Variability in competitive ability and mortality rates: The ability of transgenic coho salmon (*Oncorhynchus kisutch*) to survive in the wild.

by

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VARIABILITY IN COMPETITIVE ABILITY AND MORTALITY RATES: THE ABILITY OF TRANSGENIC COHO SALMON (*ONCORHYNCHUS KISUTCH*) TO SURVIVE IN THE WILD

BY

Wendy Vandersteen

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

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MASTER OF SCIENCE

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ABSTRACT

Coho salmon *(Oncorhynchus kisutch)* have been genetically altered to produce growth hormone without regulation, causing them to grow on average 11 times larger than control fish after one year of growth. This technology has important benefits for the aquaculture industry, but the environmental risk associated with the escape of transgenic fish into the wild is not known. To partially address this issue, I experimentally investigated how well transgenic salmon survived under semi-natural conditions. If transgenic salmon retain their growth advantage under natural conditions, one can predict that they must also be more effective at competing for food than wild salmon, and willing to suffer higher mortality rates while foraging. Two experiments were designed to test this hypothesis. The first tested the relative competitive ability of transgenic and control salmon using an unequal competitors ideal free distribution. A larger proportion of transgenic salmon were found at the high quantity food source, leading to the conclusion that they were more superior at securing higher quantity food resources. The second tested the relative mortality rates of transgenic and control salmon by providing them with the option to feed in the presence of a predator. There was no significant difference in mortality rates between the two groups. An individual-based population model was developed to examine the relative survival of transgenic fish in the natural environment. Results from the model indicated that under certain environmental conditions, transgenic fish had survival rates equal to the wild type individuals. My preliminary results did not provide conclusive evidence that transgenic fish would be unable to survive in the wild indicates that care must be taken to insure these growth-enhanced individuals are not released into the environment.

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General introduction

CHAPTER ONE – General introduction

Background Information

"Evolution" of transgenic fish

As the human population escalates, it becomes increasingly difficult for world food production to meet consumers' needs. Any further increase in food production, using current agricultural practices, could lead to irreparable damage to already stressed ecosystems. In particular, protein sources are not increasing at a rate matching that of the increasing human population (Dunham and Devlin 1999, Ward 1999). Harvesting of fish, an important protein source, has depleted many fish populations to a point where recovery may not be possible (Meffe 1992). These levels of harvest are not sustainable, and are an incredible strain on fish populations already stressed by habitat destruction, pollution and environmental change (Meffe 1992).

Limited increases in food production can be achieved using traditional agricultural methods. Optimization of abiotic factors and manipulation of biotic factors, such as selective breeding, have brought agricultural production to current levels. Advances in biotechnology indicate that animal production can surpass the limited global carrying capacity. Through genetic manipulation, fish species are being produced to improve the economics of fish culture. These manipulations have been targeted to include the following traits: increased growth rates, increased market size, more efficient food conversion rates, ability to utilize low cost diets, improved brood stock fecundity, reduced aggression, enhanced resistance to disease, and tolerance to freezing (Devlin et al. 1995; Hew et al. 1995). These traits lead to more efficient and affordable harvest of food without having to increase the resources necessary for production. Genetically modified organisms are stepping over boundaries that have limited productivity in the past. The full consequences of this revolution have yet to be determined. According to Meffe (1992), the use of biotechnology to address food production concerns and species conservation is resulting in an "increasingly intensive and essentially perpetual management of a multitude of species in a world unfit for their natural existence".

For several years, scientists have recognized the impact of growth hormone supplements on growth rates of several species of fish (Pickford and Atz 1957). Supplementation of fish with hormones was time consuming as each individual had to be injected several times in order to produce a marked increase in growth rate, although implants have now been developed that supply growth hormone for over a year. As technology advanced, researchers were able to splice growth hormone genes into the DNA of several fish species. Successful transfer of recombinant DNA was first accomplished in 1985, and by 1990 thirteen species of transgenic fish had been produced (Kapuscinski and Hallerman 1990). One concern with these first attempts was that the growth hormone genes were from an entirely different animal class, usually mammalian. Within the last few years, scientists have been able to splice piscine growth hormone genes and promoter sequences into DNA of salmon and trout (Devlin et al. 1995) and tilapia (Rahman et al. 1998), potentially increasing the effectiveness of the transgene and social acceptance of transgenic fish (Hew et al. 1995). The transgene may be more effective since it is from a closely related species, and social acceptance may be increased as manipulating genes within a species may not generate as much apprehension as manipulating genes between different animal classes.

Phenotypic expression of transgene

Some of the most commercially important, and most threatened, genera of fish are salmonids (Noakes et al. 2000). Salmon have been successfully genetically altered so that they produce growth hormone without regulation, resulting in increased growth rates. On average, transgenic coho salmon (*Oncorhynchus kisutch*) individuals at 14 months of age are 11 times larger than control salmon, with a maximum increase of 37 times having been observed (Devlin et al. 1994). This increased growth rate in such a commercially important species may have important consequences for the aquaculture industry (Lakra and Das 1998). Since these fish grow faster, they can be harvested in less time than regular fish. Transgenic technology would also allow desired traits to be transferred into other fish species allowing phenotypic traits to be developed much faster than traditional selection and breeding methods (Hew et al. 1995). Improvements in aquaculture would decrease the pressure of harvest on natural populations (Dunham 1999).

Although many species of fish have successfully had a growth hormone sequence spliced into their genome, it is interesting that the salmon demonstrate the most significant increase in growth rates (Dunham and Devlin 1999). One explanation for this can be found when considering the life history of the salmon. Salmon naturally grow slowly, especially during winter when the temperature is low and food is scarce. Transgenic salmon appear to be decoupled from seasonal regulation of growth hormone production so that, given adequate food, they grow continuously and at a faster rate than control salmon. In one experiment, winter levels of growth hormone in transgenic salmon were more than forty times higher than in control salmon (Devlin et al. 1994). Hinits and Moav (1999) found that transgenic common carp (*Cyprinus carpio*) demonstrated enhanced growth relative to control individuals only under sub-optimum conditions, indicating that environmental factors must be taken into consideration when conducting experiments to test the effectiveness of the transgene. In a recent study by Devlin et al. (2001) it was discovered that transgenic wild-type trout did not demonstrate increased growth relative to fast-growing non-transgenic domestic trout. This indicates that growth enhancement can be achieved by transgenesis or selective breeding, but the effects are not always additive, suggesting that there is an upper limit to growth.

The growth advantage of the transgenic salmon is further amplified by an earlier smolt and its subsequent period of rapid growth (Dunham and Devlin 1999). Growth hormone transgenic Atlantic salmon were found to smolt successfully as early as six months of age (Saunders et al. 1998). In comparison non-transgenic Atlantic salmon smolt no earlier than at two years of age, with an average of three to five years. Transgenic salmon can be raised under conditions that would inhibit smolting in non-transgenic salmon (24 hours of light, Saunders et al. 1998).

In addition to accelerated growth, transgenic salmon differ in their morphology, behaviour and physiology. Growth-hormone transgenic salmon consume food more rapidly than non-transgenic controls in order to sustain their increased growth rate. They also have increased food conversion efficiencies (Dunham and Devlin 1999, Devlin et al. 1995), which may be partly due to the increased surface area of their intestine (Stevens and Devlin 2000; Stevens et al. 1999) as suggested by the positive correlation between surface area and growth rate of fish. Improved food conversion efficiencies are not unique to salmon. Transgenic tilapia have food conversion efficiencies 33% higher than control tilapia (Rahman et al. 1998). Growth-enhanced salmon have an oxygen uptake

60% greater than that of control salmon (Stevens et al. 1998) correlating with an increase in gill surface area (Stevens and Sutterlin 1998). Transgenic coho salmon were found to be superior competitors compared to non-transgenic controls (Devlin et al. 1999). The competitive ability of transgenic fish will be discussed further in Chapter 2.

Trade-offs between growth and mortality

Enhanced growth does not come without any costs. There are in fact several trade-offs between growth and mortality that may explain the evolution of submaximal growth rate in the early life history of fish (see Mangel and Stamps 2001; Conover and Schultz 1997).

To maintain increased rates of growth, fish must make trade-offs between foraging effort and risk of predation (Magurran 1993; Abrahams and Dill 1989). Fish with high growth rates due to hormone supplement were found to be more willing to risk increased predation in order to forage (Abrahams and Pratt 2000; Abrahams and Sutterlin 1999; Dunham et al. 1999; Jönsson et al. 1996). To maintain their increased rate of growth, transgenic salmon may have to shift the optimal trade-off between growth and mortality risk to a more "high-gain/high-risk" phenotype (Johnsson 1993). This concept is discussed further in Chapter 3.

A trade-off may also exist between growth rates and cell function at maturity. With an increased growth rate, cells and tissues may be incapable of functioning at maximum efficiency. Transgenic coho salmon were found to be inferior swimmers when compared with non-transgenic controls (Farrell et al. 1997). Transgenic salmon swam no faster than smaller controls of the same age, and slower than older controls of the same size. One explanation for this observation is that some physiological systems may be deprived in order to support the enhanced growth (Farrell et al. 1997). When comparing individuals of the same size, fast-growing fathead minnows (Kolok and Oris 1995) and rainbow trout (Gregory and Wood 1998) swam less efficiently than the slow-growing fish.

There may be compromises between enhanced growth and somatic development of the fish. Acromegaly has been noticed in some growth-enhanced Pacific salmon, resulting from excess growth of bone due to high levels of growth hormone (Devlin et al. 1995; Ostenfeld 1998). These individuals have enlarged heads due to overgrowth of cartilage in the cranial and opercular regions. The abnormality became more severe with size and age, causing difficulty in feeding and reduced ventilation. Such abnormalities can also reduce swimming ability (Ostenfeld et al. 1998) and may ultimately impair the ability of the transgenic salmon to escape predators. Due to reduced viability of salmon expressing acromegaly, there may be limitations to the extent of growth stimulation that can be applied to fish (Devlin et al. 1995). Similar morphological abnormalities have also been observed in transgenic tilapia (*Oreochromis niloticus*), although to a lesser extent (Rahman et al. 1998, Rahman and Maclean 1999).

The heightened energetic demands of maintaining a high growth rate may have a negative impact on the immune system and the ability of fish to survive physiological stress such as starvation or low oxygen levels. Johnsson et al. (2000) found that growth-hormone treated brown trout had lower condition factor and lipid content in the liver and muscles compared to untreated fish, when reared in the wild. This would reduce the ability of the fish to survive periods of starvation.

Environmental risk

Before transgenic salmon can be used commercially, several socio-economic issues must be addressed. One concern is the potential environmental risk posed by the growth-enhanced fish should they ever escape into wild populations. Generally, survival and growth are positively correlated (Johnsson et al. 1996), but survival may be threatened if the heightened capacity of some physiological properties (allowing the enhanced growth) occurs at the expense of other physiological properties or at an increased risk of predation. It is quite possible that not all traits mediated by the growth hormone are advantageous. Since salmon have not naturally evolved to grow faster, it leads us to believe that some traits mediated by the growth hormone are indeed disadvantageous. This would indicate an evolutionary trade-off in the optimum level of growth hormone production. As conflicting views indicate, it is difficult to predict the potential environmental risk because there are unlimited scenarios for what could happen should mixing of transgenic and wild individuals occur.

Perceptions of ecological risk vary. Transgenic fish may pose an ecological risk by increasing the fitness of the population and thereby giving rise to niche expansion and impact on other fish communities (Kapuscinski and Hallerman 1990). Alternatively, transgenic fish may decrease the fitness of the wild population by passing genes that are deleterious in the wild (Howard and Muir 1999, Muir and Howard 2001). Further extension of Howard and Muir's model (1999) demonstrates that there are very broad conditions under which transgenic fish with a mating advantage and viability disadvantage may invade natural populations, reduce their fitness and potentially cause their extinction (Hedrick 2001). Knibb (1997) argues that large-scale releases of transgenic fish may pose a potential risk to wild populations, but small-scale releases are not a concern, as natural selection will remove deleterious genetic changes from the wild population. Guillén et al. (1999) found transgenic tilapia to have a lower feeding motivation and dominance status than controls. Guillén et al. (1999) also determined that the growth hormone had no biological activity in non-human primates and no health effects on human volunteers, leading to the conclusion that transgenic tilapia are safe for culture and consumption in Cuba.

In the event that genetically altered salmon are used in aquaculture, it is recommended that care be taken to ensure that all individual fish are sterilized or that rearing facilities are completely segregated from natural bodies of water (Kapuscinski and Hallerman 1990). Triploid induction is one method used to make fish sterile, however presence of spermatozoa in the testes of transgenic male tilapia lead to the proposal that only female growth-enhanced fish should be produced in order to minimize environmental risk through reproduction (Razak et al. 1999). Should accidents occur, the transgenic salmon will at least not mix their genetic material with native salmon stocks. In addition, growth-enhanced transgenic animals may have reduced reproductive fitness. Transgenic male tilapia produced low amounts of sperm, or were completely sterile (Rahman 1999, Rahman et al. 1998). The females also did not demonstrate reproductive behaviour. However, there was no obvious impairment of reproductive ability of transgenic Atlantic salmon (Hew et al. 1995). Although the risk of genetic mixing may be low, the consequences could lead to species extinction (Muir and Howard 1999) due to increased mating advantage and decreased viability of the transgenic individuals. Throughout this study, I will be focusing on the consequences of morphological, behavioural and physiological differences between transgenic and wild salmon and not on the consequences of genetic mixing.

