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Effect of food deprivation on oxygen consumption and body composition of growth-enhanced transgenic Atlantic salmon (*Salmo salar*)

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Abstract

The influence of food deprivation on the rate of oxygen consumption and the rate of mobilization/utilization of energy reserves in F_2 generation growth-enhanced transgenic Atlantic salmon were compared relative to their non-transgenic counterparts, over a pre-smolt weight interval of 8 to 55 g.

Throughout most of the 8 weeks of food deprivation, transgenic fish exhibited a greater rate of oxygen consumption compared to control salmon, but also exhibited a more rapid decline in oxygen consumption as starvation progressed. Consequently, depending on initial weight and length of food 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.

Although transgenic fish demonstrated the ability to reduce their metabolic rate during starvation, as also observed in the non-genetically modified control salmon, their persistence in maintaining a higher metabolic rate, combined with their lower initial endogenous energy reserves, suggests that the likelihood of growth-enhanced transgenic salmon achieving maximum growth or even surviving outside intensive culture conditions may be lower than that of non-transgenic salmon.

Keywords: Transgenic; Atlantic salmon; Oxygen consumption; Body composition; Starvation; Growth hormone

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1. Introduction

In addition to the regulatory requirements relating to food and health issues, a number of environmental concerns must be addressed before transgenic technology can enter commercial production. Although commercial implementation of this technology will likely employ reproductively incapable fish (female triploids), there remains some concern that such fish will (should they escape) pose a threat to natural populations through competition for food, space and/or perhaps by becoming direct predators. A more basic understanding of the bioenergetics associated with life-history related events, such as food deprivation, of growth-enhanced transgenic fish is required to better assess the relative fitness of such fish outside of an intensive culture system.

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. 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 impacts is in part dependent upon the fitness of transgenic individuals to cope with changes in food abundance. 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 larger daily feed intake (Cook, 1999; Cook et al., 2000a). Abrahams and Sutterlin (1999, 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. Transgenic Atlantic salmon have also been found to exhibit higher metabolic rates than controls under simulated aquaculture conditions (Stevens et al., 1998). The following experiment employing extended periods of food deprivation was conducted to determine if the higher metabolic rates in feeding and growing transgenic fish (Stevens et al., 1998; Cook, 1999), relative to control fish, would persist in the absence of food and result in a more rapid depletion of body energy reserves resulting in tissue breakdown and eventually death. Details on the growing conditions of the experimental fish prior to onset of food deprivation can be found in Cook et al. (2000a).

2. Materials and methods

2.1. Experimental fish

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

In fall 1989, the growth hormone (GH) transgene was microinjected (approximately 10^6 copies per egg) through the micropyle into the cytoplasm of fertilized, non-water-

activated salmon eggs (Shears et al., 1992). Milt from one of the fast growing transgenic males arising from the injected eggs (P_1 — Parental generation), which sexually matured in the fall of 1991, was crossed with a non-transgenic female. A fast growing, transgenic female (F_1) resulting from this mating was crossed with a non-transgenic male in the fall of 1996 resulting in the F_2 transgenic fish used in the present study. Also in the fall of 1996, pooled non-transgenic milt and eggs from the same Saint John River stock were used to generate non-transgenic control fish.

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

In 1996 the above progeny resulting from the cross between a transgenic female (F_1) and a non-transgenic male exhibited a bimodal size distribution at the fingerling stage in June, a phenomenon not usually seen until the first autumn of growth (Thorpe, 1977; Thorpe et al., 1980). Consequently, the two modes could be separated into two groups based on fork length above and below 8.0 cm, with 50% of total population in each mode, which is typical of Mendelian segregation of an allelic insert on a single chromosome. This separation was later confirmed by the exclusive presence of the transgene in the upper modal as revealed using polymerase chain reaction. The transgenic fish used in the present experiment were taken from the upper modal group from the 1996 spawning.

Well water was used at all stages of the experiment with properties as follows: hardness as $CaCO_3$ was 150 mg/l, pH 7.6, and salinity 4‰; water temperature was maintained at 12.6 ± 0.03°C (S.E.M.) and lighting within the hatchery mimicked the natural photoperiod.

