wood, 1996) and used as an energy source by the brain.

Jobling (1980) reported that body lipid was the major storage reserve utilized by plaice (*Pleuronectes platessa* L.) starved for 35 days. Similar results have been reported in African catfish (Hogendoorn, 1983) and rainbow trout (Reinitz, 1983). Leatherland and Nuti (1981) noted that starvation stimulated mobilization of lipid reserves and that plasma free fatty acid levels were significantly higher in rainbow trout deprived of food. Body protein is conserved at the expense of stored lipid (Shearer, 1994). Additionally, while some lipid is required to maintain the structure and function of cellular membranes (Weatherley and Gill, 1987), it is essentially an energy reserve. Protein in fish, however, is predominantly muscle tissue and required for locomotion in locating and capturing prey when available. From a bioenergetic perspective, it is more economical to catabolize fat; 1 g will release approximately 9.5 kcal of energy compared to only 4.5 kcal from protein (Eckert *et al.*, 1988). For fish which do not have large body lipid reserves, as observed in the transgenic salmon in this experiment, the switch from body lipid as the major energy source (when most of the lipid reserves are utilized) to body protein would occur at an earlier stage than in fish which have large lipid reserves. Consequently, muscle tissue would be catabolized thereby putting the fish at a physiological disadvantage with respect to non-transgenic fish in locating and capturing prey as well as escaping predators. Also, as muscle protein is catabolized for energy, less amino acids are available for utilization by vital organs such as the heart. The nutritional history and genetic disposition of a fish (energy storage and metabolism) will influence survival under conditions of food deprivation.

A seemingly unlikely effect of starvation is an increase in plasma growth hormone (GH) levels (Sumpter *et al.*, 1991). Under conditions of food deprivation, GH receptors are resistant to GH binding; therefore GH is unable to induce insulin-like growth factor

production (IGF) (Olivereau and Olivereau, 1997). Consequently, GH is directed away from growth promotion and into regulating catabolism of lipid reserves (Wagner and McKeown, 1986). The actual endocrine pathways in growth enhanced transgenic fish remain unclear. While Du *et al.* (1992) reported that plasma GH levels in growing transgenic Atlantic salmon were not significantly higher than in controls, Devlin *et al.* (1994) found levels in growing transgenic coho salmon (*Oncorhynchus kisutch*) to be 40-fold higher than those measured in their non-genetically altered counterparts. If the transgenic fish in the present study had elevated GH levels compared to control fish before being subjected to food deprivation conditions, persistently high GH levels during food deprivation could account for their higher lipid catabolism.

The absolute ash content significantly increased with time of starvation in the experimental group of transgenic fish. This would be plausible if values were expressed as a percent of dry matter rather than as an absolute weight as was used in this experiment. However, it is highly unlikely that a food deprived transgenic fish absorbed enough minerals from the surrounding water to effect a significant rise of body ash. There is typically only 2% ash per unit wet body weight in salmonids (Shearer, 1994). Failure to take representative fish samples could have caused transgenic ash content to increase after food deprivation.

At low temperatures during the winter, when food is extremely scarce, and in combination with their elevated metabolic rates with respect to control fish, the severity of starvation on growth enhanced transgenic fish and their probability of survival is questionable. An antifreeze gene promoter normally drives the expression of an antifreeze gene to produce antifreeze peptides which aid fish in resisting low water temperatures (Fletcher et al., 1985). However, when an antifreeze gene promoter is geared towards

producing GH as in transgenic fish, GH may be produced year round, particularly during the winter when water temperatures are low. As GH will affect a fish's metabolism, the metabolic rate of these genetically-modified animals will likely be significantly higher compared to control fish during winter conditions. Consequently, the endogenous energy reserves of transgenic fish will be consumed more rapidly than their non-genetically altered counterparts.

Under hatchery conditions where feed is provided at near optimal levels to promote growth, there is little need for mobilization of endogenous energy reserves. Although fed to satiation 3 times per day before being subjected to food deprivation, the transgenic fish had lower fat reserves compared to the controls. In a more natural environment, transgenic salmon would likely have even lower lipid stores than those observed under culture conditions. Throughout the spring, summer, and fall months, when water temperatures are at their highest, juvenile salmon in fresh water typically forage on aquatic and terrestrial insects (Scott and Scott, 1988). Brett *et al.* (1969) found that fish reared at high temperatures (>20°C), where the maintenance energy requirement was highest, were unable to consume enough energy to accumulate body lipid. Transgenic fish may be unable to secure enough food to meet their metabolic requirements at such high temperatures. Additionally, Abrahams and Sutterlin (in press) demonstrated juvenile transgenic salmon will spend significantly more time feeding in the presence of a predator to meet their appetite demands. High mortality of transgenic fish which may escape from aquaculture facilities would reduce any potential environmental impact.

4.5 Conclusion.

The inability to secure enough food to meet basic energy requirements is a common

risk even to poikilotherms which are noted for their tolerance to withstand extended periods of food deprivation. The metabolic response of fish to prolonged periods of food deprivation will significantly influence the prospects of survival. Although transgenic fish demonstrated the ability to reduce their metabolic rate during starvation as observed in the non-transgenic salmon, their persistence in maintaining a higher metabolic rate, combined with their lower endogenous energy reserves, suggests that the probability of growth enhanced transgenic salmon achieving maximum growth or even survival outside intensive culture conditions may be lower compared with that of non-genetically modified salmon.

5.0 GENERAL DISCUSSION

5.1 Objectives.

The objective of the present study was to compare the bioenergetics of F_2 generation growth enhanced transgenic Atlantic salmon with non-genetically modified salmon over a weight range representative of the fresh water smolt production stage. Production factors such as growth rate, rate of oxygen utilization and feed conversion efficiency are all critical parameters that influence production costs in a commercial aquaculture setting. Significant departures by transgenics from regular salmon, in any of these parameters, could affect the cost of production for transgenic salmon. It is imperative that such differences be elucidated as part of the ongoing effort to commercialize this new technology. Also, seldom has the opportunity arisen to study the effects of a single gene on the physiological bioenergetics of a vertebrate animal.