The only way to know with certainty the consequences of interaction between growth-enhanced and wild salmon is to rear the two populations together under natural conditions. This cannot be attempted due to unknown risks and consequences should unplanned accidents occur. Once genetically modified organisms are released into the environment, there is no way to call them back. We must make do with the resources we have, and ensure that the experiments are planned with utmost care to minimize variability. In this manner, we will be able to use this information to construct simulations on the consequences of species interaction, although these simulations can never be assumed to be fact (Kareiva et al. 1996). The actual scenario will probably involve both fitness increasing and fitness decreasing components, although it is not known with certainty what the net outcome of these components will be.

Research Objectives

Current research has not yet conclusively determined if transgenic fish are capable of maintaining their enhanced growth under natural conditions with the presence of predators and intermittent food resources. Transgenic fish provide a unique opportunity to explore how the fitness consequences of maintaining enhanced growth constrain the evolution of fish to grow at submaximal rates. Since hormones have multiple effects on phenotypic traits, it becomes obvious that evolution does not occur on a trait-by-trait basis, but instead incorporates trade-offs between a suite of traits (Ketterson and Nolan 1999). Through exploring animal design, it will also become increasingly clear as to whether the transgenic fish are capable of surviving under natural conditions and thereby pose a threat to wild fish populations.

Overview of research

The first step in exploring animal design and the level of ecological risk is to obtain as much information as possible on the phenotypic qualities of growth-enhanced transgenic salmon. Once this information has been obtained, it is still difficult to predict the outcome should the transgenic and wild individuals mix. At this point, a computer model would be useful in simulating the consequences of the phenotypic traits under varying environmental conditions. A combination of experimental data and computer models is a powerful tool for studying the dynamic nature of interacting species (Pascual and Kareiva 1996). It must be stressed that the results of a study such as this cannot be taken at face value, they must merely be used as guidelines to potential outcomes. Real data always involves variability, or error, and therefore some uncertainty in their representation of population behaviour. It is important to consider this source of variability when drawing conclusions.

Muir and Howard (2001) consider six fitness components by which natural selection could alter the frequency of a transgene released in a population: juvenile viability, adult viability, age at sexual maturity, female fecundity, male fertility, and mating advantage. Juvenile viability is often considered to be the most important fitness component (Clutton-Brock 1988) and was found to follow only age at sexual maturity as the most important fitness components affecting the spread of a transgene in a population (Muir and Howard 2001). This study will examine competition and mortality of transgenic and control salmon, which are two important factors contributing to juvenile viability. Knowledge of the influence of growth enhancement on foraging and anti-predator behaviour will enable us to predict relative juvenile viability, and consequently the ability of transgenic salmon to survive in the wild.

Through two laboratory-based experiments, I compared the relative competitive ability and mortality risk of transgenic and wild salmon. In the first experiment, relative competitive abilities were studied using the ideal free distribution as a tool: high food concentration and low food concentration. Competition and the results of this experiment are the topic of Chapter 2. For the second experiment, a rearing system was designed with three distinct habitats: no food, no predator; low food concentration, no predator; and high food concentration, predator. Transgenic and control individuals were placed together in the system and the relative mortality risk was observed as the individuals competed for food resources in the presence of a predator. Chapter 3 discusses the impact of predation on growth and survival, and the results of the mortality experiments.

The experimental data were then used within a computer model that accepts transgenic and wild individuals into a population and tracks their growth and survival as they complete their first year of life. This model was not used to make confident predictions about the potential impact of transgenic salmon but merely as a framework to explore the environmental conditions under which transgenic fish may experience increased fitness relative to wild fish. Chapter 4 outlines the model development and simulation of the results. Through the use of experimentally obtained data and computer modelling, I attempt to predict whether the transgenic salmon are able to maintain their growth advantage under natural conditions and what environmental conditions are necessary in order for the transgenic fish to maintain their growth advantage. Ultimately, this information will assist in predicting the ability of transgenic salmon to experience increased fitness under natural conditions relative to wild populations.

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CHAPTER TWO

Differences in competitive ability between growth-enhanced transgenic and non-transgenic coho salmon (*Oncorhynchus kisutch*)

CHAPTER TWO – Differences in competitive ability between growth-enhanced transgenic and non-transgenic coho salmon (*Oncorhynchus kisutch*)

Introduction

Competition for limited resources, such as food and space, is a fundamental aspect of population dynamics. Intraspecific variation in competitive ability has been documented in coho salmon (see Chapman 1962; Rosenau and McPhail 1987 for example) and is an important factor in the population dynamics of piscine species. Increased competitive ability incurs both costs and benefits to the dominant individual, and can have a profound influence on the behaviour and growth of subordinate individuals. Due to observed behavioural, physiological and morphological differences between growth-enhanced transgenic and non-transgenic coho salmon, transgenic salmon are expected to be superior competitors compared to non-transgenic fish, which could severely alter the population dynamics of wild salmon populations.

Growth-enhanced transgenic salmon are expected to have increased competitive ability compared with non-transgenic fish. Several characteristics may be involved in making the transgenic fish better competitors. In comparing size-matched transgenic and non-transgenic fish, the transgenic fish have increased appetite relative to the non-transgenic fish (Devlin et al. 1999) in order to meet their higher metabolic requirements due to enhanced growth and increased activity. The transgenic fish therefore have a stronger need to find food, and it is therefore assumed that this will lead to their more intense foraging behaviour. Superior competitive ability tends to increase fitness of fish by enabling enhanced growth in the more competitive individuals. Variability in competitive ability is related to intraspecific variability in size-at-age (Yamagishi 1974, Li and Brocksen 1977). This enhanced growth can be attributed to several factors arising as a consequence of superior competitive ability. More competitive individuals are able to secure the majority of limited food resources, and perhaps even displace subordinate individuals from the stream (Chapman 1962; Fausch 1984; Metcalfe 1986). This asymmetry in distribution of resources will amplify differences in size and growth rate (Doyle and Talbot 1986).

Ideal free distribution theory can be used to test the relative competitive abilities of fish. In an ideal free distribution, individuals are distributed so that none would benefit by switching sites (Fretwell 1972; Fretwell and Lucas 1970), and the proportion of individuals at each site matches the proportion of resources in each site (input-matching, Parker 1974). The ideal free distribution theory has been further developed by considering the situation where not all individuals are equal but instead differ in competitive ability (Parker and Sutherland 1986, Sutherland and Parker 1985). In an unequal competitors ideal free distribution, the more competitive individuals are expected to occur more frequently at the higher quantity food resources. Coho salmon have been found to distribute themselves according to an unequal competitors ideal free distribution (Grand 1997; Grand and Dill 1997), therefore this methodology is appropriate to test whether transgenic fish are superior competitors relative to non-transgenic individuals.

Objectives

If transgenic salmon are to retain their growth advantage under natural conditions, it is predicted that they must be more effective at competing for food than wild salmon, in order to meet their increased metabolic requirements. To test this hypothesis, I measured the spatial distribution of the transgenic and control individuals in a system made up of two habitats, one with a high concentration of food and one with a low concentration of food. According to an unequal competitors ideal free distribution, the more competitive individuals are expected to occur more frequently at the high food source (Sutherland and Parker 1985).

Methods

Experiment Subjects

Two groups of juvenile coho salmon (*Oncorhynchus kisutch*) were used in this study: an experimental group and a control group. The experimental group was produced from a cross of transgenic and wild type salmon so that it consisted of a mix of transgenic and non-transgenic fish. The control group consisted of 100% non-transgenic fish. The experimental salmon were fertilized February 12, 2000 and grew continuously at 8°C. The eggs began to hatch on April 23. On May 2, approximately 450 alevins were transferred to 15°C water. The fry were ponded on May 12 and maintained in 15°C water. The control group was ponded on May 1, and maintained at approximately 8°C. The fish in the experimental and control groups were size-matched due to manipulation of fertilization date and rearing temperature. The mean mass of the fish in the experimental group (0.413 g) was not significantly larger than the mean mass of the control group (0.408 g), as determined by a t-test ($t = -0.194$, $df = 118$, $p = 0.847$). Size-matched fish were chosen so that differences in competitive ability could not be attributed to differences in size (Huntingford et al. 1990).

Experiment Set-up

Two identical glass aquaria (60 cm long x 21.75 cm high x 18.75 cm deep) were used in this experiment. Each tank had two plastic feeding tubes placed 5 cm from each end of the tank that allowed food (NutraPlus Starter Mash produced by MooreClark) to be placed into the tank at a consistent 2:1 ratio (0.10 g and 0.05 g, respectively). For details on the design of the feeding system, see Abrahams (1989). These food amounts were chosen by determining a level of food that was completely consumed by the fish as it was dispensed by the feeders; this amount then became the ration for the low food source. The location of the high and low feeder within the tank (left or right side) was assigned randomly each day, and remained the same throughout the day. The photoperiod within the experiment room was maintained at a 12 hour cycle, with the lights coming on at 7:00 and turning off at 19:00. The building was completely dark at night as all windows were covered.

Experiment Procedure

A total of 120 fish were used in this experiment, which consisted of 60 individuals from the experimental group and 60 individuals from the control group. At the start of the experiments, transgenic and control fish were the same size so individuals to be used in the experiments were selected randomly. By day six, the transgenic salmon demonstrated such rapid growth that the largest control individuals had to be selected to ensure that size matching was maintained. Before being used in the experiment, all fish were identified with a small, numbered acetate tag painted with acrylic paint (as in Abrahams and Sutterlin, 1999) and allowed a minimum of two days recovery. During the recovery period, both the experimental and control groups were placed together in the same tank at 15°C and were fed with the same automated feeders used during the experimental trials.

Five individuals from the experimental group and five individuals from the control group were placed into each tank in the late afternoon and allowed to adjust to the system overnight. The following day, three trials were run at 9:00, 12:00 and 15:00. Prior to each trial, the fish were removed from the tanks and a complete water change was done. Food was placed into the glass flasks before the fish were returned to the tanks. Once the fish were placed back into the tank, they were allowed two hours to re-acclimate before trials were initiated. Each trial lasted 25 minutes and a remote control video camera was used to film the tanks during this entire period. Two groups were filmed simultaneously each day and were labelled as Tank A and Tank B. In total, 12 groups of fish were used for this experiment.

Since the genetic make-up of the five individuals from the experimental group was not known, these individuals were preserved after the experiments for later PCR analysis. The DNA was extracted using standard phenol-chloroform-isoamyl extraction. The PCR analysis was completed by Carlo Biagi at West Vancouver Laboratory. Hereafter, the experimental group has been separated into two categories: the transgenic group and the sib-control group. The transgenic group consists of 100% transgenic fish, while the sib-control group consists of non-transgenic fish that are siblings to the transgenic fish. Statistical analysis on these three groups still found no significant difference in mass between the transgenic and control groups. However, the mean mass of the sib-control group was significantly lower than that of the transgenic group, demonstrating the start of a bimodal size distribution characteristic of a mixed group of transgenic and non-transgenic siblings (Figure 2.1, Table 2.1).

Spatial analysis

Spatial analysis of the trials was completed by viewing the videotapes. Analysis of the videotapes provided data on the location of each individual during the trials. For each trial, video clips were captured every 30 seconds. Each 5-second clip was observed closely to obtain the spatial distribution of competitors at the high and low food sources. A detailed description of the methods used for the spatial analysis can be found in Abrahams & Dill (1989). Not every trial could be analyzed mostly due to problems identifying the individually marked fish within the tank. Refer to Table 2.2 for details on which trials could not be analyzed.