2.2. Protocol

The oxygen consumption and body composition of food-deprived juvenile transgenic and control salmon, at weight increments over a weight range of approximately 8–55 g, were evaluated using the following methods. Six hundred and sixty transgenic salmon, average weight 9.42 ± 0.09 g, were randomly distributed to 12 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 12 additional tanks for a total of 55 fish per tank. The fish were acclimated for 3 weeks in the experimental tanks. Fish were fed to satiation three times per day on a commercial feed of the following analyzed (on a dry matter basis) composition: 92.40% dry matter, 55.69% protein, 18.57% lipid and 8.17% ash. Mean weights at the start of the experiment were 13.72 ± 0.21 and 6.98 ± 0.07 g for transgenic and control fish, respectively. Fish in three transgenic and three control tanks were fasted for 24 h prior to the first oxygen consumption measurement. A non-invasive protocol was developed whereby the fish rearing tanks were temporarily converted to metabolic respiration chambers.

2.3. Respirometer design

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

At the start of the experiment and every 2 weeks thereafter, the rearing tanks were converted to flow-through respiration chambers and the oxygen consumption of the contained fish were measured. In each respirometer, a rubber stopper was placed in the external standpipe, to cause the water level to rise above the tank upper rim. A foam gasket on the rim of the tank allowed for the Plexiglas cover to be sealed airtight. Clamps were 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. Water flow rates to each individual tank were individually adjusted to facilitate a decline in oxygen concentration (due to fish respiration) to approximately 60% water saturation, a level well above the critical concentration limiting oxygen uptake (Stevens et al., 1998). Oxygen levels were measured using an Oxyguard Handy Mark 4 oxygen sensor (Point Four Systems, Port Moody, British Columbia, Canada). The entire process of converting a growth tank into a respiration chamber generally took less than 5 min with minimal stress to the fish. Each respirometer contained a small submersible pump to maintain a slow circular current. After each tank was sealed, an acclimation period of approximately 24 h commenced before the first oxygen consumption measurements (Brett and Zala, 1975). Water flow rates to each tank and dissolved oxygen concentrations (DO) in the inflow and outflow water were measured every 4 h over a 24-h period and the means were used to calculate the rate of oxygen consumption. The respiration tanks were unsealed after the last oxygen measurements were taken and the fish biomass measured.

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

$$V_{O_2} = \frac{(\Delta[O_2] \times Vw)_{\text{fishtank}} - (\text{BOD} \times Vw)_{\text{blanktank}}}{N}$$

where V_{O_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), $[O_2]_{\text{inflow}}$ and the outflowing drain water, $[O_2]_{\text{outflow}}$, Vw is the flow rate (l/h), N is the number of fish in the tank, and BOD is the biological oxygen demand (mg O_2/l) of a tank without fish.

To control 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 oxygen consumption rate by the 'blank' tank was subtracted from the calculated fish oxygen consumption to obtain a true representation of the fish's metabolism.

As fasting progressed, oxygen consumption rates were measured every 2 weeks until the fish lost approximately 15% of their initial wet body weight. Subsequent to each oxygen measurement, subsamples of five fish per tank were euthanized for analysis of body composition and gross energy content.

The fish remaining in the nine tanks of transgenic and control fish 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 (Cook, 1999), the time at which control fish of comparable size to transgenic fish were deprived of food was considerably delayed. This procedure continued until the oxygen consumption and body composition of fish in the 12 tanks of transgenics and the 12 tanks of controls were being monitored under conditions of food deprivation.

2.4. Fish carcass preparation and chemical analysis

Frozen whole fish were autoclaved for 20 min at 120°C, homogenized with a known volume of distilled water, lyophilized, then equilibrated to room humidity, weighed, and further homogenized to a fine powder. Samples were analyzed for dry matter, protein and ash using standard methods (Association of Official Analytical Chemists (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.

A Student's *t*-test was used to reveal the significance of 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 demonstrated by multiple regression analysis, using 95% as the critical level for significance. A test for common slope was used to compare coefficients in regression equations for transgenic fish and control fish.

