

Population effects of growth hormone transgenic coho salmon depend on food availability and genotype by environment interactions

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Environmental risk assessment of genetically modified organisms requires determination of their fitness and invasiveness relative to conspecifics and other ecosystem members. Cultured growth hormone transgenic coho salmon (*Oncorhynchus kisutch*) have enhanced feeding capacity and growth, which can result in large enhancements in body size (>7-fold) relative to nontransgenic salmon, but in nature, the ability to compete for available food is a key factor determining survival fitness and invasiveness of a genotype. When transgenic and nontransgenic salmon were co-habitated and competed for different levels of food, transgenic salmon consistently outgrew nontransgenic fish and could affect the growth of nontransgenic cohorts except when food availability was high. When food abundance was low, dominant individuals emerged, invariably transgenic, that directed strong agonistic and cannibalistic behavior to cohorts and dominated the acquisition of limited food resources. When food availability was low, all groups containing transgenic salmon experienced population crashes or complete extinctions, whereas groups containing only nontransgenic salmon had good ($72.0 \pm 4.3\%$ SE) survival, and their population biomass continued to increase. Thus, effects of growth hormone transgenic salmon on experimental populations were primarily mediated by an interaction between food availability and population structure. These data, while indicative of forces which may act on natural populations, also underscore the importance of genotype by environment interactions in influencing risk assessment data for genetically modified organisms and suggest that, for species such as salmon which are derived from large complex ecosystems, considerable caution is warranted in applying data from individual studies.

The quantum change in phenotype associated with some growth-enhanced transgenic fishes (1–4) has prompted considerable scientific and public debate centering on potential ecological impacts, which could arise if such fish were used in aquaculture and accidentally escaped into natural ecosystems (5). One aspect of ecological risk assessment of genetically modified organisms requires evaluation of their fitness relative to nontransgenic individuals in populations (6–8). For growth-enhanced fish, potential fitness advantages have been observed such as shortened life cycles, increased feeding capability and dominance, and reduced times fish are at risky prey sizes vulnerable to predation (9–12). However, negative pleiotropic consequences have also been observed: growth hormone (GH) transgenic salmon are more willing to risk predation in pursuit of food needs, have morphological abnormalities, and have suppressed immune function (9, 13–16). Indeed, it is the force of such negative selective factors that has probably limited growth rates in the wild (17).

Growth enhancement in GH transgenic salmon is associated with increased intake of food resources (9, 10), but it is unknown whether such fish simply possess greater scope for growth as food availability allows, or whether they possess obligatorily elevated metabolism and behavior that demands a sustained high input of food to support. Food availability, which is influenced by short-term seasonal and long-term regime shifts in ecosystem productivity, is a key compo-

nent affecting survival fitness in nature, as not all animals within a population obtain equal access to food resources, particularly under situations of low food availability or high population densities (18–20). Thus, the issue of whether altered behavioral and physiological phenotypes of transgenic fish would allow them to compete effectively in environments with different food availabilities is critical. In such competitive situations, increased feeding motivation associated with GH transgenic fish (9, 10) is anticipated to increase the probability of accessing available food resources, resulting in size advantages and development of dominance status for transgenic salmon within cohorts (11). In nature, larger coho salmon fry are known to dominate smaller salmon and displace them downstream into less favorable habitat (20, 21). Indeed, such behavioral differences among organisms are recognized as critical factors determining the invasiveness of nonindigenous species (22, 23).

In the case of growth-enhanced transgenic salmon, experimental releases to nature are not desirable because such animals and their progeny cannot be reasonably recovered from the wild and could cause inadvertent ecological impacts, and sterility techniques are not yet completely effective (24). Consequently, assessments are performed in contained research laboratory facilities. To examine the competitive abilities of GH transgenic coho salmon, we have examined growth and survival fitness of fry in different population structures consisting of 100% transgenic salmon (TT), 100% nontransgenic salmon (NN), or an equal mixture of the two genotypes (TN). Competitive and noncompetitive environments were established among treatments by providing known food amounts, at (i) *N*-satiating levels (sufficient to satiate pure nontransgenic populations), (ii) high levels (exceeding nontransgenic fish requirements), or (iii) low levels (below that required to fully support the population). We have undertaken the experiments in simple laboratory environments to allow the variables to be manipulated precisely with the objective of assessing competitive interactions between transgenic and nontransgenic salmon fry and to examine the degree to which genotype by environment interactions may influence the reliability of data generated for risk assessments.