Statistical analysis

To address the effect of genetic make-up on the behaviour of the fish, I compared the proportion of transgenic and control fish feeding at the high food source. To determine if the transgenic fish were over-represented at the high food source, I calculated the proportion of individuals feeding at the high food source that were transgenic. From this, I subtracted the proportion of transgenic fish in the entire tank. I obtained the mean difference between the proportion of transgenic fish feeding and the proportion of transgenic fish in the entire tank for each group, and then compared these values to an expected difference of 0 with a t-test. So, if the transgene has no effect on feeding behaviour, we would expect that the difference between the proportion of transgenic fish feeding at the high food source, and the proportion of transgenic fish in

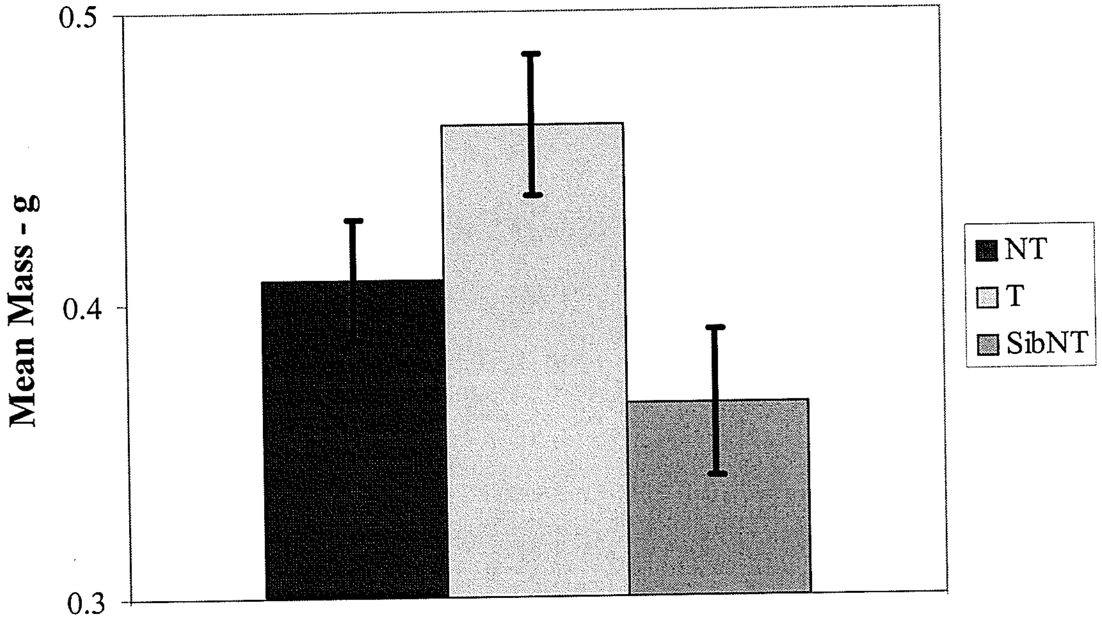

Figure 2.1. Mean mass of groups used in the competition experiments.

Mean mass of control (NT), transgenic (T), and sib-control (SibNT, siblings to the transgenic individuals) groups used in the competition experiments. The SibNT group had a significantly lower mean mass than the T group (Table 2.1). There was no significant difference in mean mass between the NT and T groups, or between the NT and SibNT groups.

Table 2.1. ANOVA results for mass comparison.

Statistical analysis of the three groups of fish used in the competition trials: transgenic (T), sib-control (SibNT, siblings to the transgenic fish), and control (NT). The mean mass of the sib-control group was significantly lower than the transgenic group. However, there was no significant difference in the mean mass of the transgenic and control groups.

ANOVA

		Sum of Squares	Df	F	Sig.
Mass	Between Groups	0.138	2	3.172	0.046
	Within Groups	2.547	117		
	Total	2.686	119		

Multiple Comparison – Bonferroni

		Mean Difference	Standard Error	Sig.
NT	T	-0.0532	0.033	0.329
	SibNT	0.0425	0.033	0.601
T	NT	0.0532	0.033	0.329
	SibNT	0.0957	0.038	0.04
SibNT	NT	-0.0425	0.033	0.601
	T	-0.0957	0.038	0.04

Table 2.2. Competition experiment design listing trials that were analyzed.

For the trials that were not analyzed, "not clear" means that I was unable to identify individually marked fished and "no T" refers to one group in which PCR analysis identified no transgenic fish.

	Tank A			**Tank B**		
	9:00	**12:00**	**15:00**	**9:00**	**12:00**	**15:00**
27-May	not clear	✓	✓	damaged tape	✓	not clear
28-May	✓	✓	✓	no T	no T	no T
29-May	✓	✓	✓	✓	✓	not clear
30-May	not clear	✓	✓	not feeding	✓	✓
31-May	✓	✓	✓	✓	not clear	not clear
1-Jun	✓	✓	✓	✓	✓	✓

the entire group, should be 0. I also determined the proportion of each fish type (transgenic, sib-control, and control in this case) feeding at the high food source, relative to the total number of each type feeding.

To assess the influence of time (time of day and time within trial), I calculated the proportion of transgenic and non-transgenic fish feeding. Repeated measures analysis of variance was used to assess the impact of time of day and time within trial on the proportion of fish feeding. This was necessary since the data represent repeated measures on the same individuals.

Results

Genetic Effects

Of the fish feeding at the high food source, a greater proportion were control fish (42%) as opposed to transgenic fish (36%). These means were found to be significantly different (t-test, $t = -3.114$, $df = 19$, $p = .006$). However, of the fish feeding at both the high and low food sources, a greater proportion of transgenic fish (72%) fed at the high food source, relative to the sib-control (69%) and control (62%) groups (Figure 2.2).

If the transgene has no affect on feeding behaviour, we would expect that the difference between the proportion of T fish feeding at the high food source, and the proportion of T in the entire group, should be 0. Instead, I found that the transgenic group were significantly over-represented at the high food source (Figure 2.3, $t_{10} = 2.566$, $p = 0.014$). This result is consistent with the prediction of the unequal competitors ideal free distribution that superior competitors should be over-represented at the highest quantity food sites.

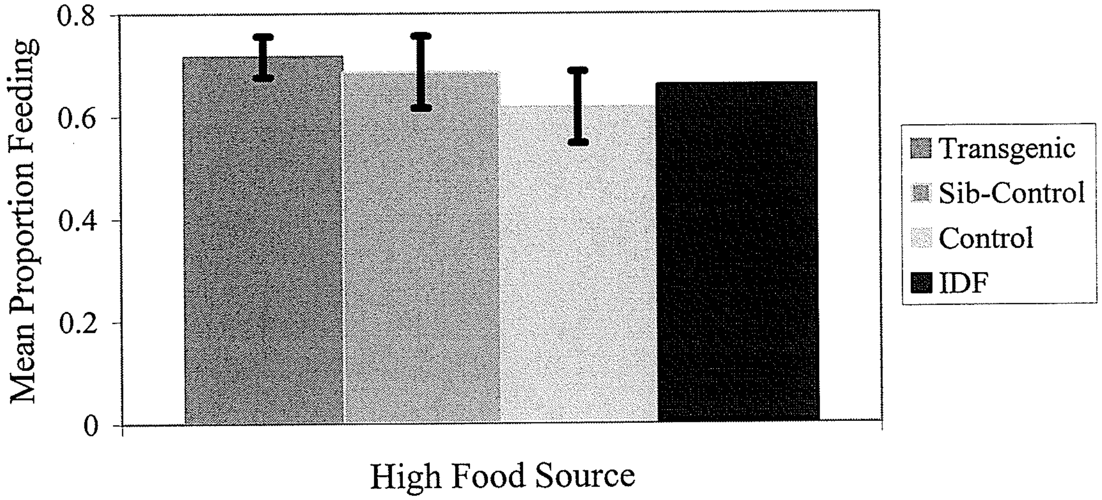

Figure 2.2. Proportion of each group feeding at the high food source.

Mean proportion of transgenic, sib-control (non-transgenic siblings of transgenic fish) and control fish feeding at the high food source from 12 different groups. The IDF bar represents the expected ideal free distribution of the fish. Error bars represent one standard error.

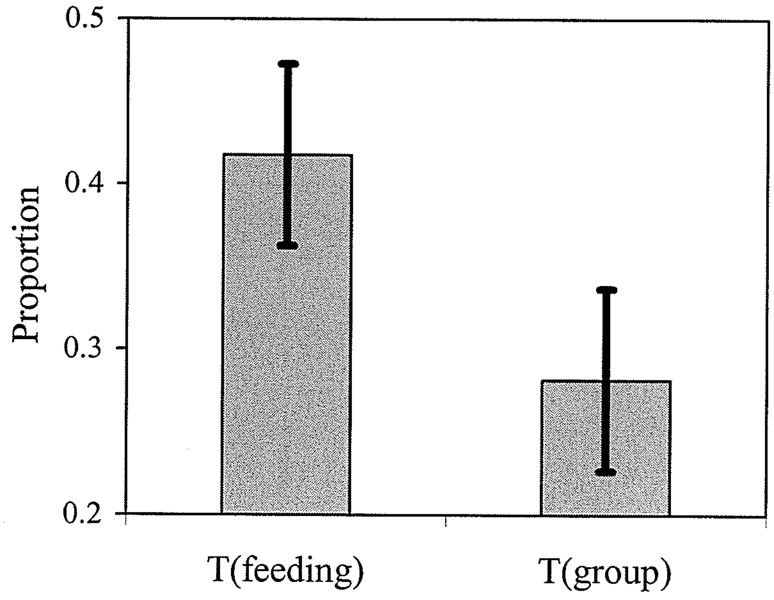

Figure 2.3. Proportion of T feeding vs. proportion of T in tank.

Mean proportion of transgenic fish feeding at the high food source – T (feeding) – compared to the proportion of transgenic fish in the entire tank – T (group) – for a total of 25 trials. The transgenic fish were significantly over-represented at the high food source ($t_{10} = 2.566$, $p = 0.014$). The error bars represent the standard error.

It was interesting to note that the sib-control group had a mean proportion that was intermediate between the transgenic and control groups, although these means were not significantly different (Figure 2.2, $t_{45} = 0.375$, $p = 0.709$). Figure 2.2 also illustrates the expected result of the spatial analysis if the fish distributed themselves solely on the 2:1 ratio of the food sources, according to an ideal free distribution. Although the separate groups do not conform exactly with the expected distribution predicted by an ideal free distribution theory, when the mean

proportion of transgenic fish (0.716 and 0.284 at the high and low sites, respectively) is averaged with the mean proportion of control fish (0.617 and 0.383 at the high and low sites, respectively) the groups conform exactly to the ideal free distribution (0.667 and 0.333).

Time Effects

Each group participated in a trial at 9:00, 12:00 and 15:00, for a total of three trials per group. According to a repeated measures analysis of variance, there was no significant effect of time of day on the proportions of transgenic and control fish feeding at either the high or low food sources. Analysis of the proportion of fish feeding over the twenty-minute time interval for each trial indicated no significant effect of time within the trial on the proportions of fish feeding, for both the transgenic and control groups (Table 2.3). For this analysis, the twenty-minute time period was divided into five four-minute categories. Graphical analysis demonstrated an interesting pattern however, when comparing the proportions of transgenic and control fish feeding for each time category. When the proportion of transgenic fish feeding decreases, the proportion of control fish feeding increases, and vice versa (Figure 2.4). This supports the conclusion that the

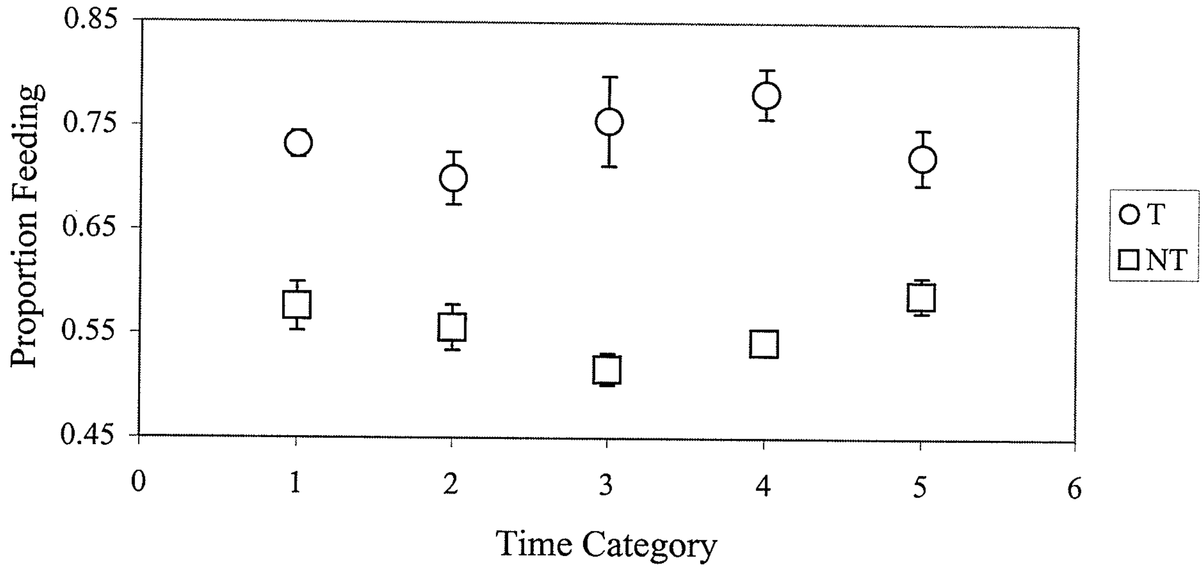

Figure 2.4. Proportion T and NT feeding over time.

Mean proportion of transgenic (T) and non-transgenic (NT) fish feeding over the twenty-minute trials. Each time category represents an average of a four-minute interval. There is no significant effect of time category on the proportion of fish feeding.

Table 2.3. Statistical analysis of competition trials.

ANOVA results for the proportion of transgenic and control fish feeding throughout the 20-minute trials at 9:00, 12:00 or 15:00. Time of day had no significant effect on the proportion of fish feeding.

		Sum of Squares	Df	F	Sig.
Transgenic	Between Groups	0.0939	2	0.621	0.538
	Within Groups	35.825	476		
	Total	35.919	478		
Non-Transgenic	Between Groups	0.0737	2	0.576	0.563
	Within Groups	30.472	476		
	Total	30.546	478		

transgenic fish are out-competing the control fish for food resources within the system, and indicates that they may not compete exclusively for the more desirable high food source.