5.2 Growth.

Using a "gene line" with a history of intermediate growth acceleration (Chapter 1), transgenic fish exhibited weight specific growth rates nearly 200% greater than their non-transgenic counterparts. Significantly enhanced growth rates (2- to 6-fold greater than control fish) of transgenic Atlantic salmon have been reported by Du *et al.* (1992), Fletcher *et al.* (1992) and Stevens *et al.* (1998). Exogenous hormone treatments (injections, dips, and implants) on juvenile salmonids (3-129 g) have yielded less growth acceleration (15-118%) than that produced through transgenic technology (Danzmann *et al.*, 1990). Supplementation of feed with growth hormone (GH), the easiest method in terms of exogenous hormone treatment, has yielded only modest growth rate increases (50-60%) in

coho salmon and juvenile black seabream (*Acanthopagrus schlegeli*) (McLean *et al.*, 1993; Tsai *et al.*, 1997). Based on the presmolt growth rates of transgenic salmon observed in this study, commercial salmon hatcheries, employing the same rearing regimes presently applied to rear regular salmon, could produce transgenic salmon smolts ready to enter sea water 6 to 11 months earlier than usual. This would represent a significant reduction in the cost of smolt production.

5.3 Feed intake, digestibility and conversion.

Transgenic fish exhibited enhanced appetite relative to controls throughout the entire growth period measured, with 161% greater feed intake at 14 g decreasing to 114% at 55 g. Appetite stimulation and improved feed conversion in fish treated with exogenous hormone have been reported (Markert *et al.*, 1977; Higgs *et al.*, 1979; Higgs *et al.*, 1982; Gill *et al.*, 1985). Feed, protein, and energy conversions in the present study were found not to be significantly different in the transgenic and control fish. Fu *et al.* (1998), using F_4 generation transgenic carp that grew 25% faster than controls, reported slight although significantly improved feed conversion efficiencies (FCE) for the transgenics. The transgenic carp achieved higher growth rates, when consuming low (20%) protein diets, mainly by increased feed intake with respect to control fish. When consuming high (30% and 40%) protein diets, the transgenic carp exhibited greater growth rates than control fish through improved energy conversion efficiencies. A protein-rich diet formulated for juvenile Atlantic salmon, containing 55% protein, was used in this study. Physiological changes likely occur in transgenic fish in order to accommodate the enhanced feed processing capabilities imposed by the increased appetite. For example, Stevens *et al.* (unpublished data), using F_2 generation juvenile transgenic Atlantic salmon of the same gene line as in the

current experiment, noted a 18-23% greater digestive surface area per unit weight compared to non-genetically modified controls. Whatever the factors involved, transgenic fish are capable of processing more feed per day than non-transgenic fish without sacrificing efficiencies in digestibility or the net conversion of feed to body tissue.

Growth hormone exerts its biological actions on almost every cell throughout the body, with its growth promoting effects mediated primarily through the induction of insulin-like growth factors (IGF) (Sakamoto and Hirano, 1993; Norris, 1997). Whether accelerated growth in transgenic fish is achieved from the continuous production of GH as a result of modified tissue specific expression (i.e. GH production in the liver), or results from a greater induction of IGF is unknown. Du *et al.* (1992), using growth enhanced transgenic Atlantic salmon containing a transgene similar to that used in the present experiment, reported that plasma GH levels were not significantly higher than in controls. However, the magnitude of transgene expression will likely be "gene line" specific, with each gene line different in relation to number and location of transgene incorporation into the fish's genome. Devlin *et al.* (1994), using a transgene comprised of a metallothionein-B gene promoter fused to a growth hormone gene, found GH levels in growth enhanced transgenic coho salmon (*Oncorhynchus kisutch*) to be 40-fold higher than those measured in their non-genetically altered counterparts.

The present study has shown that the quite remarkable growth rates achieved through transgenesis were not accompanied by an improvement in feed conversion; however this does not exclude the possibility that with diets formulated to meet their high metabolic requirements, transgenic fish's feed conversion might be improved.

5.4 Oxygen consumption.

Feed intake has a proportional influence on oxygen consumption, i.e., the more feed eaten, the larger the oxygen consumption (Hogendoorn, 1983; Kaushik and Medale, 1994; and Forsberg, 1997). The increase in oxygen consumption following an increase in feed intake is a consequence of the extra work due to ingestion, digestion and utilization of the feed. Fish obtain energy through the oxidation of organic compounds in the ingested feed after nutrient digestion and absorption. The routine oxygen consumption of the transgenic salmon (inclusive of the heat increment of feeding), was 1.5- to 1.7-fold greater than for their non-transgenic counterparts throughout a comparable range of body weights. This is supported by Stevens *et al.* (1998) who reported that 23-49 g transgenic Atlantic salmon containing the same transgene as used here had routine oxygen consumption rates 1.6- to 1.7-fold greater than controls of comparable body weight. The transgenic's higher metabolism in comparison to controls, as reflected by a greater oxygen consumption, likely induces physiological changes to accommodate a higher rate of oxygen consumption. For example, gill surface area was reported to be significantly greater in growth enhanced transgenic Atlantic salmon in comparison to non-transgenic salmon (Stevens and Sutterlin, in press).

The respiration chambers used in the present study were designed to simulate typical commercial rearing tanks with fish density and water temperature mimicking conditions normally encountered in commercial smolt production hatcheries. Measurements of oxygen consumption on fish in a post-absorptive state permit an indirect assessment of the amount of heat released by the physiological processes associated with nutrient assimilation and tissue maintenance. The observation that transgenic salmon in a post-absorptive state exhibited a 1.58- to 2.30- fold higher rate of oxygen consumption compared to fasting non-

transgenic fish of comparable size, is an indirect indicator that transgenic salmon have a higher energy requirement per unit body weight to meet their basal metabolic needs. This higher energy requirement will have to be met by potential commercial producers by providing the transgenic fish with more feed daily and/or a higher energy diet than what is typically used in the salmon industry. Additionally, because juvenile transgenic Atlantic salmon have the same critical oxygen level (level at which oxygen uptake by the fish is limited by dissolved water oxygen) as non-genetically modified salmon (Stevens *et al.*, 1998), commercial producers of such growth enhanced fish cannot allow dissolved oxygen levels to be reduced to lower levels and still meet the transgenic's higher oxygen demand. This higher oxygen demand will have to be met by either increasing dissolved oxygen levels, increasing water flow rates, or reducing fish stocking densities. However, when the oxygen consumption rates are integrated over time, transgenic fish require approximately 37% less total oxygen than non-transgenic fish to reach a common smolt size due to the shorter period to reach smolt size.

5.5 Body composition.

A consequence of higher metabolism in transgenic salmon was reflected in their body composition. The supply of dietary energy in a fish culture operation is tailored to provide optimal conditions for growth and feed conversion. However there is an input limit beyond which the fish does not utilize all the dietary energy and the excess is stored as fat. Hence, the amount of whole body lipid is dependent on the balance between dietary energy input and the metabolic energy demands of the fish. Body adiposity is inversely related to body moisture (Shearer, 1994); similarly the transgenic fish in the present study had higher moisture associated with the significantly lower levels of body lipid. Brett *et al.* (1969)

found that fish reared at high temperature, where the maintenance energy requirement was highest, were unable to consume enough energy to accumulate body lipid. The nature of the antifreeze transgene promoter normally is to drive the expression of an antifreeze gene to produce antifreeze peptides which aid fish in resisting low water temperatures (Fletcher et al., 1985). However, when an antifreeze gene promoter is geared towards producing GH as in transgenic fish, GH may be produced year round, particularly during the winter when water temperatures are low. Consequently, the temperature optimum for growth may be lower in transgenic fish compared to control fish. The optimal rearing temperature selected for such fish could be a tradeoff between minimizing maintenance energy requirements while maximizing GH production and growth.