Methods

WT coho salmon (*Oncorhynchus kisutch*) used in the present experiments were derived from 10 single-pair fertilizations of salmon eggs and milt obtained from mature adults from the Chehalis River, British Columbia (the strain from which the transgenic salmon were developed and subsequently maintained) (25). Transgenic coho salmon used in the present experiments were hemizygous individuals produced by crossing homozygous GH transgenic fathers containing the OnMTGH1 gene construct (strain M77) to eggs from the same females used above to produce the WT salmon. Within genotypes, salmon for experiments were selected randomly from mixtures of these crosses, thus limiting

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Abbreviation: GH, growth hormone.

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genetic differences between them to the presence or absence of the GH transgene. The transgenic genotype of a subsample of siblings from the transgenic population was confirmed by PCR by using transgene-specific primers (1).

Salmon were weighed and length measured just before the first feeding stage (a time when yolk reserves have just been depleted) and used to establish populations containing 50 individuals. The three population structure types established were (i) pure transgenic populations containing two groups of transgenic salmon (designated TT), (ii) pure nontransgenic populations (NN) containing two groups of nontransgenic salmon, and (iii) mixed populations (TN) containing one group of each genotype. Each group contained 25 fish, and the two groups within each population were differentially marked by adipose fin clipping for subsequent nonlethal identification. No statistically significant effect of fin clipping on growth or survival was detected among groups in the experiments. Each of the three population structures (each containing two subgroups) was replicated twice within each of three environmental conditions where food availability was controlled. In high-ration environments, each population received a daily feed ration equivalent to 7.5% of the total fish body weight in the population, whereas in low-ration environments, populations received a ration of only 0.75%. This latter level is one which from previous observation creates a highly competitive situation for food resources, whereas the high-ration level was chosen as an amount that would be anticipated to limit or remove competition. In *N*-satiating envi-

ronments, populations received only an amount of food that was required to satiate pure nontransgenic populations, except during the first 4 weeks, where populations received a fixed ration of 3% per day because of the difficulty in determining the amount of food consumed at this larval stage. At each feeding time, pure nontransgenic populations were fed to satiation, determined by experienced personnel observing consumption of offered feed and fish behavior. Satiation was considered complete when feeding behavior ceased and three consecutively offered food pellets reached the bottom of the environment without being consumed. Subsequently, the same quantity of food consumed by pure nontransgenic populations was offered to the other environments being restricted to the nontransgenic satiating (*N*-satiating) level ration. The amount of food (percentage of body weight per day) required to satiate pure nontransgenic populations (*N*-satiating ration) declined with fish size as expected, and averaged, between replicates, 3.25%, 2.44%, 2.01%, 1.54%, and 1.41% at the initiation of weeks 4, 6, 8, 10, and 12, respectively (overall average = 2.13%). Fish in all groups were fed three times per day with commercial salmon diets (NutriFry, Skretting, Vancouver). This combination of genotypes, population structures, and ration levels consisted of a total of 900 salmon among 36 groups.

Fish were reared in circular 22.5-liter flow-through environments with a simple substrate consisting of textured dark nylon mesh. Well water flow was delivered at a rate of 1 liter min⁻¹ kg⁻¹ fish, delivered outside of the environment and allowed to