General observations

The transgenic and control fish demonstrated obviously different feeding strategies. The transgenic fish would swim around constantly, switching back and forth between the high and low food sources. The control fish appeared to maintain a stationary position at either the high or low food source, and only moved when consuming food or if another individual chased them. Aggressive interactions between the transgenic and control fish did occur, where the transgenic fish would make a fast dash at the control fish, and the control fish would relocate. However, no quantitative measurements were made on these behavioural observations.

Discussion

The results of this experiment indicate that growth hormone transgenic coho salmon are indeed more successful at securing higher quantity food resources relative to size-matched non-transgenic fish. These results are in accord with Devlin et al. (1999) who also found that transgenic coho salmon had an increased ability to compete for food. Research on growth-enhanced transgenic tilapia (*Oreochromis hornorum*) produced somewhat similar results where transgenic individuals were found to be better competitors than their non-transgenic siblings. However, when compared with wild type tilapia, the wild individuals were better competitors relative to the transgenic and non-transgenic domestic fish (Guillén et al. 1999).

Although there was a greater number of control fish at the high food source relative to transgenic fish, a greater proportion of the unequally represented transgenic fish within the tank were feeding at the high food source. When considering both the high and low food sources, a greater proportion of transgenic fish were feeding throughout the twenty-minute trials relative to the control fish. This agrees with the assumption that the transgenic fish required more food to meet their increased metabolic requirements and maintain their enhanced growth.

Increased competitive ability could have a pronounced impact on the ecology of wild salmon populations. Impact of enhanced competitive ability may be felt as a direct effect of increased ability to compete for food and habitat resources, or as an indirect effect of the enhanced growth occurring as a consequence of the increased competitive ability.

Territory size increases with increasing fish size (Keeley and McPhail 1998; Grant and Kramer 1990). Since territory size may be involved in limiting population density in streams (Grant and Kramer 1990) the larger, transgenic fish may reduce the number of territories, and therefore the population size, that a stream may be able to sustain. Exacerbating this effect would be the enhanced appetite of transgenic fish, as hunger is theorized to be a factor in establishing the size of a territory that an individual will defend (Dill et al. 1981). As before, less subordinate, smaller fish may be displaced out of the stream.

Since growth and mortality rates are connected, factors affecting growth may indirectly increase recruitment. Fish often occupy niches that are size-dependent (Werner and Gilliam 1984). Ontogenetic niche shifts of fish influence population dynamics and community structure. Temporal differences in niche shifts could affect predator-prey interactions with other species. Increased food requirements could increase the predation pressure on other species of fish. For many species, there is selection for increased growth rate early in the life history, reducing the time spent in vulnerable size-classes (Werner and Gilliam 1984). Due to larger body size, dominant fish are able to relocate to deeper, faster moving water to access better quality foraging territories, thereby increasing their growth further. The more competitive individuals are able to shift foraging niches because they have outgrown predators whereas the smaller, less competitive fish are unable to feed in prime foraging territories due to high risk of mortality. Keeley and McPhail(1998) found that steelhead trout smaller than 5cm had proportionally larger territories compared to fish over 5cm. This suggests an ontogenetic niche shift, where fish over 5cm are able to move into deeper, faster flowing water that provides more food and hence a proportionally smaller territory is required.

In Atlantic salmon, more dominant, and hence larger, individuals can smolt and migrate from freshwater streams at only one year of age, as opposed to two or more years for subordinate, or smaller, individuals (Metcalfe et al. 1989). Large size is associated with early emigration to sea for coho salmon (Irvine and Ward 1989). Early-hatching Atlantic salmon fry that were dominant over later-hatching siblings were able to establish and maintain a size advantage that led to their increased probability of smolting at one year as opposed to two (Metcalfe and Thorpe 1992). Even though these individuals may return as small jacks, they would have a reproductive advantage. By reproducing a year earlier, these salmon would have decreased their mortality risk. Alternatively, the transgenic salmon may be larger at smolt relative to wild-type fish. Larger coho salmon smolts were found to have better survival relative to small smolts (Quinn and Peterson 1996), particularly in years when marine mortality was high (Holtby et al. 1990).

Increased competitive ability and larger body size may have an impact during spawning. Relatively smaller males have reduced access to females (Fleming and Gross 1992). Competition and body weight influence the breeding behaviour and success of coho salmon (Fleming and Gross 1992). Breeding success is increased indirectly because of enhanced growth, but it is also increased as a direct consequence of enhanced competitive ability. On the spawning grounds, space may be limited and the superior competitors are more successful at establishing their redd (van den Berghe and Gross 1989) and less likely to have it destroyed by another spawning female, as larger females dig deeper redds (van den Berghe and Gross 1984). Small females tend to experience delayed breeding and high egg loss relative to larger individuals (Fleming and Gross 1992). Intraspecific competition effects on the spawning grounds will be most significant when population numbers are high or habitat is scarce (Essington et al. 2000).

Ecological impact of transgenic fish may not necessarily be limited to intraspecific effects. Interspecific competition can also affect reproductive success, although the impact of interspecific competition is often minimized by differences in preferred spawning habitat (Essington et al. 2000). Since these preferences depend on many factors including body size, food preferences and spawning date, it is plausible that the transgenic females may have altered preferences in preferred spawning habitat, thereby increasing competitive interactions with other species of salmon.

Conclusion

The ability to successfully out compete non-transgenic fish for limited food resources could inhibit the survival of wild fish populations and maintain the size advantage of growth-enhanced individuals. Alternatively, food may be too limited to sustain the enhanced metabolic requirements of the transgenic fish, so that they may starve to death. However, even if they starve to death, they have still had an impact on the wild populations by restricting their intake of food for the period of time before the transgenic fish starved to death. Wild individuals may have suffered increased mortality due to being restricted to a smaller size, or they may have missed niche shifts that would have provided opportunities for increased growth or decreased risk of predation. Besides monopolizing limited food resources, the transgenic fish may exclude the wild fish from streams, forcing them into less profitable areas. If the transgenic fish were able to maintain their enhanced growth, there is the possibility that they would grow so much faster than the wild fish that they would occupy completely different niches, minimizing competitive interactions. Alternatively, they may be so large as to cannibalize the smaller wild fish. In addition to direct competition for food and space, the transgenic fish could cause damage during reproduction. Even if the transgenic fish are sterile, they may go through reproductive behaviour with their increased competitive ability allowing them to secure increased matings relative to wild fish. These matings harm the wild fish population as they are sterile matings that will promote no future addition to the population.

Dunham (1999) identifies foraging ability at one of the key factors in determining the fitness of transgenic fish. Given that the transgenic fish have been shown to be superior competitors relative to non-transgenic individuals, they may have the potential to have a significant impact on the survival of wild coho populations, as well as other species that may share the same habitat. Other factors that may limit the impact of enhanced competitive ability are increased predation and insufficient food resources. The next chapter will address the risk of mortality experienced by the transgenic fish, and the fourth chapter will address the levels of food required for transgenic salmon populations to experience increased fitness relative to wild populations.

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CHAPTER THREE

Relative mortality rates of transgenic and non-transgenic coho salmon in a semi-natural rearing system

CHAPTER THREE – Relative mortality rates of transgenic and non-transgenic coho salmon in a semi-natural rearing system

Introduction

Predation can have a dramatic impact on fish life history both directly and indirectly (see Mather 1998 for a review). Risk of predation will influence individual behaviour and the dynamics of the entire population. Predation risk is especially crucial when considering early life stages of fish, as mortality tends to be high during this stage. Changes in survival rates of larval fish can have large impacts on cohort survival, and therefore population recruitment. Due to the evolutionary pressures of predation over time, fish have evolved complex antipredator behaviour to minimize loss of future fitness.

Although predation has severe repercussions, antipredator behaviour is not without its costs as it takes time away from foraging or reproduction, and requires energy (Werner & Anholt 1993). Alternatively, under low resource availability, risk of predation may induce a behaviourally mediated positive effect on growth by reducing time spent foraging and consequently energy expended (Babbitt 2001). Fish must therefore respond to conflicting demands of growth and risk of mortality. Growth requires searching for and consuming food, which directly increases the risk of being spotted by a predator (Werner and Anholt 1993). Fish must make trade-offs between foraging and antipredator behaviour.

Juvenile coho salmon often forage on items carried by stream current (Chapman 1962) so that the most profitable feeding locations would be shallow areas with swift current (Fausch 1984). The characteristics of profitable feeding habitats also correlate to habitats that have a higher risk of predation due to limited cover or increased exposure to predators. The coloration and patterning of coho salmon, along with their behaviour, make the fish cryptic against the substrate of streams (Donnelly and Dill 1984). Any movement during foraging would reduce the effectiveness of this crypsis, and increase their visibility to predators. Territorial coho salmon must make trade-offs between defending their territory (and thereby maintaining access to food sources) and antipredator behaviour. Territorial defence may increase risk of predation by either increasing exposure to predators through increased movement (Martel and Dill 1995) or interfere with vigilance for predators (Milinski 1984). Increased aggressive behaviour can increase the risk of predation by reducing vigilance of fish for predators (Jakobsson et al. 1995).

Predation risk has long been recognized as a factor influencing the foraging behaviour of fish (Milinski and Heller 1978). Several studies have shown that fish are capable of assessing the level of mortality risk and food in the environment, and making behavioural decisions accordingly. According to Werner and Gilliam (1983), animals should minimize the ratio of mortality to growth in order to maximize fitness. A review by Lima and Dill (1990) lead to the conclusion that animals are capable of making behavioural decisions in response to predation, and these behavioural decisions represent trade-offs between growth and mortality.

Several factors influence the behaviour of juvenile salmonids when feeding under risk of predation. These include fish size, photoperiod, growth rate, hunger, growth hormone, experience with predator, handling time of food, competitors, life history trajectory and species of fish (see Reinhardt and Healey 1999 for review). Level of food in the environment affects the antipredator behaviour of fish as they are making a trade-off between risk of mortality and growth. Risk of predation may be accepted with an increase in profitability of the habitat, so that the numbers of fish in the risky habitat will match the input of food (Gilliam and Fraser 1987; Moody et al. 1996). Abrahams and Dill (1989) also found that spatial distribution of fish is influenced by the spatial distribution of food between habitats. Lima and Bednekoff (1999) developed the "risk allocation hypothesis" which suggests that the temporal variation in predation risk is the driving force behind antipredator behaviour. According to this hypothesis, fish should exhibit high antipredator behaviour in brief or infrequent risk situations, and should decrease antipredator behaviour as risky situations increase in length or frequency.

Fish may respond to predation risk in one of two ways: (1) adjust spatial distribution; or (2) adjust allocation of time spent in various activities (foraging, competition, and antipredator behaviour). Grand and Dill (1996) demonstrated that when risk of predation is increased in a profitable habitat with little protective cover, the proportion of fish in the poor habitat, with cover, will increase. When studying the spatial distribution of fish in response to predation, risk is not the only variable involved in determining the final distribution. Other factors such as competitive ability and population density may also impact movement of fish. Grand and Dill (1999) compared the effects of competitive ability and density on spatial distribution of fish under risk of predation. Competitive ability may be positively or negatively correlated with mortality risk, such that the best competitors may experience the highest risk of predation, or the lowest risk of predation. If mortality risk is independent of population density, the competitive type experiencing the highest risk of mortality will distribute in the safer, less productive site. If mortality risk is dependent on population density, and the best competitors have the highest mortality risk, both types will occur in the risky, productive habitat. If the poor competitors experience the highest mortality risk, both types will occur in the safer, less productive site.

When resources are abundant or risk of predation increases, fish will tend to reduce their activity (Anholt and Werner 1998). Animals, under increasing risk of predation, are expected to decrease the proportion of time spent active and foraging speed (Werner and Anholt 1993; Leonardsson and Johansson 1997). Atlantic salmon will forage at night to reduce their risk of predation and, concurrently, their growth rate (Metcalfe and Fraser 1997). When exposed to a predator, territorial coho salmon reduced their movement and aggressive behaviour, but still maintained their territories and some level of aggression (Dill et al. 1981, Martel and Dill 1993, Martel 1996). Mortality risk can have a dramatic impact on a foragers' time allocations (Abrams and Schmitz 1999). Utne et al. (1997) found that perch responded to risk of predation by decreasing the amount of time spent foraging in the risky patch. Brown (1999) found that fish respond to predation risk by adjusting either the time spent in the risky patch or time spent in vigilance for predators.

