

# Growth hormone transgenic salmon pay for growth potential with increased predation mortality

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Recd 26.11.03; Accptd 23.02.04; Published online 07.04.04

**Recent advances in gene technology have been applied to create fast-growing transgenic fish, which are of great commercial interest owing to their potential to shorten production cycles and increase food production. However, there is growing concern and speculation over the impact that escaped growth hormone (GH)-transgenic fish may have on the natural environment. To predict these risks it is crucial to obtain empirical data on the relative fitness of transgenic and non-transgenic fish under nature-like conditions. Using landscaped stream aquaria with live food and predators, we show that the predation mortality of newly hatched GH-transgenic coho salmon fry (*Oncorhynchus kisutch*) is much higher than in non-transgenic conspecifics, and that this difference is amplified when food abundance decreases. The growth rate of transgenic and non-transgenic fish is similar at high food levels, whereas transgenic fish grow more slowly than non-transgenic fish when food abundance is reduced. Our results suggest that the fitness of young GH-transgenic coho salmon in the wild will be determined by both predation pressure and food availability.**

**Keywords:** trade-off; coho salmon; *Oncorhynchus kisutch*; semi-natural environment; transgenic; growth hormone

## 1. INTRODUCTION

The possibility to enhance the growth of fish by transgenesis of growth factor genes may allow for the production of marketable products in shorter periods of time, and with lower production costs. Not surprisingly, companies within the aquaculture industry are seeking permission to farm growth hormone (GH)-transgenic salmon (Stokstad 2002). However, millions of farmed salmon escape every year from aquaculture seapens (McGinnity *et al.* 2003), indicating that transgenic salmon grown in conventional facilities would probably escape into the wild. Although transgenic fish could be made sterile, large numbers of adults could still have adverse effects on the environment, and sterilization methods are not yet completely effective (Devlin & Donaldson 1992). It is therefore important to evaluate the potential impacts that GH-transgenic fish

may have on the ecosystem before any farming of these strains is allowed (Reichhardt 2000).

If the increased food conversion and growth potential (Devlin *et al.* 1994; Cook *et al.* 2000) of GH-transgenic salmon were realized in the wild, their large size at a given age could increase their competitive ability (Devlin *et al.* 1999), reduce their susceptibility to predators (Juanes *et al.* 2002) and increase their reproductive potential (Fleming 1996). Under such conditions and in the absence of counteractive selection, transgenic individuals could soon spread and replace non-transgenic fish in populations. However, the fact that many animals, including fish (Ali *et al.* 2003), are capable of catch-up growth suggests that growth rates in nature are normally kept below the physiological maximum. This indicates that the fitness advantage of rapid growth is balanced by costs (Arendt 1997).

In studies using laboratory apparatus, GH-transgenic salmon are more active (Stevens *et al.* 1998) and appear more risk-prone (Abrahams & Sutterlin 1999; Sundström *et al.* 2003) than non-transgenic fish, which could potentially increase their mortality from predation in the wild. In contrast to their wild counterparts, genetically modified strains have not experienced selection in the wild and should therefore be less adapted to natural conditions (Knibb 1997). However, there are numerous examples where exotic species have invaded and replaced native species without previous selection under local conditions (Stockwell *et al.* 2003). Theoretically, the introduction of novel genes may carry transgenic organisms to higher fitness peaks that wild organisms are unable to reach through natural selection owing to significant fitness valleys between adjacent peaks.

So far, to our knowledge, transgenic salmon have been studied only under artificial laboratory conditions and it is difficult to extrapolate previous results to predict their performance in the wild. It is therefore essential to obtain data on the fitness of these GH-transgenic and non-transgenic fish under more natural conditions. Fitness estimates partition into two main components, survival and reproductive output, with many physiological and behavioural factors influencing each of these or both. Here, we examine, in near-natural environments, how variation in food abundance and predation risk affects the juvenile survival and growth of GH-transgenic coho salmon (*Oncorhynchus kisutch*) relative to non-transgenic conspecifics. By using first-feeding fry, we minimized pre-experimental effects of the hatchery environment (Sundström *et al.* 2003) and focused on a critical life-history stage when competition is intense and only 1–10% of the fry would be expected to survive the first month after emergence under natural conditions (Elliott 1994).