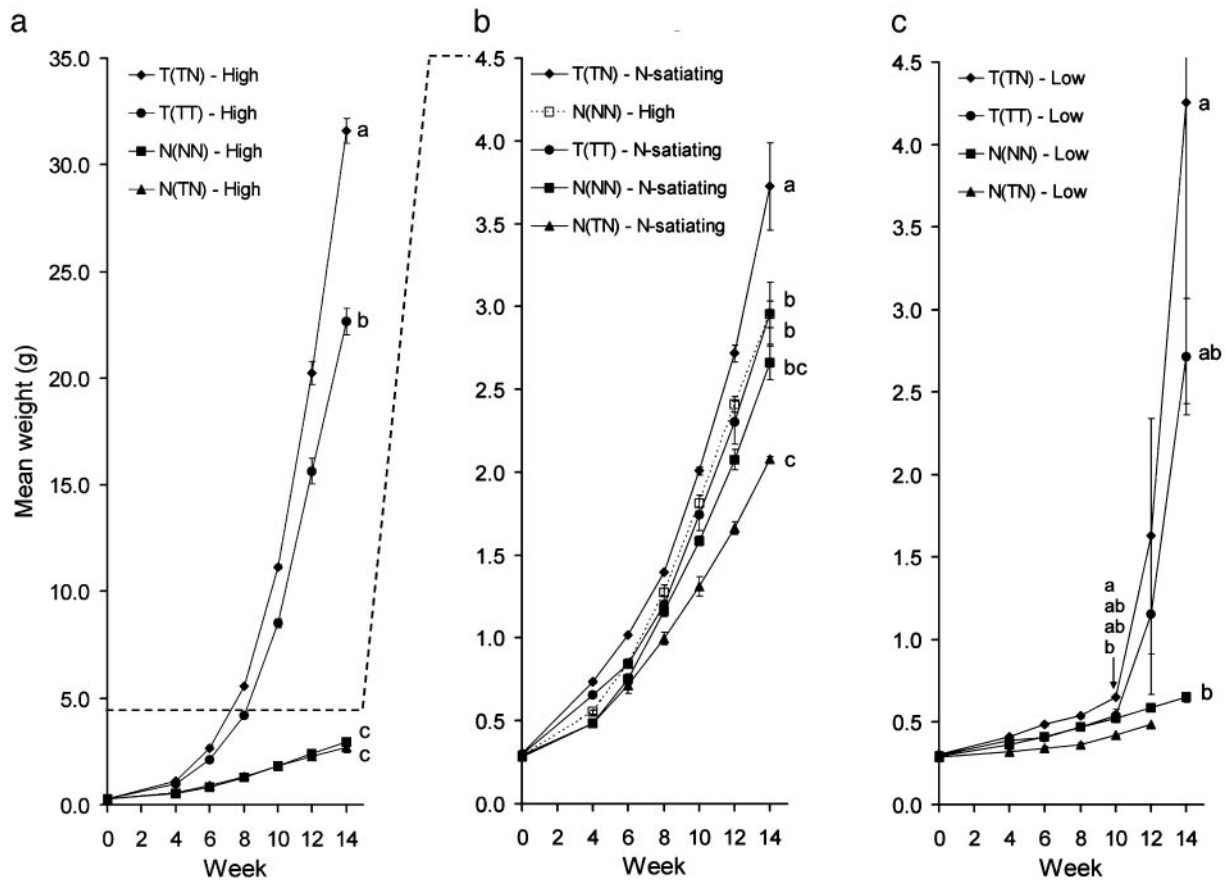


Fig. 1. Weights of transgenic (T) and nontransgenic (N) salmon groups grown in pure transgenic (TT), nontransgenic (NN), or mixed (TN) populations. Environments were provided with different ration levels: (a) high, 7.5% body weight per day; (b) *N*-satiating, sufficient to satiate nontransgenic populations; and (c) low, 0.75% body weight per day. Intergroup means and associated standard errors are shown, and statistical relations significance of differences in growth among groups within panels are indicated by different letters. Group descriptors: N(NN), nontransgenic salmon from pure nontransgenic groups; N(TN), nontransgenic salmon from mixed groups; T(TN), transgenic salmon from mixed groups; T(TT), transgenic salmon from pure transgenic groups. Suffixes (high, *N*-satiating, low) of group descriptors indicate ration level. In *b*, data from *a* for N(NN)-High are also shown for reference.

flow through mesh ports on the sides to minimize flow effects between habitats. Growth (weight and length) and survival was examined in each population initially at week 4, followed by biweekly measurements until week 14. Specific growth rates for weight (percentage gain per day) were calculated by $SGR = (\ln W_2 - \ln W_1) / \text{growth interval (days)} \times 100$. Condition factor was calculated as $CF = \text{weight (grams)} / \text{length (cm)}^3 \times 100$. Statistical relationships among groups were determined by ANOVA followed by Tukey's test to distinguish groups with the program SIGMASTAT (Systat Software, Point Richmond, CA). When unequal variances were encountered, data were normalized by \ln transformation. If \ln transformation did not normalize the data, then the Mann-Whitney rank sum test was used to examine differences between populations. Survival analysis was performed by using Kaplan-Meier log-rank method, followed by the Holm-Sidak multiple-comparison method, to determine pairwise relationships (SIGMASTAT). Statements of significant difference were made if $P < 0.05$, unless multiple comparisons were being performed, in which case a Bonferroni correction was applied.