Due to increased metabolic demands of enhanced growth, transgenic salmon are expected to demonstrate an increased risk of mortality in order to feed. Predation risk will have less of an influence on an individual's behavioural decisions if they gain a greater fitness advantage from the increased food relative to other individuals (Abrahams and Dill 1989). In the case of transgenic salmon, increased food will support their increased growth rate leading to an increased fitness advantage. Factors such as the metabolic state of an individual determine how much mortality risk that individual will accept in order to find food (Magurran 1993). Hungry fish or fish experiencing competitive interference for food are less responsive to predators (Milinski and Heller 1978; Dill et al. 1981, Chick and Van Den Avyle, 2000), and will travel greater distances (increasing exposure to predators) when hungry (Dill et al. 1981), while well-fed or slow-growing and mature fish have demonstrated increased antipredator behaviour (Reinhardt and Healey 1999, Utne et al. 1997). More specifically for coho salmon, weight-compensating individuals will decrease antipredator behaviour by foraging in the risky, more productive, patch (Damsgård and Dill 1998). Abrahams and Pratt (1999) found that growth rates are an accurate predictor of antipredator behaviour where fish with high growth rates are more willing to risk predation in order to feed. Transgenic Atlantic salmon demonstrated increased rates of movement, and spent more time feeding in the presence of a predator, relative to non-transgenic fish (Abrahams and Sutterlin 1999). Transgenic catfish are more susceptible to predation compared to non-transgenic individuals (Dunham et al. 1999). After attacks by a predator, growth hormone treated rainbow trout resumed feeding earlier and fed closer to the water surface compared to untreated individuals, thereby increasing their consumption of food at an increased risk of being consumed by a predator (Johnsson et al. 1996).

Besides increased metabolic demands, transgenic salmon may have an adjusted phenotype making them more susceptible to predation. Johnsson (1993) predicted that selection for large body size under reduced predation pressure (such as in a hatchery) will shift the optimal trade-off between growth and mortality in the favour of a "high gain –

high risk" phenotype. Domestication in steelhead trout has been found to increase the level of risk fish are willing to take in order to forage for food (Johnsson and Abrahams 1991). Artificial selection, in addition to growth hormone, has been found to reduce antipredator behaviour (Johnsson et al. 1996). If allowed to feed under natural conditions in the presence of predators, the transgenic salmon may undergo a higher rate of mortality as a consequence of their increased appetite and corresponding growth rate. Presence of predators may limit the success of transgenic salmon in the wild.

Objectives

If transgenic salmon are to maintain their growth advantage under natural conditions, it is predicted that they would be willing to suffer higher mortality rates while foraging. The prediction generates the hypothesis that the transgenic fish will have higher mortality rates relative to control fish when foraging in a semi-natural rearing system. Although contained within the laboratory, the rearing conditions for this experiment were designed to simulate natural conditions. A rearing system was produced with three distinct habitats: (1) no food, no predator; (2) low food concentration, no predator; and (3) high food concentration, predator. Transgenic and wild individuals were placed together in the system. The relative mortality was observed as the individuals competed for food resources in the presence of a predator.

Methods

Experiment Subjects

All fish used in this experiment were sampled from a mixed family of transgenic and non-transgenic coho salmon. For each trial, two groups were sampled from this family: an experimental group and a control group. Each group consisted of 50 randomly selected individuals. The experimental group was the group of fish that was placed into the rearing system, while the control group was merely sampled and preserved. The novel aspect of this experiment was that the transgenic and non-transgenic fish were both size and age-matched, since they were so young.

The predators used in these experiments were hatchery reared cutthroat trout (*Oncorhynchus clarki*) with a mean mass of 100.1 g (SD = 15.97). Since the predators were hatchery reared, they had to be trained to capture and consume live prey. To accomplish this goal, I began feeding the trout coho salmon alevins. The alevins were placed into the tank so that they would fall in front of individual trout. Once all trout were consuming the alevins, I then began feeding them coho salmon fry. These were initially placed into the tank right in front of individual trout. Once the trout were effective at capturing and consuming the fry when placed in their immediate vicinity, I added the fry to the tank at the end opposite to the location of the trout. In this manner, I was able to establish predators effective at capturing and consuming live prey.

Experiment Set-up

Three round tanks 1 m in diameter were connected with shower drains that were 10 cm at the opening and 5 cm in the centre. The tanks were connected in a triangular configuration (Figure 3.1) so that each tank was connected to the other two, and a fish in

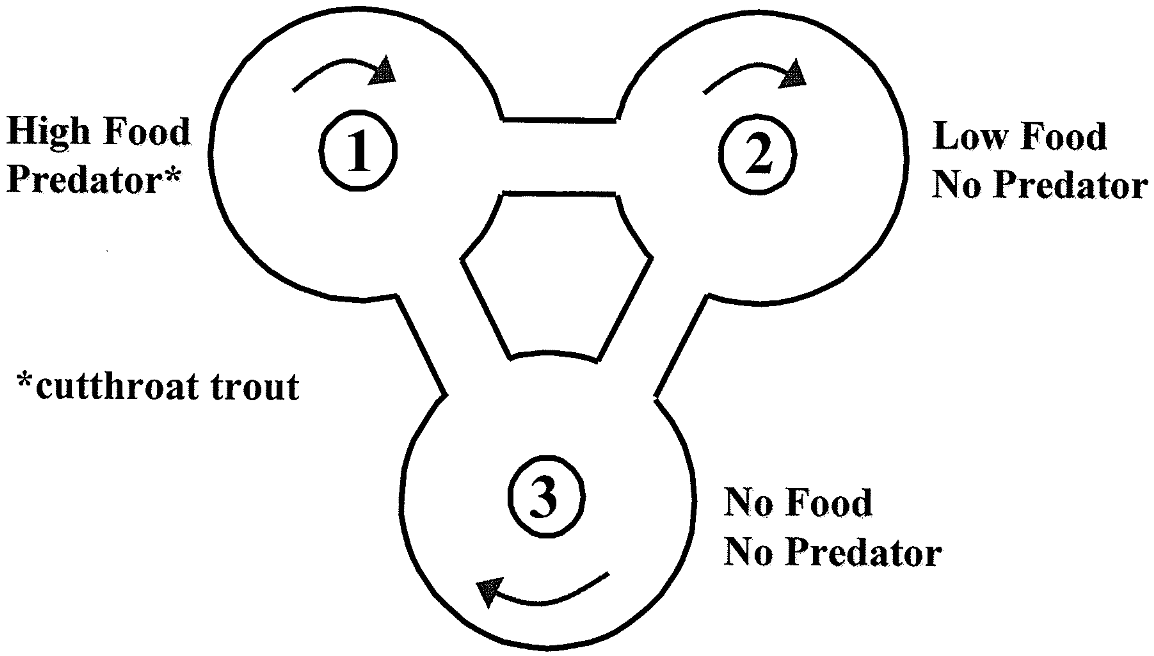

Figure 3.1. Tank set-up for the mortality experiments.

any one tank had equal access to the other two tanks. A 20 L pail was placed upside down in the centre of each tank to form a raceway around the perimeter. In each tank, the water depth was 30 cm with an approximate flow of 10 cm/s (maintained by adjusting the rate of water change) and a temperature of 11°C. Although the system had a continual supply of fresh water, there was no risk of the fish escaping from the system. The system followed strict requirements ensuring containment of transgenic fish (with the use of screen traps). The high food, predator habitat contained four predators that were free to capture and eat any salmon in the habitat.

Twice as much food was placed into the high food habitat compared with the low food habitat (0.2 and 0.1 g, respectively, of Nutra Plus Starter mash). The riskier habitat (with a predator) was chosen to be more profitable (high food concentration) so that there would be a conflict in whether to risk mortality for increased food. If the safe and risky habitats were both as profitable, there would be no reason for the salmon to risk mortality (Ludwig and Rowe 1990; Grand and Dill 1999). Werner et al. (1983) have observed that in natural communities, the richer habitat incurs a higher predation risk. Additionally, this arrangement should tend to segregate the individuals proportionately throughout the system.

Experimental Procedure

To ensure that the fish would distribute among the three habitats, a control trial without food or predators was run. Ten individuals were placed into the system at habitat 1 and were kept there overnight. In the morning, two individuals were in habitat 1, three in habitat 2 and five in habitat 3. This demonstrated that the salmon were indeed capable of distributing themselves within the system. A group of fifty individuals was randomly selected from the mixed group of transgenic and non-transgenic salmon. These individuals were weighed and frozen for later PCR analysis to estimate the initial ratio of transgenic to non-transgenic fish. Another group of fifty individuals (the experimental group) was randomly selected, weighed and placed into the system at habitat 1. Within two days, the predators had consumed approximately 50% of the individuals in the tank. At this time, the remaining individuals were removed from each habitat, weighed and preserved for PCR analysis. The fish were weighed before and after the experiment to ensure that size-selective mortality was not the explanation for any observed differences in mortality between the two groups. This experiment was conducted a total of three times.

Results

Once the PCR analysis was completed, I calculated the proportion of transgenic fish in the six groups (the three groups that acted as controls and the three groups that were placed into the rearing system). Contrary to what was expected, the mean proportion of transgenic salmon in the groups that were not placed into the system (53%) was not significantly different from the mean proportion of surviving transgenic salmon (75%) in the experimental groups (Figure 3.2, $\chi^2 = 0.352$). In fact, the survival of transgenic fish within the system was slightly higher than the survival of non-transgenic controls.

Figure 3.2 depicts the size-distribution of the fish before, and after, the mortality experiments. This graph demonstrates the typical bimodal size-distribution of a mixed group of transgenic and non-transgenic fish, with the non-transgenic fish being those

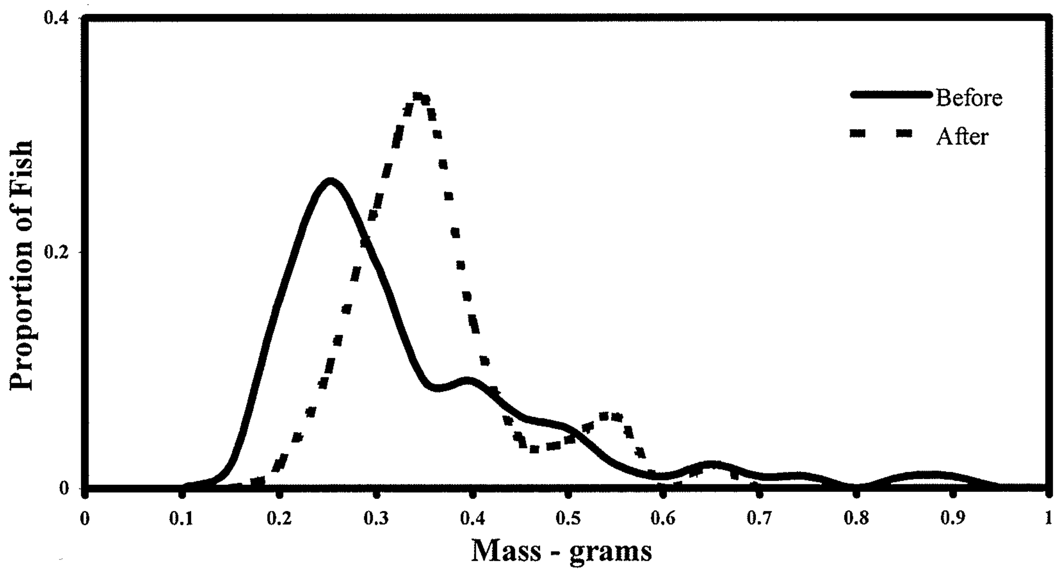

Figure 3.2. Size-distribution of fish.

Size-distribution of the salmon before and after the three mortality experiments. There was a significant difference in the size distributions, when pooled into six categories and compared with a G test (G = 11.86, d.f. = 5, p = 0.037).

individuals under the first portion of the distribution, and the transgenic fish being those individuals under the second, and much wider, portion of the distribution. When you compare the size-distribution of the fish before the experiment to the size-distribution of the fish after the experiment, you can see a slight shift in the size-distribution, as well as a "cropping" of the distribution at either end. This indicates that there may be selective mortality for the smallest, and the largest, individuals which would help to explain why there is no observed difference in mortality between the transgenic and non-transgenic fish (the transgenic fish would be the largest individuals and the non-transgenic fish would be the smallest individuals). Statistical analysis indicates a significant differencebetween these distributions ($G = 11.86$, d.f. = 5, $p = 0.037$). For this analysis, the data were grouped into six categories.

Discussion

In this study, the transgenic coho salmon did not experience higher levels of mortality relative to non-transgenic controls. This suggests that there may be a range of growth rates that provide the same level of fitness, as suggested previously by Mangel and Stamps (2001). Mangel and Stamps (2001) used a life-history model to demonstrate that individual trade-offs in growth and mortality allow equal fitness for individual variation in growth rates.

It would be useful to have tracked the location of the fish within the system, so data could be obtained on habitat use for the transgenic and control fish to determine if the transgenic fish are spending more time in the risky habitat, but experiencing decreased mortality relative to the control fish. A dynamic optimization model developed by Grand

(1999) illustrated that the largest fish of a cohort would favour the high-risk habitat relative to the smallest fish, when mortality is size-dependent.