In mammals, the secretion of insulin, glucocorticoids and leptin (in concentrations proportional to total body adipose stores) will inhibit the neuronal signaling mediated by hypothalamic neuropeptide Y, a feed intake stimulant (Schwartz and Seeley, 1997; Shearer *et al.*, 1997). Although the exact mechanisms have not been clearly elucidated in fish, Jobling and Miglavs (1993) reported an inverse relationship between feed intake and body lipid deposits in 8 g juvenile Arctic char (*Salvelinus alpinus*); as body lipid deposits increased, feed intake decreased. Because body lipid of transgenic fish remained relatively low in comparison to control fish, it is unlikely that this negative feedback loop operated during this study. While both the transgenic and control fish were fed to satiation three times a day, perhaps this feeding level never approached the maximum daily intake the transgenics were capable of processing. Consequently, a feeding regime may have to be developed to meet the specific energy requirements of growth enhanced transgenic salmon in order to optimize growth rate.

Lipids are required to maintain the structure and function of cellular membranes and threshold mesenteric lipid levels must be reached before the sexual maturation process in fish can begin (Rowe *et al.*, 1991). Although both fast growing Growth enhanced transgenic salmon and trout undergo normal sexual maturation and produce viable gametes within hatchery settings, under condition of food scarcity (such as in the wild), reproduction may be suppressed.

At all given body weights throughout the experiment, the transgenic fish had significantly lower protein and ash contents than the controls. This was surprising in that most species of fish (irrespective of feeding levels above maintenance and growth rates), body protein and ash are dependent solely on body size (Shearer, 1994). The fast growing transgenic salmon in the present study deposited protein at rates (weight of protein deposited/day) considerably greater than the control fish but had approximately 10% less protein at any given body weight. Increased rate of protein deposition exhibited by the transgenic fish compared to control fish was likely related to their ability to assimilate more feed daily, i.e., larger daily feed intake (Chapter 2) and larger digestive surface area (Stevens *et al.*, unpublished data). Contrary to the results obtained in the present study, Fu *et al.* (1998) found a slight, although significantly higher, whole body protein content in transgenic carp over non-genetically modified controls. However, the carp used in that study were only growing 25% faster than controls and Fu *et al.* (1998) expressed body composition as a percent of wet body weight and did not compensate for differences in body weight resulting from dissimilar growth rates.

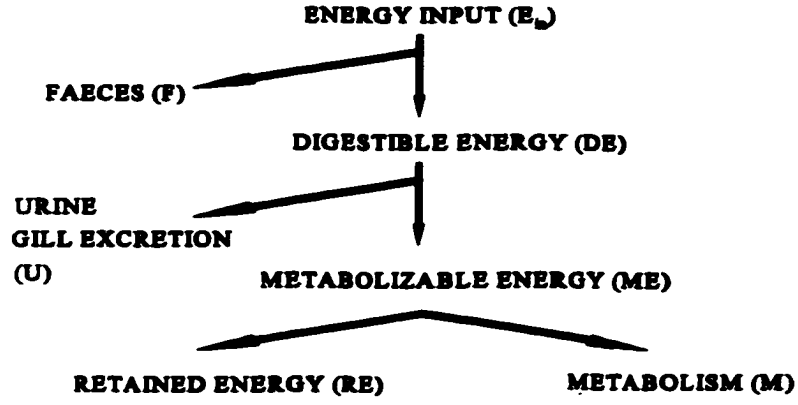
Commercial feed companies, in collaboration with nutrition researchers, attempt to develop least-cost feed formulations that will maximize growth and minimize energy losses,

while still maintaining an acceptable product with respect to body composition. The experimental diet used here contained 55% protein and 18% lipid, typical of the levels found in commercially formulated juvenile Atlantic salmon diets. Optimal amino acid, lipid, energy, vitamin, mineral, etc., requirements for growth enhanced transgenic fish will likely have to be quantified before their full growth potential may be realized.

5.6 Energy Budget.

A simple energy budget (Figure 5.1) was used as a means of comparing differences in energy gains and losses by transgenic and control fish.

Figure 5.1. Energy Budget: Utilization and partitioning of dietary energy in fish.



The dietary energy intake, E_{in} = F+U+RE+M; where F is energy lost to the fish in the form of faeces, U is total energy lost in urine or excreted via the gills, RE is retained energy, i.e., growth, and M is energy lost to metabolism (maintenance, SDA, activity).

As an example, energy budgets for a 14 g transgenic and a 14 g non-transgenic fish to grow to 55 g were calculated using data collected throughout this study. Total energy consumed (feed) was not significantly different between transgenic and control fish. Transgenic and control energy digestibility levels were 81% and 84%, respectively.

Therefore, corresponding energy loss via the faeces were19% and 16% of the total energy intake. A 7% energy loss via urine and gill excretion was assumed for both experimental groups (Brett and Groves, 1979). Retained energy, in the form of 'growth', represented 37% and 40% of respective total energy intakes for transgenics and controls, respectively. Under aerobic conditions, the amount of heat produced is related to the amount of oxygen consumed. A Q_{ox} value is used to convert oxygen consumption into heat production, with the consumption of one gram of oxygen being associated with the release of 3.24 kcal of energy (Elliott and Davison, 1975). Using this Q_{ox} value and the respiration data, the transgenic and control metabolic energy expenditures to grow from 14 g to 55 g represented 19% and 27% of their total energy intake, respectively. Consolidating this information, transgenic and control energy budgets are as follows:

$$\text{Transgenic: } E_{in} = F(19\%) + U(7\%) + RE(37\%) + M(19\%) = 82\%$$

$$\text{Control: } E_{in} = F(16\%) + U(7\%) + RE(40\%) + M(27\%) = 90\%$$

When all sources of energy loss and gain are added together they should equal 100% of the energy intake. In the above examples this is not the case. The most likely source of error was an overestimation of feed intake by both experimental groups of fish. The difference between feed fed to the fish and the excess feed syphoned out was assumed to be eaten. However, if feed was able to escape down the drain as a result of deficiencies in experimental tank design and protocol, this would give an overestimation of feed intake by the fish. This would lead to an underestimation of feed conversion efficiency; as to why the transgenic energy budget exhibited a discrepancy of 18% while the controls exhibited only

10% discrepancy remains unclear.