3. Results

3.1. Oxygen consumption

The relationship between body weight and time of starvation for transgenic and control fish is shown in Table 1. The oxygen consumption (mg O_2/weight of fish) of transgenic and control fish, in relation to initial body weight and duration of starvation (Fig. 1), was best described by multiple regression with initial body weight and starvation time as independent variables (Table 2). Initial weight represents the weight of the fish at zero time of starvation. The rates of oxygen consumption of both transgenic and control fish declined as starvation progressed, 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). Transgenic fish had significantly higher rates of oxygen consumption than control fish (of comparable body weights) initially (zero time starvation) and

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

throughout most of the period of food deprivation. However, the slopes of oxygen consumption in relation to initial body weight and time of starvation (Fig. 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.

3.2. Body composition

The body composition and energy content of transgenic and control fish, in relation to initial body weight and duration of starvation (Figs. 2–6), was best described by multiple regression with initial wet body weight and starvation time as independent variables (Table 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 control fish (Figs. 2–6). With the exception of ash content, all other body components in food-deprived fish decreased to significantly ($P < 0.05$) lower levels than could be expected in fed fish (zero time food deprivation) of the same body weight. 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 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 3).

4. Discussion

4.1. Oxygen consumption

As in the fed state (Cook et al., 2000b), food-deprived GH transgenic salmon maintained higher rates of oxygen consumption than control fish. Both transgenic and non-genetically modified Atlantic salmon exhibited depression in average oxygen consumption rate when subjected to starvation conditions. As the oxygen consumption of these fish is dependent on body weight, it is not surprising that a decrease in body weight (due to starvation) would result in a concomitant reduction in the rate of oxygen

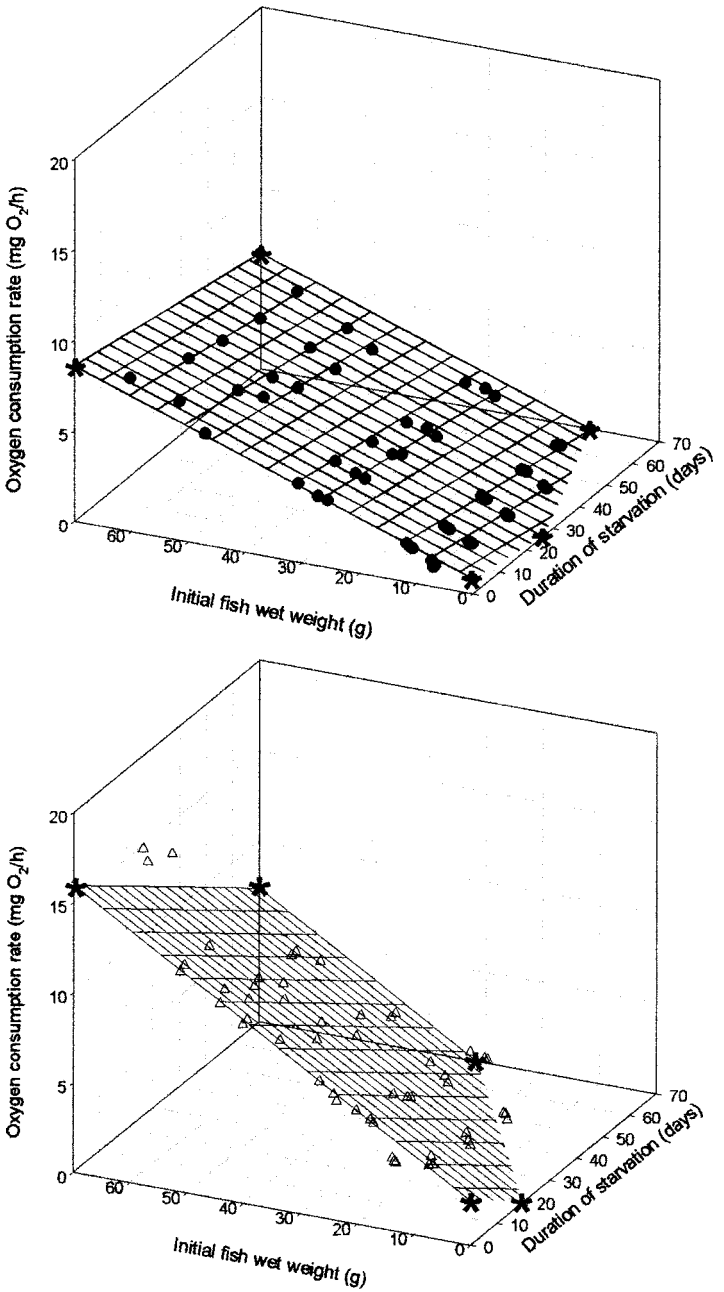

Fig. 1. Oxygen consumption rate (mg O_2 /h) in relation to initial body weight (g)[day = 0] and duration of starvation (days) of transgenic Atlantic salmon (open triangles) and controls (solid circles). Data is presented with fitted multiple regression (mesh plane) and the symbol '*' depicting axis interception by regression plane.