Johnsson (1993) found that large trout are willing to risk increased predation in order to feed, and compares these results to the contradicting results of Grant and Noakes (1987) who argue that increasing body size should increase antipredator behaviour. I think that both results are possible, but you must consider a temporal factor as well. If the juvenile fish are still growing and attempting to reach a certain size that will enable them to smolt successfully, one would expect them to risk increased mortality in order to reach that size. Once individuals have reached the size necessary for smolt, they would be expected to protect that investment, and reduce their exposure to predators. Small Atlantic salmon parr that are not smolting will maintain a state of adaptive anorexia over the winter (Metcalfe and Thorpe 1992), while larger individuals that will smolt in spring continue to feed, indicating that fish are capable of making foraging decisions according to long-term energy requirements, in addition to meeting short-term needs (Bull et al. 1996). Following premises of the Asset Protection Principle, large coho salmon should only accept the same level of mortality as small fish if they are either hungrier or they receive a greater growth benefit (Clark 1994). Reinhardt and Healey (1999) have demonstrated that juvenile coho salmon are responsive to photoperiod cues, and their own body size, when adjusting their feeding behaviour.

This experiment has not considered temporal variation in predation risk that would more closely approximate the conditions found in the natural environment. The observed behavioural pattern when experiencing a constant risk of predation may differ from situations where predation risk varies over time. Lima and Bednekoff (1999) introduced the "risk allocation hypothesis" which predicts that optimal foraging behaviour will depend not only on the absolute level of predation risk, but also on the context in which it is experienced. In other words, fish experiencing brief, intense periods of high predation risk will allocate a great proportion of time and resource to antipredator behaviour during those periods, and will forage during the low-risk periods. In light of the risk allocation hypothesis, contrary to the majority of studies that provide a brief, intense period of high-risk, this study provided a more continuous period of high-risk. Therefore, the risk of predation may be overestimated in this study, as the constant source of predation may have required vigilance and other anti-predator behaviour that could not be sustained continuously. It is unlikely that fish would find these conditions in the natural environment.

Alternatively, viewed from the perspective of resource levels within the system, predation risk may have been underestimated. The high-risk / high-food habitat may have had such high levels of food that the fish could reduce their activity to minimize predation, and still consume a high amount of food. Anholt and Werner (1998) found in their experiments that mortality of tadpoles was lower at higher food levels, due to reduced activity when foraging for food. If risk-taking behaviour is indeed state dependent (Damsgård and Dill 1998) the mortality rate experienced by the transgenic fish will depend on their hunger level. However, given the current understanding of transgenic fish, they are expected to maintain a continual search for food and not demonstrate reduced activity such as would be expected for the control fish. This is one of the ways they are able to maintain their enhanced growth relative to the control fish.

There is evidence from these experiments that the group experienced size-selective mortality for both the smallest and the largest individuals, however the results were not significant. Most often, we intuitively think of the smallest fish as being more susceptible to predation. Rice et al. (1993) found that higher growth rates among fish larvae cohorts generally led to higher survival rates, with the size-distribution of the survivors being larger than what would be expected by extrapolating growth rate, indicating that the fastest growing, and hence larger individuals are more successful at avoiding predation. However, there are documented cases in which the larger fish were selected by the predator. Quinn and Kinnison (1999) found that brown bears selectively preyed upon larger sockeye salmon. Large fish size may be detrimental at times if predators preferentially attack the larger individuals (Litvak and Leggett 1992). Paradis et al. (1999) found evidence of counteracting components of vulnerability to predators in a model they developed to test for size-selective mortality.

Potential ecological impact

Without increased mortality rates, the transgenic salmon have the real potential to successfully survive in the wild, provided they could consume enough food to maintain their growth rates. The transgenic fish, with a potential to successfully out-compete non-transgenic fish and similar rates of mortality when size-matched, could lead to competitive displacement of the wild fish if they were able to maintain a size advantage and thereby experienced decreased levels of mortality (Werner and Gilliam 1984).

This experiment only provided information on the relative survival rates of the transgenic and control fish with no indication of relative growth rates. So, it is still not known whether the transgenic fish sacrificed enhanced growth in order to reduce their mortality. They may have fed in the productive, high-risk habitat but merely experienced lower mortality due to slight size differences or altered behaviour. Alternatively, they may have sacrificed food in order to avoid the predators. Ludwig and Rowe (1990) predicted that an increase of predation risk in one habitat would serve to decrease the body size throughout the population. This would mean that the transgenic fish would be consuming less food than required in order to maintain their enhanced growth. On experiments conducted on tadpoles, the effect of predation risk on growth was found to vary depending on the level of food in the environment (Babbitt 2001). When food levels were high, an increase in predation risk decreased growth of the tadpoles. However, the opposite effect was observed when food resources were low. In this situation, increased predation risk actually increased growth of the tadpoles. One explanation for this is that the tadpoles were able to utilize their limited food resources more efficiently by decreasing their activity in response to the increased risk of predation. It is not known if the transgenic fish would respond in this manner, and what the crucial level of food would be to replicate this observation.

If the transgenic fish are initially able to outgrow their predators, high risk of predation may increase the growth advantage of transgenic fish by reducing the competition for food resources in high-food / high-risk habitats. Werner et al. (1983) found that in a population with different size-classes, where the small fish were susceptible to predation by bass and the larger fish were not, presence of the bass increased growth of the larger fish due to reduced competition for resources in the high-food habitats. Further experiments are required to determine if the transgenic fish are able to maintain a growth advantage even under high predation relative to wild fish, or if their growth rates would drop as low as those of the native fish populations.

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CHAPTER FOUR

Individual-based population model using dynamic optimization

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Model Development

One of the biggest problems in doing research with transgenic organisms is attempting to predict the ability of the organism to invade a natural environment strictly from the results of laboratory experiments. It is not possible to do field experiments with these genetically manipulated organisms due to risk of escape. Some theoretical interpretation is required to make the leap from the laboratory to the wild. It has been suggested that a combination of laboratory experiments and the development of computer models may assist in forming more reasonably accurate predictions in a natural setting (Kareiva et al. 1996, Kareiva 1989). Dynamic state variable models are a means of conceptualizing constraints and trade-offs in ecology (Clark and Mangel 2000, Mangel and Clark 1988). More specifically related to the issue of the ecological impact of growth-enhanced salmon on wild salmon populations, dynamic state variable models provide a method for incorporating behavioural decisions made by individuals during foraging in order to predict the relative fitness, or survival success, of the growth-enhanced fish.

Overview of Model

This computer model combines literature derived performance data for transgenic and non-transgenic coho salmon with some estimated values to predict what effect these interacting components may have on the survival of the transgenic fish in relation to the non-transgenic fish. For the model, the focus is on juvenile viability, or an estimate of the probability of survival to smolt. This stage was chosen as it is the stage with the most available experimentally derived information regarding growth, survival and behaviour. For example, there is a lack of estimates of mortality of Pacific salmon during the marine phase of their life, and certainly no information pertaining to daily or weekly time increments as would be generated within a computer simulation (McGurk 1996). In addition, the juvenile stage is generally thought to have the greatest impact on overall fitness (Clutton-Brock 1988).

Fish in this simulation have three foraging options available to them: hiding, territorial and active foraging. Foraging strategies are an appropriate focus, as they determine food consumption, which then determines the variability in growth. Variability in food consumption had been found to be a major determinant in the variability in fish growth. The three foraging strategies correspond similarly to three behavioural options outlined by Puckett and Dill (1985): territorial, nonterritorial and floater. Territorial fish defend a feeding area in which it spends most of its time. Nonterritorial fish do not defend an area but are occasionally aggressive, while floater fish exist in spaces between territories of other fish. In my model, the active foraging strategy is slightly different from the floater strategy description used by Puckett and Dill (1985), and was developed after observing the foraging behaviour of transgenic fish within the laboratory. In this model, actively foraging fish can be aggressive to other fish and spend their time moving throughout a range, which may include territories of other fish, in search of food. A hiding strategy was chosen as a third option to make a sharp contrast in predation rates and food availability, relative to the territorial and active foraging strategies. As Puckett and Dill (1985) also indicate, an individual fish may not adopt one strategy permanently, but may spend a portion of time within each strategy on a daily basis. Although coho salmon may also alter foraging behaviour by selecting larger or smaller prey according to the level of mortality risk in the environment (Dill and Fraser 1984), this will not be considered in this model in an effort to maintain simplicity to facilitate interpretation of the results.

Energy costs for the three foraging strategies were as follows: active foraging > territorial > hiding. Intuitively, territorial behaviour will impose more energetic costs in relation to hiding behaviour, since the fish are actively defending a territory. Although territorial behaviour is costly with aggressive interactions, aggressive displays and feeding forays, the active foraging strategy incurs a higher energetic cost. This is because the fish are constantly in search of food and may be required to participate in aggressive interactions should they infringe on another individual's territory. Energetic gains for the three foraging strategies follow the same pattern as energy costs: active foraging > territorial > hiding. The more profitable habitat is generally considered to be the riskier habitat (Ludwig and Rowe 1990) to ensure that there is a conflict between foraging and predation.

Success of individuals within a dynamic optimization model is defined by the terminal fitness function. In this particular simulation, the terminal fitness function was chosen to be the probability of survival from smolt to adult, which is used as a measure of future reproductive success (Figure 4.1). Body size and condition factor of the fish are the states that affect the terminal fitness function, or probability of survival. It has been long known that survival of Pacific salmon smolts to adult is positively correlated with body size (Foerster 1954; Ricker 1962). More specifically, Holtby et al. (1990) found a

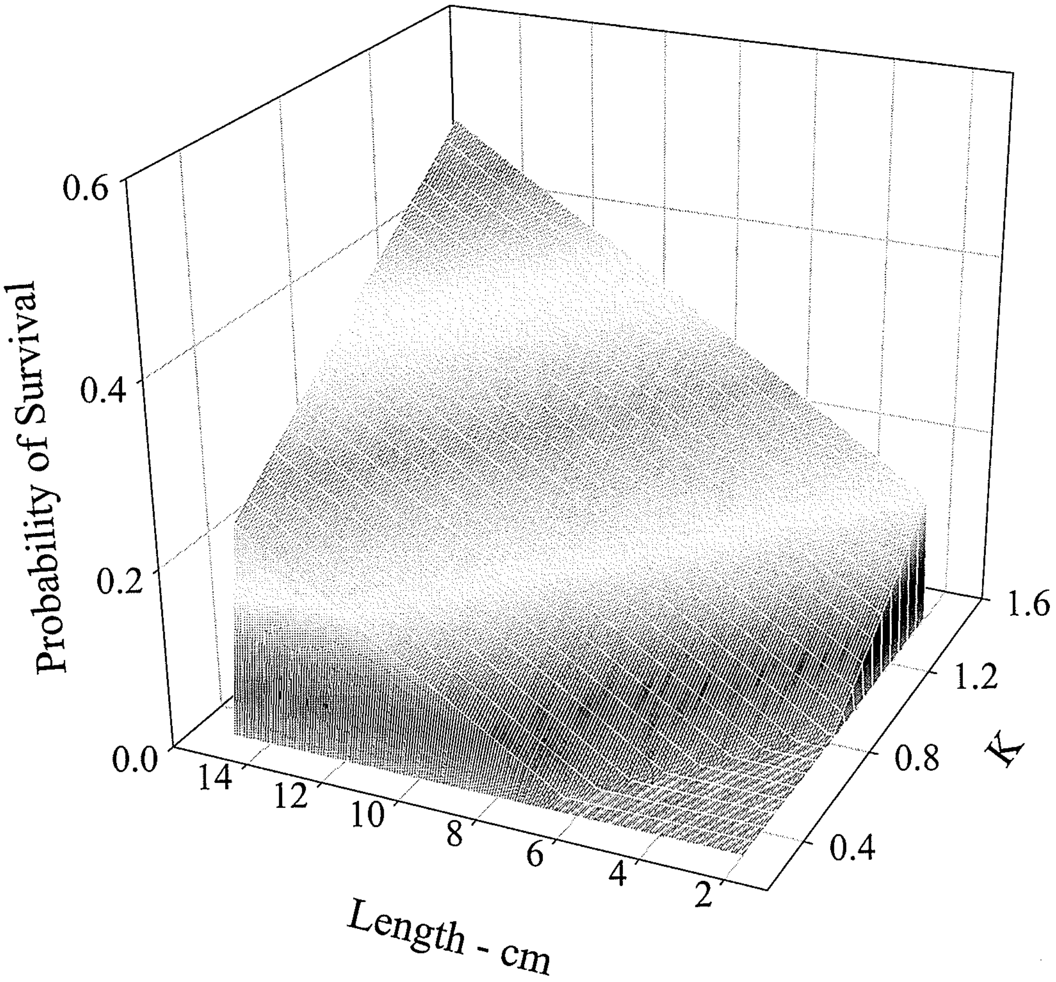

Figure 4.1. Terminal fitness function.

Terminal fitness function showing the relationship between K (condition factor), length of the fish, and the probability of surviving smolt (Labelle et al. 1997; Mangel 1996; Quinn and Peterson 1996; Holtby 1990).

significant positive correlation between body size and survival to adult for coho salmon. A positive correlation was also found between body size and over-winter survival for juvenile coho salmon (Quinn and Peterson 1996).

The model has two main components: the dynamic optimization model and the forward iteration. The dynamic optimization model works backward through time and serves to define the foraging strategy decisions an individual should take in order to maximize its fitness. One foraging strategy is chosen for each combination of size, condition and time remaining to until the end of the life-stage (winter in this simulation). The forward iteration uses the results from the dynamic optimization model to work forward through time, and simulates the daily foraging decisions of an individual over the defined time period. The forward iteration simulates the natural environment and determines if the fish is consumed by a predator, or is able to find food and grow. Refer to Table 4.1 for further information on the variables included in this individual-based population model.