## 2. MATERIAL AND METHODS

The study was conducted between 23 March and 13 April 2003 at Fisheries and Oceans Canada's West Vancouver Laboratory (WVL). The facility has multiple containment screen systems and is specially designed to prevent the escape of genetically modified organisms into the natural environment. Non-transgenic fish were offspring from wild parents from the Chehalis River, BC, Canada, and were incubated at WVL. Transgenic fish were initially produced by microinjecting eggs from the same wild strain with the gene construct OnMTGH1 that drives overexpression of type I salmon GH (Devlin *et al.* 1994). Strains were subsequently crossed at each generation with wild Chehalis River fish to maintain a wild genetic background. Experimental transgenic fish used in this study were F<sub>3</sub> generation progeny derived from a cross of homozygous males of one founder line (M77) with the same Chehalis River wild females used

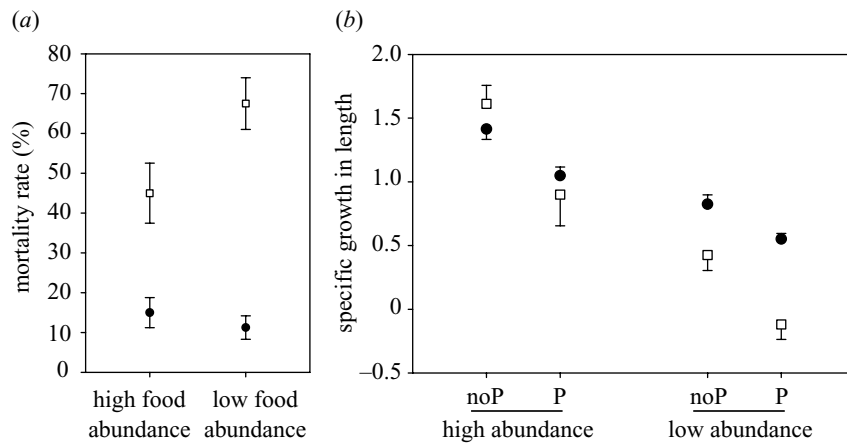


Figure 1. Mortality rates (a) and specific growth rate in length (b) of non-transgenic (filled circles) and GH-transgenic (open squares) coho salmon fry that were fed every day (high abundance) or every third day (low abundance) in the presence of a predator (P) or absence of a predator (noP). In (a) only mortality from tanks with predator (P) are shown.

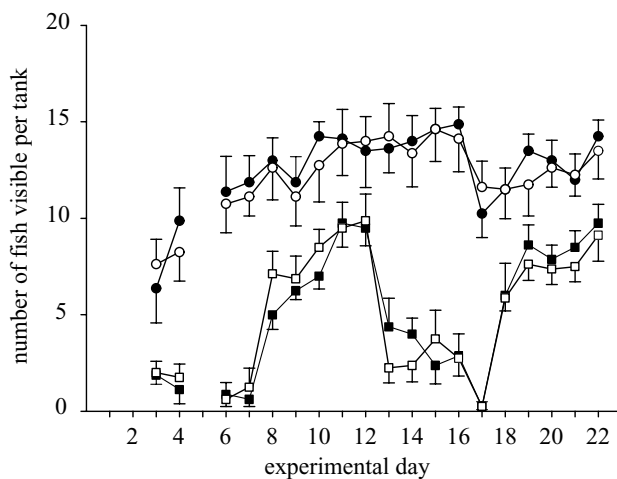


Figure 2. The average numbers of fish visible in experimental tanks. Circles indicate treatment without predator present and squares indicate treatment with predator present at days 3–6 and 13–17. Filled symbols indicate high food ration treatments (every day) and open symbols indicate low food ration treatments (every third day). The number of observed fish at days 9 and 17–21 are underestimated owing to poor visibility in the water.

above to produce non-transgenic offspring. This cross yields 100% transgenic offspring and allows the use of early developmental stages (first-feeding fry) that are of known genotype. Thus, the two genotypes analysed in these experiments were half-sibs, and differed essentially only by the presence or absence of the OnMTGH1 transgene from strain M77.