Table 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 where: $Y = b_0 + b_1 \times \text{BW} + b_2 \times \text{Time}$ and 'b_0', 'b_1' and 'b_2' are regression coefficients

Experimental group	b_0	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. However, in both experimental groups, the subsequent level of oxygen consumption was lower than observed in fish of comparable body weight measured in a post-absorptive state. 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. The elevated oxygen consumption observed in the food-deprived transgenic salmon relative to control fish is probably not entirely due to their greater activity as Stevens et al. (1998) also recorded higher metabolic rates under conditions of controlled swimming.

A commonly observed response by fish during periods of food shortage is a metabolic depression, as reflected by lower oxygen consumption rates. 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 (30–60 g) (Jobling, 1980) and African catfish (*Clarias lazera*) (1–97 g) (Hogendoorn, 1983). Determining the degree to which this response is adaptational or obligatory involves consideration of a number of factors. 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 a homoeostatic mechanism involving a ''set point'' that assesses stored energy reserves, the likely ''historic duration'' of the food shortage, and determines what energy might be allocated to activity. Because transgenic fish had an initial lower body energy content than control fish and a higher oxygen consumption rate, this energy ''set point'' would have been reached first by the transgenic fish (irrespective of the influence of the transgene) and may explain why their rate of oxygen consumption declined more rapidly than that of control fish during the period of food deprivation.

4.2. Body composition

During food deprivation the two major body constituents, lipid and protein, have differential rates of mobilization with lipid initially being exploited as the main energy

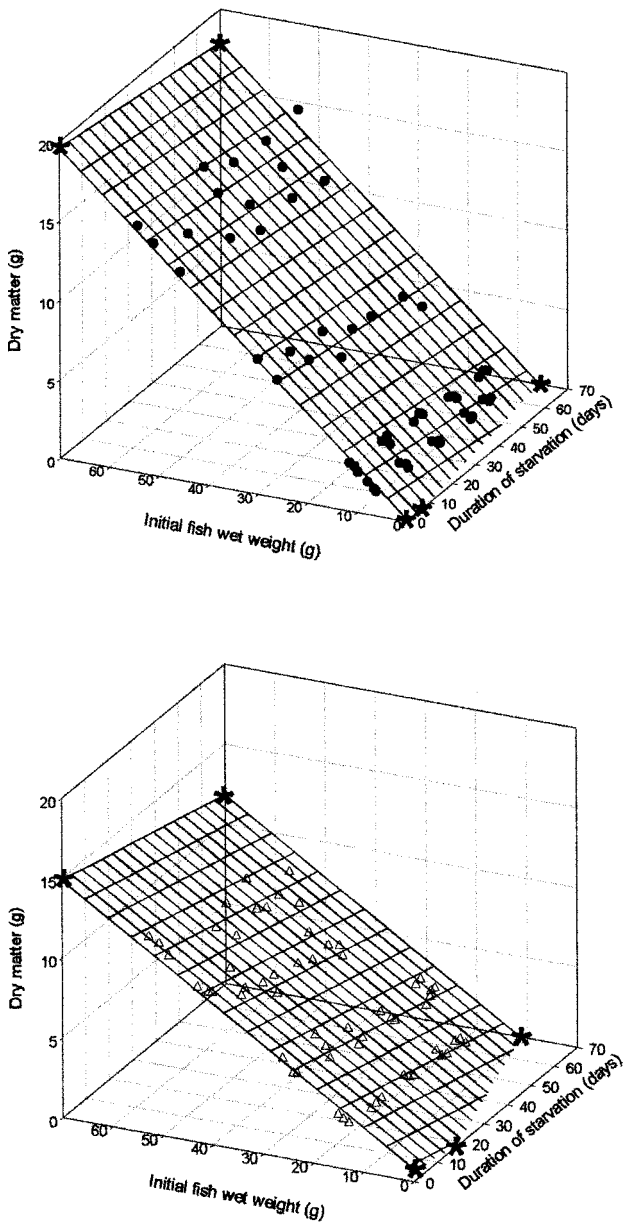

Fig. 2. Dry matter content (absolute weight in g) in relation to initial body weight (g)[day = 0] and duration of starvation (days) of transgenic Atlantic salmon (open triangles) and controls (solid circles). Data is presented with fitted multiple regression (mesh plane) and the symbol '*' depicting axis interception by regression plane.