5.7 Feed deprivation.

Under extended periods of feed deprivation, energy required for the survival and maintenance of vital physiological processes originates exclusively from the degradation of body stores. The rates at which a fish utilize oxygen and deplete body reserves can be used as an index of energy utilization during such periods of starvation. Throughout most of the approximately eight weeks of feed deprivation, transgenic fish exhibited a greater rate of oxygen consumption compared to control salmon, but also a more rapid decline in oxygen consumption throughout the period of starvation. Consequently, in some circumstances (small fish under prolonged food deprivation), the rate of oxygen consumption of transgenic fish declined to a point where it equalled or was less than that of the control fish.

The rates of mobilization of the body's main constituents, lipid and protein, were different between the two experimental groups. Transgenic fish depleted body lipid and protein at a faster rate than did the controls. Additionally, in both groups, lipid was catabolized faster than was protein. Growth hormone levels generally increase during starvation but are directed away from growth promotion and into regulating lipid reserve catabolism (Wagner and McKeown, 1986). Presumably, if growth enhanced transgenic fish had elevated GH levels while actively growing, and if this condition prevailed during feed deprivation, it is possible that these fish have a more efficient protein sparing ability by using a greater quantity of lipid as their energy source; until the growth hormone levels in this particular transgenic gene line are elucidated, this remains only speculation. The transgenic fish in the present study did mobilize a proportionally larger amount of lipid than protein in comparison to control fish during food deprivation. Although none of the fish died in these

experiments, it is not known how far protein can be depleted before death ensues. Presumably transgenic fish, with their lower body protein and higher metabolism than control fish, would reach critical conditions and die sooner.

5.8 Starvation budget.

Using the same Q_{ox} value described in section 5.6, the amount of oxygen consumed by, for example, a 55 g transgenic or control fish starved for 60 d was converted into the total energy utilized over the same amount of time.

Transgenic: 13.52 g O_2 consumed × 3.24 kcal/g O_2 = 43.82 kcal burned

Control: 8.62 g O_2 consumed × 3.24 kcal/g O_2 = 27.92 kcal burned

Total carcass depletion of energy measured using bomb calorimetry (total carcass energy at the start of the experimental period - total carcass energy after 60 d starvation) was 21.30 kcal and 11.69 kcal for transgenics and controls, respectively. Energy depletion as measured using the respirometers was more than 2-fold higher than energy depletion as measured from the changes in body composition. Body composition was similar to that previously reported for juvenile Atlantic salmon (protein 17%; lipid 4-9%; ash 2.0-2.5%) (Higgins and Talbot, 1985; Poston, 1991). Hence it is unlikely the compositional data reported in the present study deviated by a magnitude large enough to account for the incongruity seen in the energy budgets. The calculated total energy depletion of a 55 g non-transgenic Atlantic salmon deprived of feed for 60 d, using energetic models based on oxygen measurements (Bureau, unpublished data), was 42 kcal. While this value is slightly higher than the energy loss by the non-transgenic salmon in the present study as calculated through oxygen consumption,

the discrepancy is not of a large magnitude. Consequently, the discrepancy between energy loss calculated by oxygen consumption and that calculated by bomb calorimetry remains unresolved.

5.9 Conclusion.

In many species of terrestrial livestock, the genetic gains in growth attained using traditional methods of selective breeding have resulted in simultaneous improvements in feed conversion efficiency. The theoretical basis for a similar expectation in fish has been presented by Gjedrem (1997). His 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. Even if true, improvements based on traditional selection and transgenic manipulation differ markedly in approach in that selective breeding involves the time-consuming partitioning of a large number of favorable genes related to growth while the transgenic approach (as applied in the fish used in this study) selectively targets a master gene process (hormone production) and immediately inserts a single functional gene (with controlling promoter) with the hope that the animal will be able to subsequently coordinate physiological processes accordingly.

Gjedrem (1997) documented growth rate improvements of 5% per generation using selective breeding, while salmon derived from GH gene injected eggs commonly exceed 100% improvement in a single generation. The transgenic experimental subjects used throughout the present experiment possessed the physiological plasticity to accommodate such a magnitude of growth acceleration with few effects other than an enhanced appetite, a lean body, and an elevated metabolism. While this study has improved the understanding

of the energy requirements of transgenic fish as well as provided fundamental information necessary for cultivating these fish, additional research is required to reveal any other physiological effects of growth transgenesis.

APPENDIX A

Table 1
Weight and specific growth rate (SGR) of growth enhanced transgenic Atlantic salmon (*Salmo salar*) and controls fed to satiation three times/day on a commercial diet.

Control			Transgenic		
Julian day	Wet wt.[a] (g)	SGR (% body wt./day)	Julian day	Wet wt. (g)	SGR (% body wt./day)
201	6.64	1.95	201	13.79	3.84
201	7.00	1.75	201	13.42	4.01
201	6.95	1.74	201	13.21	4.31
201	6.85	2.31	201	13.62	3.81
201	6.99	2.18	201	14.81	5.02
201	7.32	2.06	201	13.21	4.19
201	7.13	2.08	201	12.70	4.15
201	6.82	1.90	201	15.15	4.03
201	7.27	2.14	201	14.41	4.45
215	8.72	1.95	215	23.60	3.84
215	8.94	1.75	215	23.53	4.01
215	8.87	1.74	215	24.15	4.31
215	9.47	2.31	215	23.23	3.81
215	9.49	2.18	215	29.91	5.02
215	9.77	2.06	215	23.75	4.19
215	9.54	2.08	215	22.70	4.15
215	8.90	1.90	215	26.64	4.03
215	9.81	2.14	215	26.85	4.45
215	8.72	0.99	215	23.53	3.56
215	8.94	1.62	215	23.23	3.67
215	8.87	1.29	215	29.91	2.10
215	9.47	1.67	215	23.75	3.54
215	9.49	0.88	215	22.70	3.58
215	9.77	1.20	215	26.85	2.74
215	9.54	1.12	229	38.72	3.56
215	8.90	1.24	229	38.81	3.67
215	9.81	1.26	229	40.15	2.10
229	10.02	0.99	229	38.98	3.54
229	11.21	1.62	229	37.49	3.58
229	10.63	1.29	229	39.38	2.74
229	11.97	1.67	229	38.81	2.83
229	10.74	0.88	229	38.98	2.71

[a]Wt represents the mean fish wet body weight

Table 1 con't:

Control			Transgenic		
Julian day	Wet wt. (g)	SGR (% body wt./day)	Julian day	Wet wt. (g)	SGR (% body wt./day)
229	11.56	1.20	229	37.49	2.42
229	11.16	1.12	243	57.71	2.83
229	10.59	1.24	243	56.94	2.71
229	11.71	1.26	243	52.62	2.42
229	10.02	1.89			
229	11.21	1.14			
229	10.63	1.27			
229	11.97	1.34			
229	10.74	1.54			
229	11.71	1.93			
243	13.05	1.89			
243	13.15	1.14			
243	12.70	1.27			
243	14.45	1.34			
243	13.32	1.54			
243	15.35	1.93			
243	13.05	1.67			
243	13.15	1.22			
243	12.70	1.36			
243	14.45	1.58			
243	13.32	1.42			
243	15.35	1.31			
257	16.48	1.67			
257	15.60	1.22			
257	15.37	1.36			
257	18.03	1.58			
257	16.24	1.42			
257	18.44	1.31			
257	16.48	2.06			
257	15.60	2.54			
257	15.37	2.22			
257	18.03	2.40			
257	16.24	2.18			
257	18.44	2.21			
272	22.43	2.06			
272	22.83	2.54			
272	21.44	2.22			
272	25.83	2.40			

Table 1 con't:

Control			Transgenic		
Julian day	Wet wt. (g)	SGR (% body wt./day)	Julian day	Wet wt. (g)	SGR (% body wt./day)
272	22.51	2.18			
272	25.67	2.21			
272	22.43	1.04			
272	22.83	1.34			
272	21.44	1.34			
272	25.83	1.33			
272	22.51	1.79			
272	25.67	1.47			
285	25.67	1.04			
285	27.18	1.34			
285	25.51	1.34			
285	30.70	1.33			
285	28.42	1.79			
285	31.07	1.47			
285	25.67	0.72			
285	28.42	0.79			
285	31.07	1.06			
299	28.38	0.72			
299	31.76	0.79			
299	36.06	1.06			
299	28.38	0.92			
299	31.76	1.14			
299	36.06	1.11			
313	32.28	0.92			
313	37.26	1.14			
313	42.13	1.11			
313	32.28	1.18			
313	37.26	1.29			
313	42.13	1.07			
326	37.61	1.18			
326	44.06	1.29			
326	48.39	1.07			
326	37.61	1.02			
326	44.06	0.71			
326	48.39	1.00			
348	47.04	1.02			
348	51.47	0.71			
348	60.24	1.00			

Table 2
Body composition and energy content per fish wet weight of control Atlantic salmon (*Salmo salar*) fed to satiation three times/day on a commercial diet.

Wet wt.[a] (g)/fish	Dry matter (g)/fish	Protein wt. (g)/fish	Lipid wt. (g)/fish	Energy (kcal)/fish	Ash wt. (g)/fish
6.18	1.61	0.99	0.40	10.3	0.14
6.56	1.68	1.03	0.44	10.6	0.14
6.82	1.82	1.09	0.45	11.4	0.16
7.42	2.01	1.21	0.56	12.7	0.17
7.88	2.10	1.31	0.51	13.5	0.18
9.32	2.60	1.52	0.71	16.8	0.20
9.78	2.64	1.61	0.68	17.0	0.21
10.44	2.88	1.78	0.81	18.7	0.21
10.88	2.94	1.67	0.82	18.9	0.23
11.00	3.03	1.79	0.86	19.7	0.22
11.44	3.14	1.82	0.84	20.4	0.24
13.30	3.67	2.09	1.25	23.9	0.28
13.63	3.66	2.16	1.07	23.8	0.27
26.06	7.49	4.48	2.27	49.6	0.51
29.90	8.64	5.09	2.70	57.4	0.61
45.64	13.16	7.87	3.84	85.6	0.96
50.98	14.73	8.82	4.57	95.8	1.14
54.20	15.57	9.63	4.28	100.9	1.19

[a]Wt represents the mean fish wet body weight

Table 3
Body composition and energy content per fish wet weight of growth enhanced transgenic Atlantic salmon (*Salmo salar*) fed to satiation three times/day on a commercial diet.

Wet wt.[a] (g)/fish	Dry matter (g)/fish	Protein wt. (g)/fish	Lipid wt. (g)/fish	Energy (kcal)/fish	Ash wt. (g)/fish
12.94	2.88	1.97	0.56	17.0	0.22
14.02	3.15	2.13	0.59	18.8	0.24
15.04	3.30	2.27	0.61	20.2	0.25
19.84	4.49	3.04	0.88	27.7	0.29
23.2	5.36	3.60	1.08	32.3	0.36
23.98	5.44	3.67	1.15	33.7	0.35
24.48	5.70	3.77	1.17	34.5	0.36
24.92	5.75	3.88	1.16	35.0	0.35
26.08	6.18	4.12	1.38	37.8	0.47
35.64	8.31	5.51	1.69	51.1	0.57
36.18	8.45	5.54	1.81	53.2	0.57
38.88	9.22	5.97	2.09	57.5	0.55
40.12	9.50	6.21	2.07	63.1	0.56
40.78	9.49	6.33	2.19	59.2	0.60
43.00	9.68	6.30	2.23	60.0	0.55
48.74	11.31	7.71	2.50	71.0	0.68
50.68	12.01	8.52	2.60	75.9	0.75
52.74	12.25	8.16	2.41	77.5	0.73

[a]Wt represents the mean fish wet body weight

Table 4
Body composition per unit wet weight of control Atlantic salmon (*Salmo salar*) fed to satiation three times/day on a commercial diet.

Wet wt.[a] (g)/fish	Dry matter (%)	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
6.18	26.11	73.89	16.09	6.42	2.34
6.56	25.60	74.40	15.63	6.73	2.17
6.82	26.64	73.36	16.04	6.58	2.28
7.42	27.07	72.93	16.27	7.52	2.29
7.88	26.67	73.33	16.64	6.47	2.24
9.32	27.85	72.15	16.31	7.58	2.18
9.78	26.94	73.06	16.48	6.95	2.15
10.44	27.62	72.38	17.02	7.72	2.03
10.88	26.99	73.01	15.32	7.53	2.15
11.00	27.58	72.42	16.27	7.84	2.02
11.44	27.43	72.57	15.94	7.36	2.11
13.30	27.59	72.41	15.70	9.37	2.07
13.63	26.83	73.17	15.88	7.85	1.98
26.06	28.73	71.27	17.20	8.71	1.96
29.90	28.90	71.10	17.04	9.03	2.05
45.64	28.83	71.17	17.25	8.42	2.09
50.98	28.90	71.10	17.31	8.97	2.23
54.20	28.72	71.28	17.77	7.90	2.20

[a]Wt represents the mean fish wet body weight

Table 5
Body composition per unit wet weight of growth enhanced transgenic Atlantic salmon (*Salmo salar*) fed to satiation three times/day on a commercial diet.