Results

At high ration levels (Fig. 1a), pure populations of GH transgenic salmon grew much more rapidly than nontransgenic controls, with a 1.78-fold greater growth rate ($SGRs = 4.78 \pm 0.09\%$ SE vs. $2.67 \pm 0.01\%$ SE per day, respectively) and 7.7-fold increase in mass over the course of the experiment. Growth of transgenic fish was further enhanced ($SGR = 5.17 \pm 0.02\%$ SE per day) in mixed populations containing slower-growing nontransgenic salmon, resulting in an 11.6-fold greater mass [com-

pare T(TN) and N(TN)]. However, the growth of nontransgenic animals did not differ whether they were raised in pure nontransgenic (NN) or in mixed (TN) populations. Thus, in high food-availability environments, transgenic fish did not competitively interfere with the growth of nontransgenic fish within populations (Fig. 1a).

Pure populations of nontransgenic salmon did not grow slower in *N*-satiating environments (Fig. 1b) than in high-ration environments (Fig. 1a), indicating that nontransgenic appetites were fully satisfied (note change in scale between *a* and *b*). Similarly, pure transgenic populations that received the same amount of food also did not display enhanced growth relative to nontransgenic animals (Fig. 1b), indicating their growth is highly ration-dependent. However, in mixed populations, transgenic fish in *N*-satiating environments did achieve a significantly larger weight than nontransgenic cohorts (Fig. 1b) ($P < 0.05$; $SGRs = 2.69 \pm 0.01\%$ and $2.13 \pm 0.11\%$ SE per day, respectively), consistent with transgenic fish being able to more effectively compete for available food resources.

Under low ration conditions (Fig. 1c), growth rate was reduced on average 2.99-fold below that observed in *N*-satiating environments, but the same rank order of growth among groups was observed. By week 10, a significant split in weights was detected between transgenic and nontransgenic salmon grown in mixed populations under low rations. Subsequent to week 10, mean transgenic group weights increased dramatically (Fig. 1c), but this reflected a major shift in the number of animals alive within the populations.

Survival was strongly influenced by ration level and population structure. At high and *N*-satiating rations, all groups survived