Dynamic Optimization Model

The state of each individual at the beginning of each time period, *t*, is represented by its mass, $x(t)$, in grams. Mass has upper and lower physiological limits such that:

$$x_{\min} \leq x_{(t)} \leq x_{\max} \tag{1}$$

If $x_{(t)}$ falls below $x_{\min}$, the fish dies of starvation. For all simulations $t = 100$, with one time period representing one day. A time period of 100 days was chosen as an estimate of the number of days the juvenile salmon will have to feed before seasonal reduction of food resources in the fall. At the beginning of each time period, individuals can choose one of three feeding strategies. In strategy 1 the fish are hiding, in strategy 2 they are

Table 4.1. Variables used within the individual-based population model.
T and NT refer to transgenic and non-transgenic specific variables, respectively

	Values Investigated		Description	Reference
β_i	0 to 10		used to calculate rate of predation for each strategy	
λ_i	0.0 to 0.2		used to calculate probability of finding food for each strategy	
α_i	0.01 to 0.06		used to calculate metabolic costs for each strategy	
y_i	0.09, 0.18		used to calculate the increase in mass if food is found	
days	100			
handlingtime	0.5		variable used to calculate food consumed	adjusted to achieve desired levels of food consumption
iterations	1000		number of times to run through the forward iteration	trade-off between computer limitations and an acceptable level of variability
Kincrements	20		discretizes condition variable	
Kmax	1.5		maximum condition	
Kmin	0.3		minimum condition, below which the fish will starve to death	
Kopt	1		optimum condition, any excess energy will be directed to growth in length	
p_1	-0.5		variable relating length to risk of predation	adjusted to achieve predation levels as in Grand 1999
p_2	100		variable relation K to risk of predation	adjusted to achieve predations levels as in Grand 1999
Tffvar1	0.269		variable in the terminal fitness function	Mangel 1996; Holtby et al. 1990
Tffvar2	0.027		variable in the terminal fitness function	Mangel 1996; Holtby et al. 1990
Tffvar3	0.25		variable in the terminal fitness function	Labelle et al. 1997; Quinn and Peterson 1996; adjusted so that maximum survival corresponds to literature values
xincrements	200			
xmax	15		maximum length	Weatherley and Gill in Groot, Margolis and Clarke 1995
xmin	2		minimum length	Weatherley and Gill in Groot, Margolis and Clarke 1995
	T	**NT**		
γ	2.2	1	coefficient for the conversion of energy to length	Stevens and Devlin 2000
δ	1	1	coefficient for the conversion of length to energy	Brett in Groot, Margolis and Clarke 1995
costmultiplier	1	1.6	scales relative metabolic costs	Stevens and Sutterlin 1999; Stevens et al. 1998

territorial, and in strategy 3 they are actively foraging, but not maintaining a territory. The three strategies, $i = 1, 2, 3$, are characterized by four primary parameters: (1) probability of predation per time period, β_i; (2) chance of finding food per time period, λ_i; (3) energetic cost of foraging, α_i; and (4) increase in mass, if food is discovered, Y_i.

Probability of predation, as used in this simulation, includes all sources of mortality. Mortality risk is proportional to both body size, x, and foraging speed, s. Foraging speed is calculated from mass, using the relationship:

$$s_{(x)} = 2 * (x / 0.01)^{1/3} \tag{2}$$

This relationship makes use of the assumption that foraging speed is equal to 2 * L, where $L = (x / 0.01)^{1/3}$ (Brett 1971, 1975; Beamish 1978). Predation rate for each strategy is then calculated as:

$$\beta_{(x,\, i)} = \beta_i x^{p1} + (\beta_i / p_2)\, s_{(x)} \tag{3}$$

where β_i represents the strategy specific risk of mortality and p_1 and p_2 are related to such factors as changes in capture or detectability with changes in mass or speed, respectively (Werner and Anholt 1993; Leonardsson and Johansson 1997). The baseline mortality rate, β_{base}, is defined as the probability of mortality for a fish of mass $x = x_{\min}$. Variables are set so that rate of predation is inversely proportional to mass, and increases linearly with foraging speed. In the model, $\beta_1 < \beta_2 < \beta_3$, where $\beta_{base1} = 8.24 * 10^{-5}$, $\beta_{base2} = 8.24 * 10^{-4}$, and $\beta_{base3} = 8.24 * 10^{-3}$. Probability of finding food, λ, is calculated as:

$$\lambda_{(x,\, i)} = s_{(x)} \lambda_i / (1 + s_{(x)} h \lambda_i) \tag{4}$$

where λ_i is the resource density for each strategy and h is handling time (Werner and Anholt 1993; Leonardsson and Johansson 1997). Throughout the simulation, $\lambda_1 < \lambda_2 < \lambda_3$.

This model is designed to track the survival of fish from hatch until their first winter. The lifetime fitness function, $F_{(x,\, t,\, T)}$, can be defined as the maximum expected value of the terminal fitness function based on behavioural decisions taken between time t and T, when the value of the state variable is x. The terminal fitness function (Figure 4.1) is the probability of surviving smolt at size x:

$$f_{(x,K)} = \text{-tffvar1} + (\text{tffvar2})(x) + (\text{tffvar3})(K) \qquad (5)$$

where tffvar2 and tffvar3 represent the slope of the relationship between size and survival, and K and survival, respectively (Mangel 1996, McGurk 1996, Quinn and Peterson 1996; Holtby et al. 1990, and Bilton et al. 1982) For each strategy, with probability λ_i:

$$x_{1(t)} = x_{(t-1)} + \gamma(Y_i - \alpha_i)_+ - \delta(\alpha_i - Y_i)_+ \qquad (6)$$

and with probability $(1 - \lambda_i)$:

$$x_{2(t)} = x_{(t-1)} - \delta\, \alpha_i \qquad (7)$$

where γ is the conversion factor of excess food to length, and δ is the conversion factor of length to energy (Mangel & Clark, 1988). Since there are minimum and maximum values of x, x_1 and x_2 must be constrained so that if $x_1 > x_{\max}$, then $x_1 = x_{\max}$ and if $x_2 < x_{\min}$ then $x_2 = x_{\min}$.

The fish will survive period $(T - 1)$ if it is not killed by a predator, with probability $(1 - \beta_i)$, and its state variable will change from $x_{(T-1)}$ to $x_{(T)}$ as given by Eq. (6) and (7). The probability of surviving both periods $(T - 1)$ and T, if strategy i is chosen in period $(T - 1)$, is:

$$(1 - \beta_i)\,[\,\lambda_i F_{(x1i,\, T,\, T)} + (1 - \lambda i) F_{(x2i,\, T,\, T)}] \qquad (8)$$

Once this is calculated for each strategy, $F_{(x, T-1, T)}$ is the maximum of the these probabilities, relative to strategy choice. In other words, the model picks the strategy providing the maximum probability of survival. Continuing this process, the program works backward iteratively to eventually solve $F_{(x, 1, T)}$. Each iteration calculates $F_{(x, t, T)}$ and provides the optimal strategy selection, dependent on both x and t.

Forward Iteration

Using the decision matrix generated by the dynamic optimization model, the forward iteration measures growth of an individual over time. The forward iteration follows the same dynamics as the dynamic optimization model, but iterates forward in time instead of backwards. So, the individual begins at $t = 1$, and chooses the optimal feeding strategy as determined by the dynamic optimization model. If the individual is not consumed by a predator, with probability $(1 - \beta_i)$, the fish then has the opportunity to feed. If it finds food, with probability λ_i, it grows. If the fish does not find food, with probability $(1 - \lambda_i)$, it loses mass to pays costs of foraging. If the fish is not below $x_{\min}$, it moves on to $t = t + 1$. For all simulations, the number of forward iterations was set at 1000.

Sensitivity Analysis

Sensitivity analyses were conducted to examine the effect of food availability and rate of predation in the environment on the relative survival of transgenic and non-transgenic fish. For this analysis, I manipulated β (risk of predation) and λ (probability of finding food). Refer to Table 4.1 for the set values of all other parameters. For each possible combination of β and λ, I obtained the difference in ratio of transgenic to non-transgenic fish from the start of the simulation to the ratio of transgenic to non-transgenic fish remaining at the end of the simulation. By manipulating these variables, I was able to define an environment in which the transgenic salmon could survive successfully, and potentially outcompete wild salmon populations. A multiple regression analysis was applied to the data to assess which variables (β, λ or initial size) were contributing the most to the relative success of transgenic fish in the wild.

Individuals from both groups (transgenic and non-transgenic) were expected to spend less time in the high risk / high food foraging strategy (active foraging strategy) as the probability of finding food increased, or the risk of predation increased. It was predicted that the transgenic salmon would risk increased mortality for the increased probability of finding food, as defined by the active foraging strategy. Transgenic salmon should spend more time actively foraging than in territorial behaviour, relative to the non-transgenic individuals.

Results

General foraging behaviour

As predicted, fish from both groups spent a greater proportion of time in the safer, territorial habitat as risk of predation increased. In Figure 4.2, as risk of predation increased from β_{base} to 5 x β_{base} to 10 x β_{base}, both the transgenic and non-transgenic fish became less likely to choose an active foraging strategy. However, this result was only clearly obvious when the probability of finding food was high ($\lambda = 0.1$). When the probability of finding food was low ($\lambda = 0.05$), fish from both groups almost exclusively chose the high risk, high gain foraging strategy. It was also observed that as the fish in

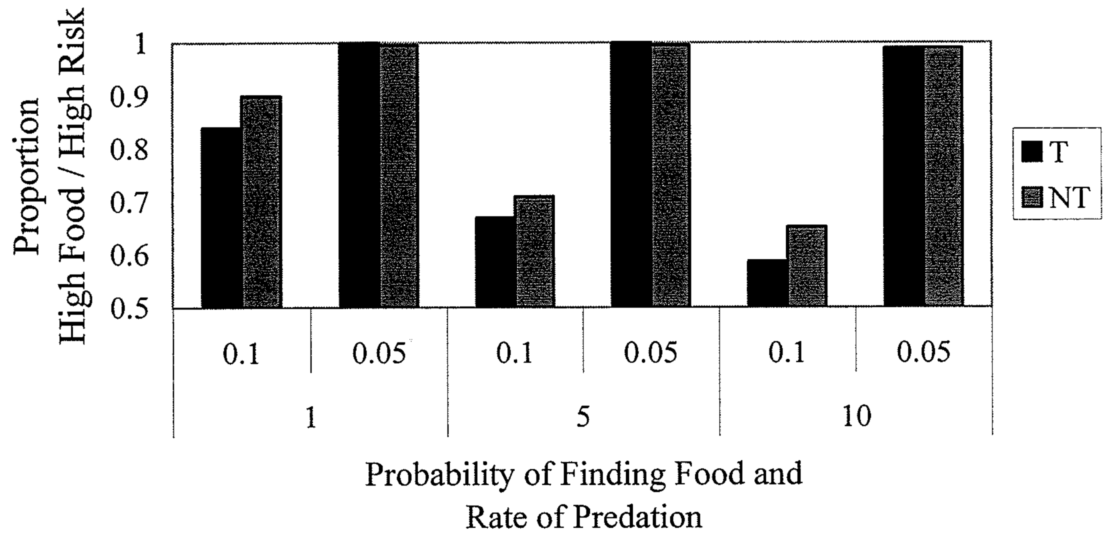

Figure 4.2. Proportion of time in high risk strategy.

The proportion of times (at t = 1) transgenic and control individuals chose the high gain / high risk foraging strategy for all possible size ranges from hatch to smolt (2 – 15 cm). The proportions were calculated for β_{base}, 5 x β_{base}, and 10 x β_{base}, representing low, medium and high rates of predation, respectively. For each predation rate, a simulation was run at both a high (0.1) and low (0.05) probability of finding food.

the simulation approached the end of the time frame for the simulation, they demonstrated an increased willingness to accept increased risk for the increased food uptake in the active foraging strategy.

Contrary to my hypothesis, the transgenic fish did not spend a greater proportion of time in the high risk, high gain active foraging strategy at high food levels (Figure 4.2). In relation to the non-transgenic fish, the transgenic fish spent approximately 5% less time in the active foraging strategy. This difference remained the same across all levels of predation compared. This result switched at low food levels, when the transgenic fish spent as much, or slightly more time in the high risk, high gain strategy.

Risk of predation

According to my hypothesis, risk of predation was expected to be one of the environmental conditions most crucial in determining the fate of transgenic fish introduced into a natural environment. As expected, risk of predation did decrease the relative survival rate of transgenic fish. At higher levels of predation, the relative survival rate of the transgenic fish decreased rapidly as predation rate increased (Figure 4.3). It was also interesting to note the increased variability in relative survival as predation rate increased, even though the number of iterations remained the same (iterations = 1000). Results of the multiple regression analysis confirm that relative survival is negatively correlated with risk of predation, and indicates that risk of predation explains 7.93% of the variation in the results of the simulation (Table 4.2).