At day 1 of the experiment, 10 non-transgenic and 10 transgenic fry at the first-feeding stage were taken directly from hatching trays and measured, marked (alternating adipose fin clip on fish genotypes) and placed in each of 32 stream tanks (i.e. 640 fry in total). No effect of clipping was apparent among groups for either viability or growth effects. Non-transgenic fish ( $34.3 \pm 0.16$  (s.e.) mm) were slightly longer than transgenic fish at the start of the experiment ( $32.9 \pm 0.15$  mm; Student's *t*-test:  $t_{62} = 6.56$ ,  $p < 0.001$ ). The experimental stream tanks (70 cm  $\times$  60 cm  $\times$  50 cm) contained coarse gravel that is normally found in the spawning area of adult wild fish, and which provided numerous crevices where fry could hide. Three large rocks or piles of smaller rocks provided further hiding places. Water from nearby Cypress Creek was used in a flow-through system, with water flow maintained at 2–4 l min<sup>-1</sup>. Water temperature varied between 4.0 and 6.5 °C and light conditions followed the natural cycle (sunrise at about 06.00 and sunset about 19.00) supplemented with artificial light between 07.00 and 18.00.

Half of the fish were fed once daily on newly hatched live brine shrimp (*Artemia* sp.) at ca. 1% of fish biomass per tank (high abundance), whereas the other half were fed on the same amount every third day (low abundance). Shrimp would quickly spread throughout the tank and were available to be fed on for several hours post-feeding. At days 4 and 7, ca. 40 black benthic worms (*Tubifex* sp.) were fed evenly to each tank. These worms would remain in the gravel and could be fed on throughout the experiment. At days 10 and 16, five live small surface-drifting crickets (*Acheta domestica*: 2–3 mm) were given to each tank. Uneaten crickets were removed the next day. In half of the tanks a single live predator fish was introduced at day 2 and removed at day 7. A predator was again introduced into the same tanks at day 12 and removed at day 17. Predators were non-transgenic coho salmon juveniles of ca. 10 g with previous experience of feeding on salmon fry.

Every morning at 10.00 each tank was visually examined and the number of fish counted. At day 22, each tank was carefully emptied and all fish were captured. Fish were identified and measured before being returned to the restored tanks. Thereafter, post-experimentally, all fish were fed *ad libitum* three times a day for another 50 days in the absence of predators. The effects of feeding level and predation risk on specific growth in length were tested with a two-way factorial split-plot ANOVA. Tanks were plots, feeding level and predation risk were between-plot factors, and fish type was the within-plot factor (Quinn & Keough 2002). Mortality was evaluated in predatory tanks with a similar model but without predation risk as a between-plots factor.

### 3. RESULTS

In treatment groups without a predator, only one non-transgenic and two transgenic fish died out of a total of 320 animals (less than 1% mortality). In predator treatments, transgenic fry suffered higher mortality rates ( $56 \pm 5.6$  (s.e.)%) than non-transgenic fry ( $13 \pm 2.4$ %;  $F_{1,14} = 72.0$ ,  $p < 0.001$ ). This mortality difference was most pronounced at the low food abundance (figure 1a; type  $\times$  food interaction  $F_{1,14} = 6.7$ ,  $p = 0.022$ ). Stomach content analysis of predator fish after their removal confirmed that fry mortality was caused by predation. The number of predated fish was not correlated with the average initial length of the group (non-transgenic:  $r = -0.28$ ,  $n = 16$ ,  $p = 0.29$ ; transgenic:  $r = 0.104$ ,  $n = 16$ ,  $p = 0.7$ ), indicating that the higher predation on transgenic fish was not related to size effects.