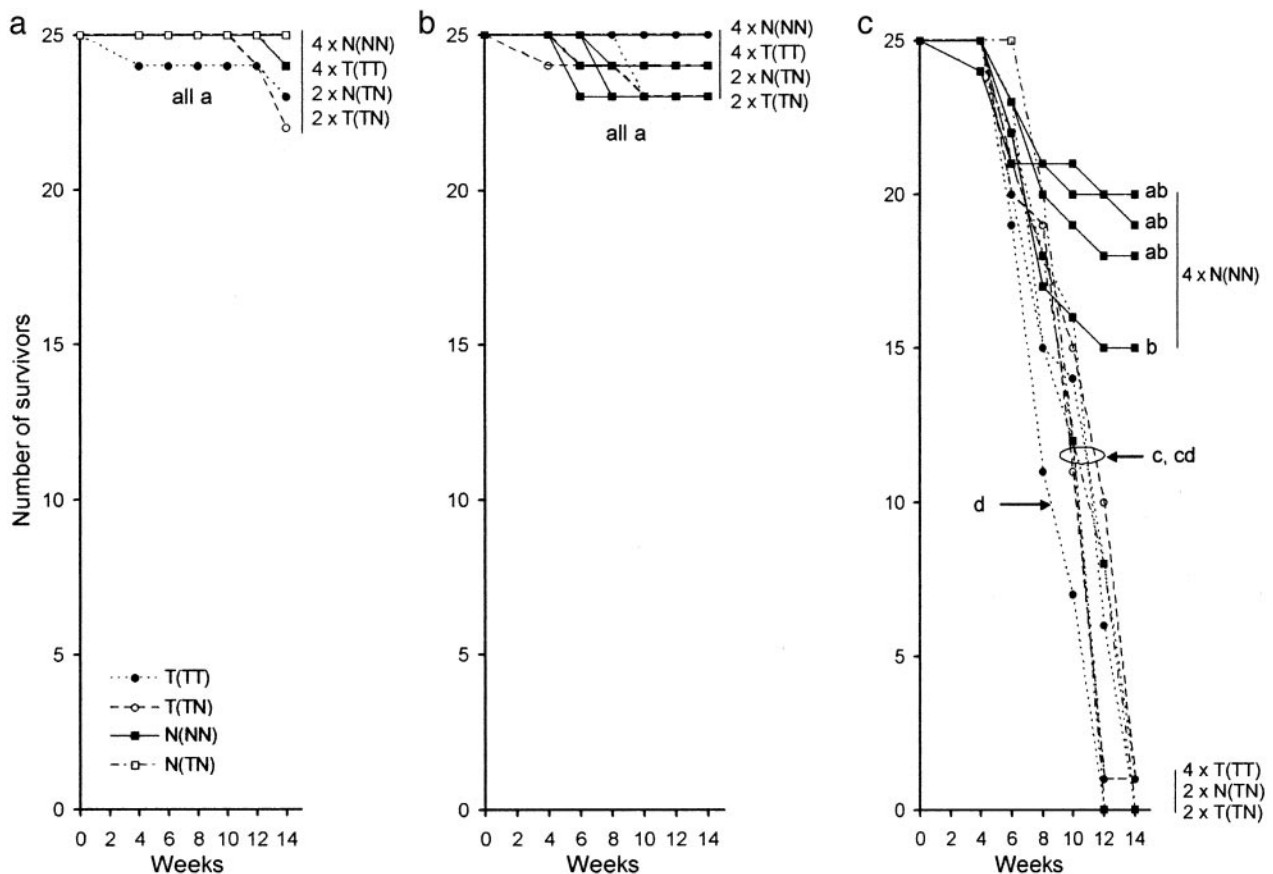


Fig. 2. Survival of groups as described in Fig. 1. Group descriptors are as in Fig. 1, and the legend in *a* applies also to *b* and *c*. In some cases, groups have not been individually distinguished for clarity, and the number within each statistically similar experimental group is shown. Statistical relations significance of differences in survival of groups across all panels are indicated by different letters. Values within circles are not statistically different.

equally well (average 96.8%), irrespective of genotype or whether they were in pure or mixed populations (Fig. 2*a* and *b*). However, in environments with low food availability, which create highly competitive conditions, significant differences were observed among genotypes and among population types (pure vs. mixed). Survival of nontransgenic salmon in low-ration pure populations did not differ among groups and was not significantly reduced from nontransgenic salmon in high and *N*-satiating ration environments in three of four groups (Fig. 2*c*). In contrast, all groups containing transgenic salmon in low-ration environments ultimately experienced population crashes or complete extinctions by week 14 of the experiment. These groups contained either all transgenic individuals or mixtures of transgenic and nontransgenic salmon, and survival was significantly reduced ($P < 0.002$ in all cases) relative both to nontransgenic animals under low rations and to all other groups in high or *N*-satiating environments. In populations containing transgenic salmon, the survival of transgenic and nontransgenic groups was equally reduced, except for one pure population of transgenic salmon, which showed greater mortality (significance identifier *d* in Fig. 2*c*).