source. Because of the greater rate of oxygen consumption rate of transgenic fish relative to control fish, their fat and protein was mobilized more rapidly in response to

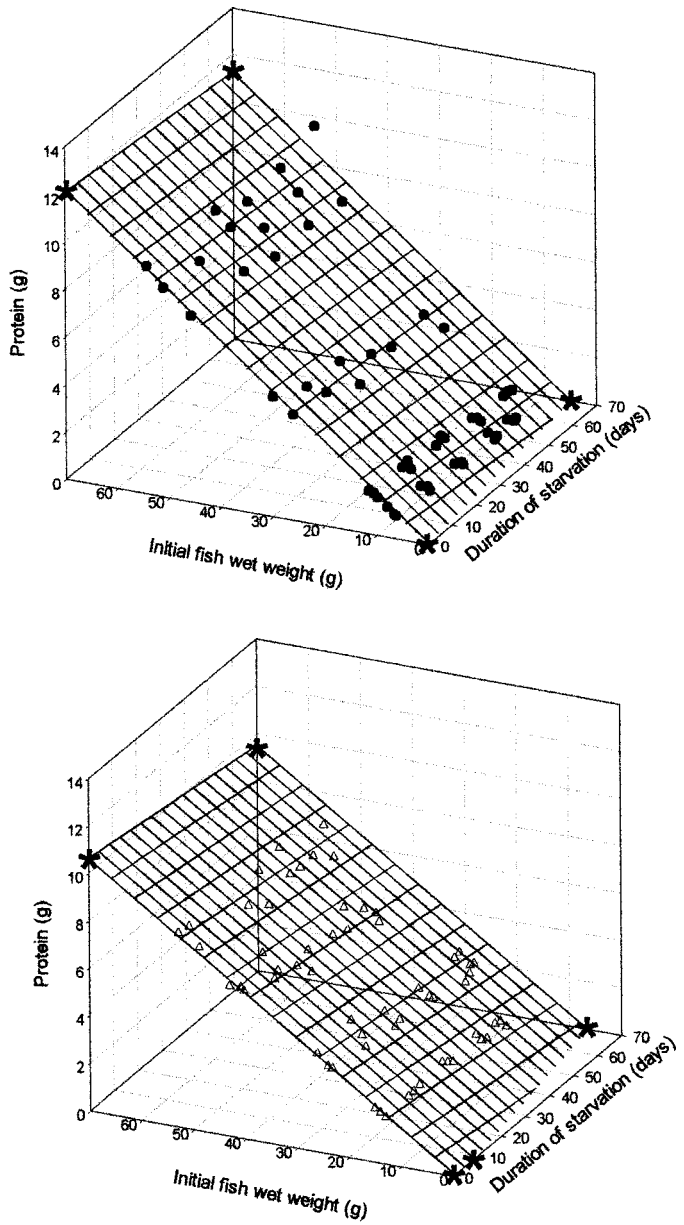

Fig. 3. Protein content (absolute weight in g) in relation to initial body weight (g)[day = 0] and duration of starvation (days) of transgenic Atlantic salmon (open triangles) and controls (solid circles). Data is presented with fitted multiple regression (mesh plane) and the symbol '*' depicting axis interception by regression plane.

food deprivation. Both transgenic and control fish depleted body lipid more rapidly than protein. For example, a 55-g transgenic fish, starved for 60 days, lost 38% more lipid