Wet wt.[a] (g)/fish	Dry matter (%)	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
12.94	22.28	77.72	15.20	4.36	1.72
14.02	22.46	77.54	15.20	4.18	1.69
15.04	21.94	78.06	15.08	4.06	1.66
19.84	22.62	77.38	15.31	4.42	1.44
23.20	23.12	76.88	15.50	4.67	1.55
23.98	22.70	77.30	15.29	4.78	1.46
24.48	23.28	76.72	15.41	4.76	1.46
24.92	23.07	76.93	15.58	4.66	1.42
26.08	23.69	76.31	15.79	5.29	1.80
35.64	23.32	76.68	15.45	4.73	1.59
36.18	23.37	76.63	15.32	5.01	1.58
38.88	23.70	76.30	15.35	5.37	1.41
40.12	23.69	76.31	15.47	5.16	1.39
40.78	23.28	76.72	15.52	5.36	1.48
43.00	22.50	77.50	14.65	5.18	1.28
48.74	23.20	76.80	15.82	5.13	1.40
50.68	23.70	76.30	16.82	5.13	1.48
52.74	23.23	76.77	15.47	4.57	1.39

[a]Wt represents the mean fish wet body weight

APPENDIX B

Table 1
Mean routine oxygen consumption (VO_2), with inclusion of the heat increment associated with feeding, for growth enhanced transgenic Atlantic salmon *(Salmo salar)* and controls.

Control		Transgenic	
Wt.[a] (g)	VO_2 (mg O_2/h)	Wt. (g)	VO_2 (mg O_2/h)
6.62	1.61	12.70	4.60
6.64	1.53	13.11	4.47
6.85	1.70	13.21	4.84
6.88	1.59	13.21	4.73
6.95	1.63	13.42	4.95
6.99	1.75	13.43	4.54
7.00	1.45	13.78	4.99
7.13	1.72	13.79	4.96
7.26	1.77	13.79	4.91
7.32	1.86	14.10	4.93
7.27	1.76	14.41	4.71
8.72	1.59	14.81	5.03
8.87	1.62	15.15	4.79
8.94	1.75	22.70	6.99
9.47	1.93	23.23	7.75
9.49	1.85	23.53	7.28
9.77	1.91	23.60	7.17
9.81	1.95	23.75	7.26
10.63	2.01	24.15	7.36
11.16	2.39	26.64	7.70
11.21	2.16	37.49	11.19
11.97	2.39	38.72	11.21
11.56	2.49	38.81	12.20
13.05	2.92	38.98	11.31
13.32	3.14	39.38	10.99
15.35	3.54	40.15	11.27
15.37	3.31	52.62	19.40
15.60	3.80	56.94	18.97
16.24	3.62	57.71	19.76
16.48	3.43		
18.03	3.86		

[a]Wt represents the mean fish wet body weight

Table 1 (Con't)

Control		Transgenic	
Wt. (g)	VO_2 (mg O_2/h)	Wt. (g)	VO_2 (mg O_2/h)
18.44	3.75		
21.44	4.44		
22.43	4.72		
22.51	4.95		
22.83	4.65		
25.51	4.89		
25.67	5.26		
25.83	5.38		
27.18	5.07		
28.38	5.08		
30.70	5.66		
31.76	6.26		
32.28	5.71		
36.06	7.02		
37.26	7.01		
37.61	7.26		
42.13	7.53		
44.06	8.75		
47.04	8.23		
48.39	9.36		
51.47	9.97		
60.24	10.69		

Table 2
Mean routine oxygen consumption (VO_2) for growth enhanced transgenic Atlantic salmon (*Salmo salar*) and controls fasted for 24 h.

Control		Transgenic	
Wt.[a] (g)	VO_2 (mg O_2/h)	Wt. (g)	VO_2 (mg O_2/h)
6.62	1.24	13.11	3.78
6.88	1.12	13.43	3.83
7.26	1.45	13.79	4.01
10.59	1.91	23.60	6.61
11.16	2.01	24.15	6.96
11.56	2.11	26.64	7.53
25.51	3.72	39.38	10.23
27.18	3.85	40.15	9.91
30.70	4.35	52.62	18.67
47.04	6.16	56.94	17.99
51.47	7.72	57.71	18.65
60.24	8.46		

[a]Wt represents the mean fish wet body weight

Table 3
Mean weight specific routine oxygen consumption (MO_2), with inclusion of the heat increment associated with feeding, for growth enhanced transgenic Atlantic salmon (*Salmo salar*) and controls.

Control		Transgenic	
Wt.[a] (g)	MO_2 (mg O_2/kg/h)	Wt. (g)	MO_2 (mg O_2/kg/h)
6.62	243.91	12.70	362.26
6.64	230.07	13.11	340.79
6.85	248.39	13.21	358.02
6.88	231.16	13.21	366.55
6.95	234.01	13.42	369.06
6.99	250.15	13.43	338.06
7.00	207.59	13.78	361.96
7.13	241.70	13.79	359.89
7.26	243.75	13.79	356.29
7.27	241.83	14.10	349.59
7.32	254.52	14.41	327.04
8.72	182.76	14.81	339.94
8.87	182.62	15.15	316.28
8.90	211.76	22.70	308.05
8.94	195.74	23.23	333.44
9.47	204.01	23.53	309.47
9.49	194.69	23.60	303.62
9.77	195.96	23.75	305.64
9.81	198.82	24.15	304.95
10.59	219.53	26.64	289.04
10.63	189.33	37.49	298.54
11.16	213.92	38.70	289.42
11.21	192.49	38.81	314.39
11.56	215.72	38.98	290.03
11.97	199.48	39.38	279.04
13.05	223.43	40.15	280.61
13.32	236.05	52.62	368.74
15.35	230.58	56.94	333.13
15.37	215.41	57.71	342.37
15.60	243.47		
16.24	222.99		
16.48	207.85		

[a]Wt. represents the mean fish wet body weight

Table 3 (Con't)

Control		Transgenic	
Wt. (g)	MO_2 (mg O_2/kg/h)	Wt. (g)	MO_2 (mg O_2/kg/h)
18.03	213.99		
18.44	203.26		
21.44	207.24		
22.43	210.40		
22.51	219.70		
22.83	203.47		
25.51	191.68		
25.67	205.02		
25.83	208.37		
27.18	186.55		
28.38	178.98		
30.70	184.24		
31.76	197.17		
32.28	177.00		
36.06	194.55		
37.26	188.15		
37.61	193.00		
42.13	178.77		
44.06	198.70		
47.04	175.02		
48.39	193.43		
51.47	193.78		
60.24	177.39		

Table 4
Mean weight specific routine oxygen consumption (MO_2) for growth enhanced transgenic Atlantic salmon (*Salmo salar*) and controls fasted for 24 h.