In mixed populations under high-ration conditions, a bimodal distribution of weight classes, separated by genotype, emerged over the course of the experiment (Fig. 3*a*). In low-ration conditions, the appearance of a few large fish, invariably transgenic, emerged within each population that contained transgenic salmon (Fig. 3*c*). These faster growing fish dominated the growth of low-ration environments, where they were observed to be extremely aggressive to their cohorts. Of 224 fish that died under low-ration conditions, 197 were physically recovered and appeared, based on body damage, to have died from aggressive attacks by other fish or from malnutrition [the average weight (0.39 ± 0.01 SE grams) and condition factor (0.78 ± 0.01 SE) of recovered mortalities were both very low]. The condition factor (weight to length relationship) of surviving salmon raised in low-ration environments were lower than for fish in *N*-satiating and high-ration environments (Fig. 4*a*), until the final weeks when mean condition factors returned to normal in two transgenic groups due to loss of poorly conditioned fish. Dominant transgenic fish, as small as 1.56 grams, were observed to cannibalize 11 fish in their cohorts, and 16 fish not recovered were consumed either before or after death. Piscivorous feeding by transgenic fish continued throughout the trial, to the extent that attempted consumption of very large cohort members was observed, which resulted in deaths of predator fish (Fig. 4*b*). In contrast, larger animals with good condition factors observed under high and *N*-satiating ration conditions (Fig. 3*a* and *b*) and in pure nontransgenic populations at low rations (data not shown) did not display aggression and cannibalism of cohorts, presumably because metabolic requirements were being satisfied sufficiently.

The biomass of all populations in high and *N*-satiating ration environments paralleled growth curves due to the high survival among these groups (data not shown). However, biomass in the low-ration environments showed overall declines in all populations containing transgenic fish, whereas populations containing only nontransgenic animals continued to gain biomass throughout the experiment, albeit slowly because of limited food availability (Fig. 4*c*).

Discussion

High-food-availability conditions allow transgenic coho salmon to grow significantly faster than nontransgenic individuals when they are grown noncompetitively in separate environments (1, 12, 25). The present experiments have shown that when GH transgenic and nontransgenic salmon are reared in the same environment, and sufficient food resources are present, transgenic salmon did not competitively interfere with the growth of nontransgenic fish and did not significantly express their agonistic behavior potential

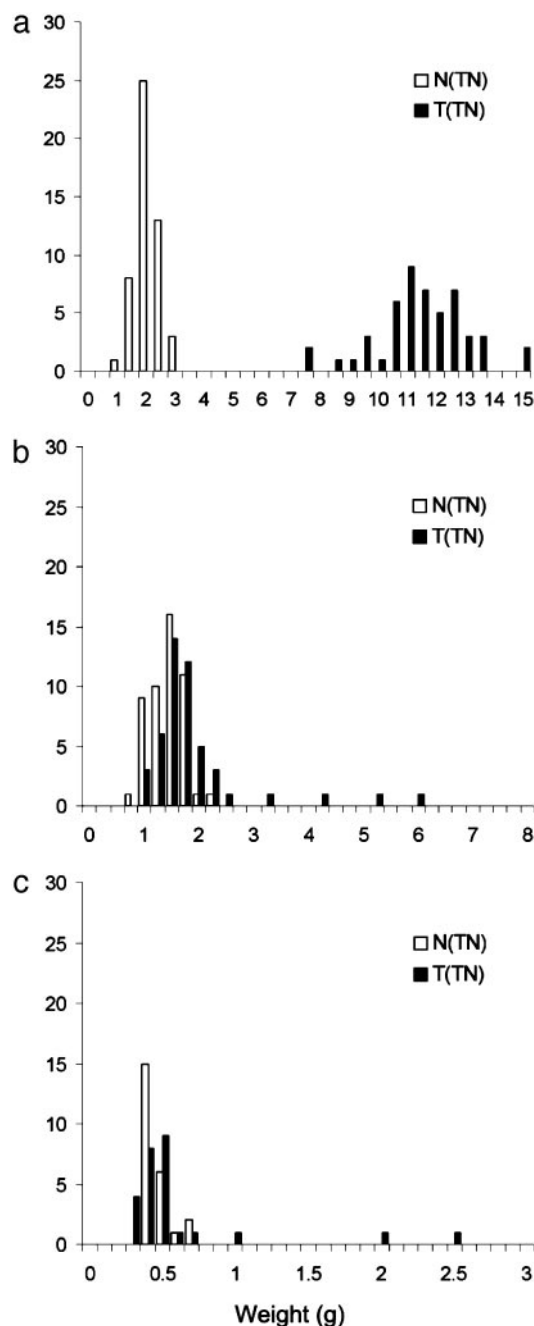


Fig. 3. Frequency histograms of salmon size classes within mixed populations of transgenic (filled bars) and nontransgenic (open bars) salmon at week 10. (a) High ration. (b) *N*-satiating ration. (c) Low ration. Group descriptors as in Fig. 1. *x* axis represents binned fish weights (g), but note that scales differ among graphs.