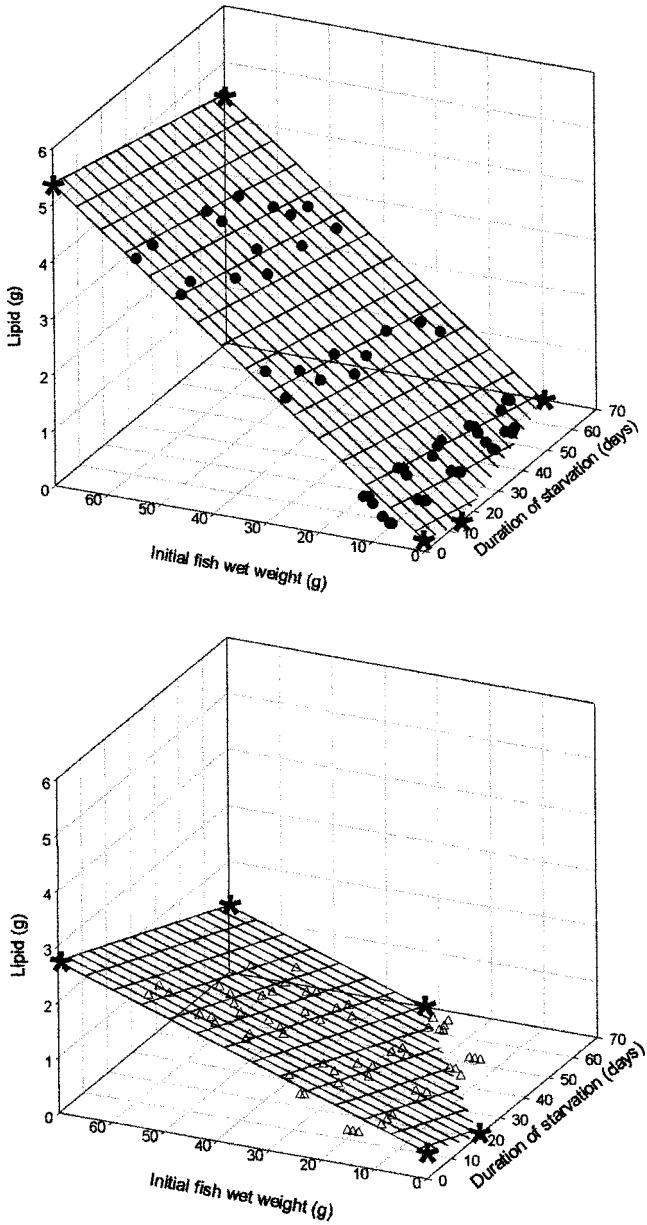

Fig. 4. Lipid content (absolute weight in g) in relation to initial body weight (g)[day = 0] and duration of starvation (days) of transgenic Atlantic salmon (open triangles) and controls (solid circles). Data is presented with fitted multiple regression (mesh plane) and the symbol '*' depicting axis interception by regression plane.