Control		Transgenic	
Wt.[a] (g)	MO_2 (mg O_2/kg/h)	Wt. (g)	MO_2 (mg O_2/kg/h)
6.62	187.36	13.11	288.16
6.88	162.10	13.43	285.40
7.26	199.31	13.79	291.02
10.59	180.34	23.60	279.99
11.16	179.77	24.15	288.07
11.56	182.82	26.64	282.80
25.51	145.98	39.38	259.86
27.18	141.67	40.15	246.93
30.70	141.77	52.62	354.73
47.04	131.03	56.94	316.01
51.47	149.90	57.71	323.14
60.24	140.50		

[a]Wt. represents the mean fish wet body weight

APPENDIX C

Table 1
Oxygen consumption rate (mg O_2/h), under feed deprivation conditions, for growth enhanced transgenic Atlantic salmon (*Salmo salar*) and non-genetically modified controls.

Control			Transgenic		
Wt.[a,b] (g)	O_2 consumption (mg O_2/h)	Time of starvation (days)	Wt. (g)	O_2 consumption (mg O_2/h)	Time of starvation (days)
6.88	1.12	0	13.11	3.78	0
6.56	0.72	14	12.12	2.01	14
5.6	0.55	28	12.25	1.42	28
5.17	0.43	42	11.36	1.16	42
6.62	1.24	0	13.43	3.83	0
6.88	0.73	14	12.92	2.45	14
6.04	0.59	28	12.44	1.64	28
5.63	0.42	42	11.72	1.47	42
7.26	1.45	0	13.79	4.01	0
7.31	0.80	14	13.03	1.95	14
6.64	0.70	28	12.7	2.04	28
6.44	0.55	42	12.35	1.47	42
11.56	2.11	0	23.6	6.61	0
11.32	1.43	14	22.43	3.71	14
10.07	1.42	28	21.15	3.46	28
9.24	1.05	43	20.3	2.61	42
8.93	0.84	56	19.06	2.11	57
11.16	2.01	0	24.15	6.96	0
10.94	1.36	14	22.43	3.86	14
9.95	1.28	28	21.48	3.46	28
9.35	1.02	43	20.79	2.96	42
9.06	0.88	56	19.38	2.15	57
10.59	1.91	0	26.64	7.53	0
10.73	1.30	14	24.71	4.24	14
9.32	1.27	28	24.29	3.46	28
8.9	1.00	43	23.23	3.58	42
8.45	0.90	56	22.38	2.36	57
27.18	3.85	0	40.15	9.91	0

[a] Wt represents the fish's wet body weight
[b] Initial body weight is at time of starvation = 0

Table 1 con't:

Control			Transgenic		
Wt. (g)	O_2 consumption (mg O_2/h)	Time of starvation (days)	Wt. (g)	O_2 consumption (mg O_2/h)	Time of starvation (days)
25.25	3.43	14	38.13	7.37	14
24.13	2.78	28	36.6	5.71	28
35.4	4.11	43	23.47	2.68	41
22.46	2.24	63	33.37	3.62	56
25.51	3.72	0	39.38	10.23	0
24.78	3.23	14	37.64	9.68	14
23.13	2.89	28	36.59	6.72	28
22.66	2.31	41	35.52	5.32	43
20.47	1.91	63	33.47	3.89	56
30.7	4.35	0	57.71	18.65	0
29.94	3.89	14	54.73	10.15	14
28.43	3.30	28	53.41	6.61	29
27.58	2.79	41	52.9	5.99	42
25.98	2.36	63	49.21	6.05	56
47.04	6.16	0	56.94	17.99	0
44.59	5.52	22	54.07	10.57	14
43.11	4.48	35	52.64	7.42	29
41.1	3.84	49	49.83	6.48	42
39.43	3.23	63	47.57	6.29	56
51.47	7.72	0	52.62	18.67	0
48.45	5.64	22	51.58	11.88	14
46.77	4.82	35	50.44	7.14	29
44.07	4.76	49	48.67	6.44	42
43.64	4.18	63	45.95	6.00	56
60.24	8.46	0			
57.96	6.89	22			
57.19	6.33	35			
54.64	5.91	49			
53.03	5.70	63			

Table 2
Body composition and energy absolute content per unit wet weight of control Atlantic salmon (*Salmo salar*) under feed deprivation conditions.

Time of starvation (days)	Wet wt.[a,b] (g)/fish	Dry Matter wt. (g)/fish	Protein wt. (g)/fish	Lipid wt. (g)/fish	Energy (kcal)/fish	Ash wt. (g)/fish
0	45.64	13.20	7.87	3.80	85.60	1.00
22	45.54	12.67	7.88	3.30	81.16	1.07
35	43.12	11.62	7.39	2.95	73.00	1.08
49	44.66	12.02	7.57	2.92	75.80	1.13
63	44.08	11.39	7.38	2.67	69.54	1.14
0	54.20	15.60	9.63	4.30	100.92	1.20
22	46.14	12.52	7.98	3.14	79.07	1.13
35	49.04	13.52	8.32	3.68	85.19	1.22
49	42.06	11.14	7.10	2.73	69.41	1.08
63	45.44	11.77	7.43	2.76	72.45	1.19
0	50.98	14.70	8.82	4.60	95.82	1.10
22	59.38	16.88	10.25	4.41	108.93	1.40
35	55.96	15.57	9.52	4.22	99.07	1.35
49	54.76	15.26	9.74	3.52	95.67	1.42
63	58.06	15.58	10.34	3.02	98.38	1.45
0	29.90	8.64	5.09	2.70	57.38	0.61
14	25.82	7.39	4.33	2.15	48.00	0.58
28	25.02	6.96	4.20	1.99	45.26	0.57
41	21.22	5.57	3.41	1.50	35.69	0.48
63	19.64	4.97	3.21	1.27	30.15	0.48
0	26.06	7.49	4.48	2.27	49.62	0.51
14	25.18	7.12	4.25	2.07	47.18	0.51
28	20.62	5.60	3.38	1.70	36.18	0.46
41	24.40	6.63	3.86	1.96	42.97	0.55
63	19.12	4.61	2.82	1.24	28.58	0.43
0	6.56	1.68	1.03	0.44	10.64	0.14
14	6.40	1.59	1.03	0.31	9.85	0.16
28	5.80	1.39	0.88	0.26	8.52	0.15
42	4.84	1.09	0.69	0.18	6.59	0.12
50	5.40	1.25	0.78	0.24	7.51	0.16
0	6.18	1.61	0.99	0.40	10.25	0.14
14	5.80	1.44	0.88	0.33	8.95	0.14
28	5.60	1.31	0.82	0.25	8.05	0.14
42	5.66	1.31	0.82	0.25	7.90	0.15