toward cohorts. Whereas very high-ration environments may occasionally occur in nature, competitive situations with either normal or limited food availability are expected to be more usual and, thus, have relevance for ecological risk assessments. In natural streams used by coho salmon, space and food availability are both limiting, and larger fry benefit from high dominance status, which allows them to defend territories and gain access to preferred feeding stations (19–21). In our experiments, when food availability for GH transgenic fish populations is at a level that fully satiates nontransgenic populations, the growth of nontransgenic and transgenic animals in separate populations did not differ. These results indi-

nistic and cannibalistic behaviors. Observations with nontransgenic salmonids have shown that hunger and growth rate are associated with increased agonistic behavior (31–33).

The present experiments have revealed that during a young life history stage, which is critically involved in the determination of the survival component of fitness, growth-enhanced transgenic salmon can cause significant effects on population numbers and biomass, size-at-age relationships, and growth of nontransgenic cohorts. Previous studies have shown that GH transgenic salmon use stored energy reserves faster than nontransgenic salmon during starvation (28), suggesting that they may be less able to withstand periods of food deprivation, with consequent overall lower fitness. However, in the current experiments, which also include behavioral interactions, the survival impacts of transgenic salmon on populations was found to act equally on cohorts independent of their genotype, indicating that it is the presence of growth-enhanced transgenic salmon in the population that dominated survival probabilities under these conditions. The emergence of dominant, cannibalistic transgenic individuals resulted in population-level effects that were mediated by interindividual behavioral and competitive interactions initiated by GH transgenic salmon, rather than from the independent metabolic states (26, 27, 29) of each fish in the population. In those cases where population crashes occurred, only GH transgenic fish of a healthy body composition remained.

The present data suggest that effects of GH transgenic salmon on natural populations would be highly dependent on food availability in local environments. Under poor conditions, the presence of GH transgenic genotypes may lead to population impacts that nontransgenic groups would be able to withstand for longer periods. Under these conditions, transgenic genotypes may be essentially eliminated from small localized natural populations, along with other nontransgenic members of the cohort. Under favorable conditions, such as high food availability or low population densities, GH transgenic genotypes would be anticipated to grow rapidly with minimal effect on survival. Indeed, experiments examining the effect of GH treatment on salmonids in nature have revealed little effect on survival under normal conditions (34). High growth conditions would favor earlier sexual maturity of transgenic genotypes (12, 25), which could facilitate GH transgene introgression into populations.

Whereas immediate effects on conspecifics or other ecosystem members are unknown under such conditions, it is possible significant numbers of GH transgenic salmon with high food requirements in populations could lead to resource shortages and effects as observed in smaller populations. Transgenic fish, through their altered behavior, could also affect natural populations by increasing time other fish spend hiding rather than foraging, changing territory sizes, altering predation risk, or altering the timing or extent of emigration to search for more favorable environments (9, 14, 20, 21, 35). However, the probability of population extinction events occurring in nature would be expected to be strongly influenced by food availability, the proportion of transgenic fish in the population, population size, habitat complexity, and predation risk effects (9, 14, 15), and, critically, may only be anticipated in small populations where the opportunity for dispersal to alternate habitat is limited.

Although it would be theoretically desirable to examine risk-assessment variables in natural ecosystems, the need for containment of GH transgenic salmon necessitates conduction of experiments in laboratory facilities. For species such as salmon with very large complex habitats, data for risk assessments are collected from simulated environments and apparatus from which phenotypic characteristics are determined and inferences made regarding potential effects, which may occur in natural riverine and oceanic habitats (9, 10, 14–16). However, strong genotype by environment interaction, as has been observed in the present experiments, underscore the importance of this factor in determining the usefulness of such data for risk assessments of genetically modified organisms (36) because impacts observed in one study environment (either natural or artificial) will have uncertain magnitude elsewhere. Consequently, although the attributes of GH transgenic salmon as observed in the present experiments are likely indicative of forces that would act on salmon populations, we caution against using such data prematurely in predictions of potential impacts until a broad understanding of the effects is known in many relevant habitats. Similar caution is also warranted for other species with complex ecological requirements.

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