(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 experi-

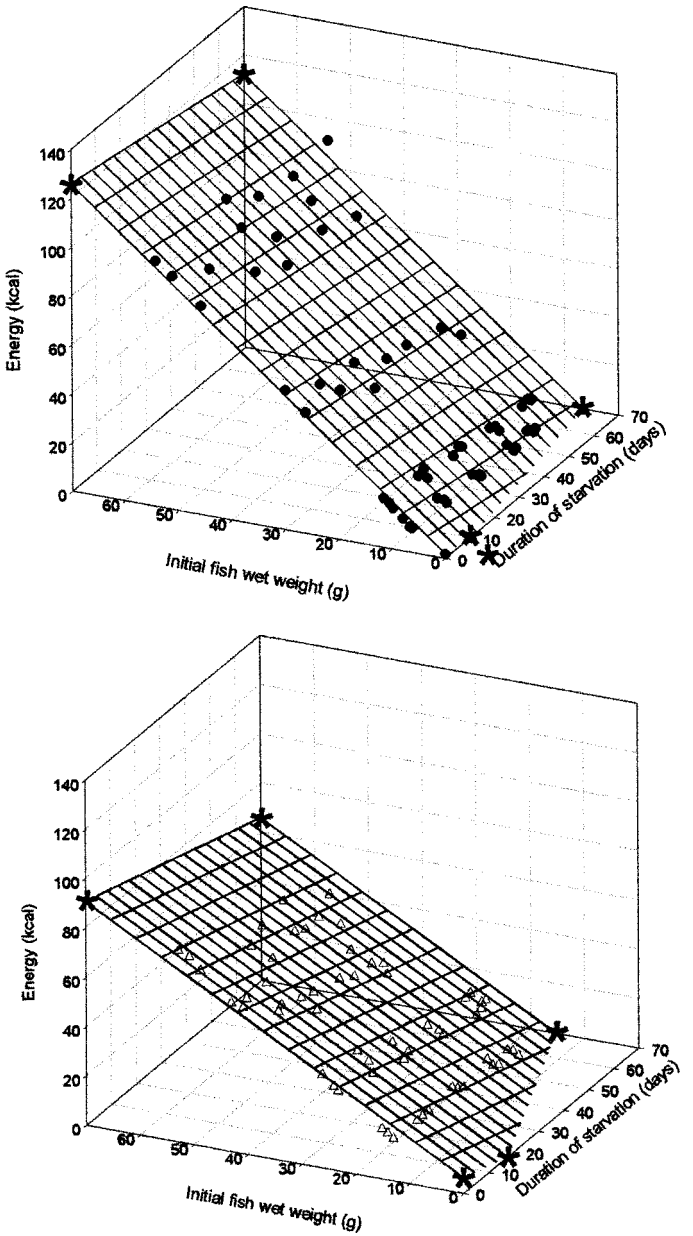

Fig. 5. Energy content (kcal) in relation to initial body weight (g)[day = 0] and duration of starvation (days) of transgenic Atlantic salmon (open triangles) and controls (solid circles). Data is presented with fitted multiple regression (mesh plane) and the symbol '*' depicting axis interception by regression plane.

mental 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

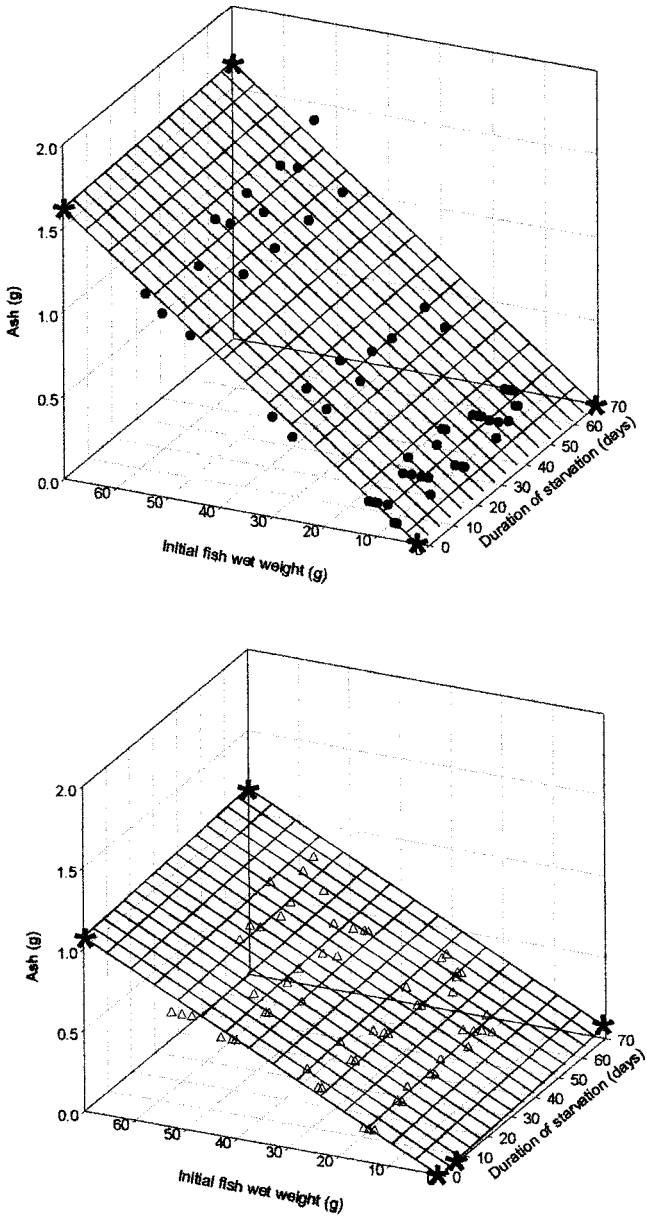

Fig. 6. Ash content (absolute weight in g) in relation to initial body weight (g)[day = 0] and duration of starvation (days) of transgenic Atlantic salmon (open triangles) and controls (solid circles). Data is presented with fitted multiple regression (mesh plane) and the symbol '*' depicting axis interception by regression plane.