[a] Wt represents the fish's wet body weight
[b] Initial body weight is at time of starvation = 0

Table 2 con't:

Time of starvation (days)	Wet wt. (g)/fish	Dry Matter wt. (g)/fish	Protein wt. (g)/fish	Lipid wt. (g)/fish	Energy . (kcal)/fish	Ash wt. (g)/fish
50	6.18	1.50	0.92	0.29	9.12	0.17
0	7.88	2.10	1.31	0.51	13.45	0.18
14	6.34	1.60	0.99	0.32	9.92	0.16
28	5.34	1.31	0.77	0.30	8.18	0.14
42	6.16	1.45	0.89	0.33	8.73	0.16
50	5.46	1.25	0.78	0.22	7.61	0.14
0	10.44	2.88	1.78	0.81	18.74	0.21
14	12.30	3.25	1.95	0.77	20.99	0.27
28	10.92	2.88	1.84	0.69	18.54	0.25
43	8.90	2.24	1.35	0.53	14.17	0.22
56	9.16	2.30	1.38	0.51	14.36	0.24
0	9.78	2.64	1.61	0.68	16.98	0.21
14	10.26	2.72	1.65	0.72	17.52	0.23
28	11.18	2.93	1.77	0.76	18.75	0.26
43	8.22	2.05	1.23	0.36	12.65	0.22
56	9.22	2.31	1.44	0.53	14.32	0.25
0	11.44	3.14	1.82	0.84	20.35	0.24
14	10.46	2.78	1.64	0.81	17.76	0.24
28	8.82	2.26	1.38	0.48	14.28	0.21
43	8.64	2.15	1.30	0.48	13.22	0.23
56	7.52	1.82	1.12	0.33	10.99	0.21

Table 3
Body composition and energy absolute content per unit wet weight of growth enhanced transgenic Atlantic salmon (*Salmo salar*) under feed deprivation conditions.

Time of starvation (days)	Wet wt.[a,b] (g)/fish	Dry Matter wt. (g)/fish	Protein wt. (g)/fish	Lipid wt. (g)/fish	Energy (kcal)/fish	Ash wt. (g)/fish
0	15.04	3.30	2.27	0.61	20.19	0.25
14	9.84	2.04	1.57	0.25	11.66	0.20
28	12.32	2.38	1.85	0.26	13.35	0.25
42	12.14	2.32	1.82	0.20	12.83	0.25
50	10.36	1.90	1.59	0.13	9.94	0.23
0	12.94	2.88	1.97	0.56	17.03	0.22
14	13.16	2.83	2.13	0.41	16.56	0.26
28	12.66	2.54	1.93	0.30	14.22	0.26
42	11.70	2.15	1.74	0.14	11.53	0.25
50	10.48	1.86	1.56	0.10	8.56	0.23
0	14.02	3.15	2.13	0.59	18.84	0.24
14	11.56	2.44	1.83	0.32	14.05	0.23
28	12.34	2.39	1.86	0.25	13.30	0.25
42	10.92	2.00	1.61	0.16	10.87	0.22
50	11.68	2.05	1.73	0.10	10.17	0.26
0	23.20	5.36	3.60	1.08	32.30	0.36
14	21.42	4.70	3.33	0.82	27.59	0.36
28	21.30	4.34	3.28	0.61	24.44	0.40
42	19.98	3.68	3.03	0.28	19.77	0.39
57	18.74	3.36	2.79	0.22	17.81	0.40
59	20.30	3.71	3.07	0.26	19.62	0.42
0	23.98	5.44	3.67	1.15	33.66	0.35
14	24.62	5.38	3.80	0.98	32.07	0.42
28	19.32	3.78	2.96	0.43	21.08	0.35
42	20.54	3.83	3.07	0.38	21.15	0.39
57	16.90	2.84	2.41	0.18	14.75	0.34
59	19.90	3.52	2.98	0.16	18.22	0.40
0	26.08	6.18	4.12	1.38	37.80	0.47
14	27.40	5.98	4.33	1.08	35.46	0.50
28	22.78	4.69	3.53	0.60	27.17	0.43
42	21.88	4.12	3.31	0.28	22.28	0.46
57	21.58	4.00	3.36	0.22	20.90	0.47
59	22.50	4.06	3.41	0.26	21.20	0.48
0	43.00	9.68	6.30	2.23	60.05	0.55

[a] Wt represents the fish's wet body weight
[b] Initial body weight is at time of starvation = 0

Table 3 con't:

Time of starvation (days)	Wet wt. (g)/fish	Dry Matter wt. (g)/fish	Protein wt. (g)/fish	Lipid wt. (g)/fish	Energy (kcal)/fish	Ash wt. (g)/fish
14	40.90	9.25	6.51	1.94	56.56	0.69
28	31.70	6.58	4.79	1.08	38.81	0.58
43	32.29	6.04	4.84	0.69	33.77	0.57
56	31.74	5.57	4.84	0.25	29.05	0.61
0	40.78	9.49	6.33	2.19	59.17	0.60
14	35.10	7.60	5.51	1.35	45.77	0.64
28	35.82	7.24	5.54	0.98	41.94	0.65
43	40.04	8.05	6.10	1.08	46.27	0.77
56	31.46	5.72	4.75	0.40	30.07	0.64
0	40.12	9.50	6.21	2.07	63.13	0.56
14	37.06	8.10	5.89	1.42	48.86	0.63
28	30.64	6.05	4.65	0.86	34.80	0.55
43	34.47	6.45	5.17	0.58	35.92	0.63
56	29.46	5.09	4.41	0.24	26.05	0.57
0	52.74	12.25	8.16	2.41	77.53	0.73
29	46.30	9.42	6.87	1.50	55.03	0.79
42	54.34	10.92	8.20	1.43	62.38	0.97
56	44.56	8.19	6.69	0.57	43.88	0.86
0	50.68	12.01	8.52	2.60	75.89	0.75
29	54.36	10.97	8.42	1.45	63.83	0.93
42	47.32	9.06	7.20	0.91	50.73	0.83
56	50.76	9.81	8.08	0.91	53.89	0.95
0	48.74	11.31	7.71	2.50	71.01	0.68
29	46.10	9.10	7.05	1.09	51.66	0.90
42	47.92	9.33	7.53	0.97	52.44	0.86
56	44.18	7.87	6.79	0.48	42.65	0.80

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