Our visual observations confirmed that fry responded to the presence of the predators by hiding (figure 2) and spending less time foraging in open water. This helps to explain why the growth of surviving fry was lower in predator tanks for both non-transgenic and transgenic fish (figure 1b;  $F_{1,27} = 25.5$ ,  $p < 0.001$ ). The predator-induced

growth reduction tended to be more pronounced in transgenic fish (type  $\times$  predator interaction  $F_{1,27} = 3.1$ ,  $p = 0.092$ ) and they also grew less with low food abundance (type  $\times$  food interaction:  $F_{1,27} = 9.9$ ,  $p = 0.004$ ), whereas with high food abundance, growth in length was similar between transgenic and non-transgenic fish. After 50 days of post-experimental *ad libitum* feeding, transgenic fish ( $53.7 \pm 0.47$  (s.e.) mm) had significantly outgrown non-transgenic fish ( $50.8 \pm 0.53$  mm; paired  $t$ -test:  $t_{30} = 4.2$ ,  $p < 0.001$ ) confirming the higher growth potential of GH-transgenic fish under more hatchery-like growth conditions.

#### 4. DISCUSSION

Our results demonstrate that GH-transgenic salmon fry suffer increased predation under near-natural conditions, which is consistent with laboratory studies showing increased risk-taking behaviour in transgenic fish (Abrahams & Sutterlin 1999; Sundström *et al.* 2003; see also Dunham *et al.* 1999). In addition, transgenic fry were unable to realize their growth potential when prey abundance was low. Thus, they were apparently not able to take advantage of their enhanced growth capacity by hiding during periods of intense predation risk and then increase foraging and growth during periods of low risk. This suggests that the behavioural plasticity of transgenic fry is limited and that their elevated activity increased energetic expenditure as well as predator exposure. The results show that transgenic fry are most successful when food abundance is high and predators are absent, whereas the combined effect of low food abundance and high predation risk is especially detrimental to their fitness. In recent field experiments, Johnsson and colleagues found that GH-implanted wild brown trout (*Salmo trutta*) can grow faster than control trout in the wild without suffering increased mortality (Johnsson & Björnsson 2001), although the effect of GH-treatment is stronger in the hatchery where food is more abundant (Johnsson *et al.* 2000). These studies differ from the present study in that older life-history stages were examined, where predation rates may be significantly reduced relative to the vulnerable first-feeding fry stage (Fleming *et al.* 2000). In addition, GH implants may not completely mimic the effect of the GH transgene.

Our results indicate that the competitive success of GH-transgenic salmon fry in nature is reliant on food availability and predation pressure in the local environment. However, the long-term impact of GH-transgenic salmon on wild populations will not be determined solely by their performance during the fry stage, but will also depend on their total lifetime fitness. Understanding all of the variables influencing reproduction and viability is critical for risk assessment as disadvantages in one component can be offset by advantages in another (Muir & Howard 1999). In addition, large numbers of escaped fish may have negative effects on wild populations even if their relative fitness is low (Reichhardt 2000). Depending on the level of introgression of transgenic fish into natural populations and the relative level of other fitness parameters, reduced survival of transgenic animals could facilitate population extinctions, as have been previously modelled (Muir & Howard 2002). However, in the absence of such counteracting fitness effects, our results suggest that GH transgenes would be rapidly eliminated from wild populations.

#### Acknowledgements

C. A. Biagi assisted in setting up the experimental apparatus and rearing the fish. This work was supported by a grant to R.H.D. from the Canadian Biotechnology Strategy. L.F.S. was funded by Stiftelsen Lars Hiertas Minne, and J.I.J. was financed by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning.

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