more energy from lipids than protein, respectively. Although protein was depleted, it is theoretically possible that it was not used strictly for energy, in that some protein could

Table 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 where: $Y = b_0 + b_1 \times \mathrm{BW} + b_2 \times \mathrm{Time}$ 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 (kcal)/fish	Control	2.043	1.771	−0.195	0.95
	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 ($P < 0.05$).

have been converted to carbohydrate through gluconeogenesis (Lauff and Wood, 1996) and used as an energy source particularly by the brain.

Jobling (1980) reported that body lipid was the major storage reserve utilized by plaice 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. It is therefore generally accepted that body protein is conserved at the expense of stored lipid during the initial phase of food deprivation and, while some lipid is required to maintain the structure and function of cellular membranes, it is essentially an energy reserve (Weatherley and Gill, 1987; Shearer, 1994). Fish protein, however, is predominantly sequestered in muscle tissue and must be reserved in salmon to support extensive feeding and reproductive migrations. Bioenergetically, it is more economical to catabolize fat in that 1 g will release approximately 9.5 kcal of energy compared to only 4.5 kcal from protein. Because the transgenic salmon in this experiment had lower lipid reserves than the control salmon, the transition from lipid to protein as the major energy source would occur at an earlier stage than in control fish having more abundant lipid reserves. Consequently, the early onset of the catabolism of muscle protein by transgenic fish could be to their physiological and ecological disadvantage relative to non-transgenic fish.

A seemingly unlikely effect of starvation is an increase in plasma GH levels in fish (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). However, the actual endocrine pathways mediating this response in growth-en-

hanced transgenic fish remain unclear. Although Hew et al. (1995) demonstrated that while novel mRNA is being transcribed in a number of non-pituitary tissues in transgenic fish, 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), however, found GH levels in growing transgenic coho salmon 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 or greater rates of GH production compared to control fish before being subjected to food deprivation, persistently high GH levels during deprivation could account for their higher rate of lipid catabolism.

The absolute ash content significantly increased with time of starvation in the 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 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) and non-representative fish samples likely account for this discrepancy.

During low winter temperatures, when food is scarce, and when transgenic salmon are also likely to have elevated metabolic rates compared to non-genetically modified salmon, starvation might be so severe as to effect the probability of survival. The particular transgene construct used in the current study employs an antifreeze promoter gene that normally drives the expression of an antifreeze gene to produce antifreeze peptides which aid fish in resisting low water temperatures (Fletcher et al., 1985). Although entirely speculative, it is possible the antifreeze promoter may be responsible for producing GH in transgenic fish, particularly during the winter, resulting in a disproportionate influence (altered temperature coefficient) on growth and metabolism.

Under hatchery conditions where optimum to maximum rations are provided, there is little need for fish to mobilize endogenous energy reserves. Although fed to satiation three times per day prior to the onset of food deprivation, the transgenic fish had lower fat reserves compared to the control fish, and in a natural environment, transgenic salmon would likely have had even lower lipid stores than those observed under culture conditions. Throughout the summer, when water temperatures are at their highest, juvenile salmon in fresh water territorially compete for forage consisting of 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, and it is possible that GH transgenic fish may be unable to secure sufficient food to meet their metabolic requirements at such elevated temperatures. Additionally, Abrahams and Sutterlin (1999, in press) demonstrated that juvenile transgenic salmon are more active than regular salmon and, in an ''attempt to satisfy'' their enhanced appetite, are ''willing to assume'' risk (greater exposure to predators) in order to obtain food.

There have been a plethora of studies on possible negative impacts of genetic introgression or direct competition by selectively bred hatchery fish on wild populations. Based on the results of this study, transgenesis, combined with techniques of sterility induction, might provide aquaculturists with the means to circumvent the intermediary stages of potentially damaging fitness.

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 rate of oxygen consumption during starvation as observed in the non-transgenic salmon, their persistence in maintaining a higher metabolic and carcass depletion 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 than that of non-genetically modified salmon.

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