Probability of Finding Food

Food was another variable hypothesized to be important in deciding the fate of transgenic salmon in the wild. Under low food levels, it is predicted that the transgenic

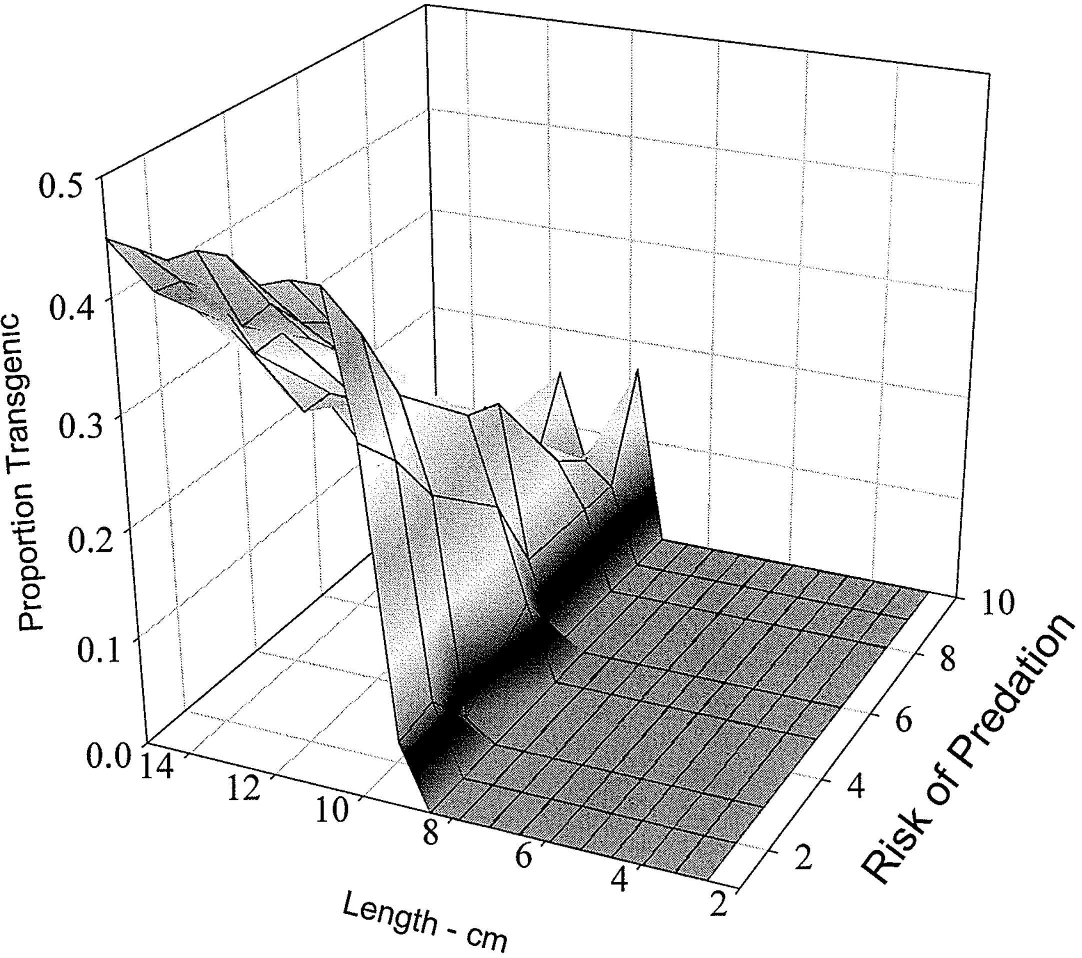

Figure 4.3. Relationship between β, size and survival.

Effect of predation rate and size of fish on the relative survival of transgenic individuals at high rates of predation, where β ranged from β_{base} to 10 x β_{base}. For this simulation, K = 1.02, λ = .05, and iterations = 1000.

Table 4.2. Multiple regression analysis of the sensitivity analysis data.

The probability of survival of the transgenic fish = $-1.120 - 0.186\beta + 0.614\lambda + 0.494x$.

Condition factor was excluded from the equation.

Multiple R	0.743
R Square	0.552

	df	Sum of Squares
Regression	3	1925.760
Residual	38459	1563.563

F = 15789.322

Sig F = .000

Variables in the Equation

Variable	t	Sig t	% Contribution
Beta	-54.099	0.000	7.93
Lambda	178.198	0.000	26.19
Length	142.781	0.000	21.08
(constant)	-43.116	0.000	
	Total Variance Explained		55.2
	Variance Unexplained		44.8

fish would not have access to the amount of energy necessary to maintain their enhanced growth and meet their increased hunger. The fish may spend more energy than they could find to consume.

From the sensitivity analysis, it was clear that the amount of food in the environment did have a positive effect on the relative proportion of transgenic fish surviving to smolt (Figure 4.4). At low food levels, individuals had to begin the simulation at a large size in order to experience fitness equal to the non-transgenic fish. As food increased, the minimum size required for equal fitness between the two groups decreased. As λ approached the maximum values tested for the iteration (maximum $\lambda = 0.20$), the relative survival of transgenic fish was at times slightly higher than 0.5, indicating that the transgenic fish experienced increased fitness relative to the non-transgenic fish. Multiple regression analysis found a positive correlation between relative survival rate and probability of finding food, with the probability of finding food explaining 26.19% of the variation in the simulation results (Table 4.2).

Effect of initial size on relative survival

From the results of the sensitivity analysis, it is obvious that the size of the fish at the beginning of the simulation has an important contribution to the relative survival of the transgenic fish. Once the fish reaches a minimum size, which varies according to the level of food and risk of predation, the relative survival rate increases rapidly. Figure 4.5 illustrates the typical growth curves maintained for the transgenic and non-transgenic fish for all simulations used in this analysis. Growth curves may vary somewhat according to the level of food in the environment or the initial condition of the fish, but in general the transgenic fish experienced a two-fold increase in growth rate relative to the non-

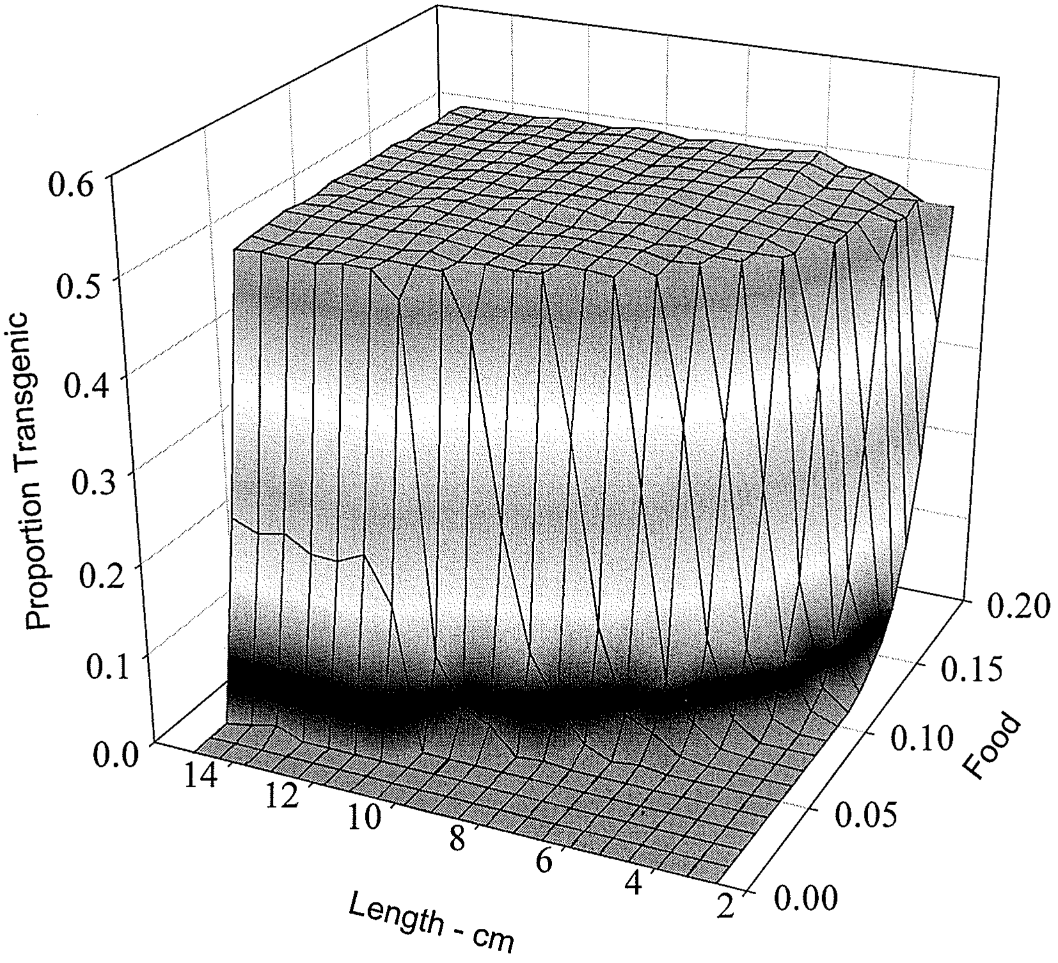

Figure 4.4. Relationship between λ, size and survival.

Influence of the probability of finding food and size of the fish on the relative survival of transgenic individuals. λ ranged from 0 to 0.2, covering the range of low to high levels of food in the environment. K = 1.02, $\beta = \beta_{base}$, iterations = 1000.

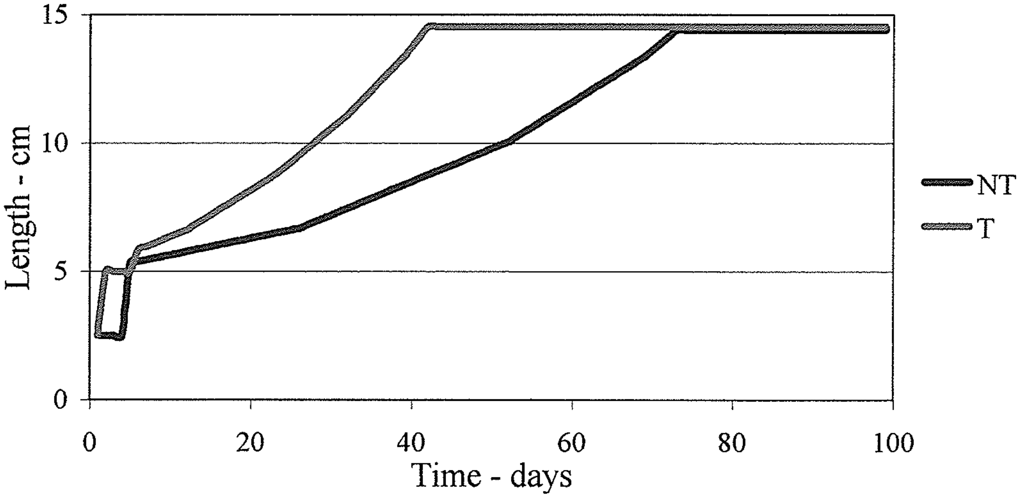

Figure 4.5. Growth curves from simulation.

Growth curves over 100 days for both a transgenic and a non-transgenic individual. $K = 1.02$, $\lambda = 0.1$, $\beta = \beta_{base}$. Transgenic fish grow approximately two times faster than the non-transgenic fish.

transgenic fish. According to regression analysis, size of the fish is positively correlated with relative survival of the transgenic fish, and accounts for 21.08% of the variation in the simulation results (Table 4.2).

Discussion

Relative survival of transgenic fish

The probability of finding food was the most important variable in this model for determining the relative fitness of transgenic fish when compared to non-transgenic individuals. At high levels of food and low levels of predation, the transgenic fish experienced a higher probability of surviving to smolt than the non-transgenic fish. The amount of food in the environment will then have a profound influence on the ability of transgenic fish to survive if they enter the natural system through either intentional or accidental release.

The initial size of the fish at the beginning of the simulation was the second largest contributor to predicting the relative survival of transgenic fish. Even under environmentally poor conditions with a high risk of predation or a low probability of finding food, the transgenic fish were able to experience similar probabilities of survival to smolt, compared with the non-transgenic fish, if their initial size was large enough. Therefore, even if an environment appears inhospitable for supporting transgenic growth, if the escaping or released fish are large enough, they may still be able to successfully survive in the wild.

Risk of predation explained the least amount of variability in relative fitness of the transgenic fish. This result does fit with the observations on behavioural foraging decisions made by the transgenic fish, relative to the non-transgenic fish, in the simulation. If the transgenic fish tend to choose the low risk / low gain territorial strategy at a higher rate than the non-transgenic fish, they will be less affected by increased rates of predation. However, the transgenic fish also received no added fitness benefit from increased rates of predation. Risk of predation appears to be impacting the transgenic and non-transgenic individuals equally. In the model, the probability of being consumed by a predator is related to β, the size of the fish, and the foraging speed of the fish. Even though the transgenic fish are choosing the less risky foraging strategy, they may experience a higher rate of predation in that strategy relative to a non-transgenic fish foraging under that strategy due to their increased activity.

When comparing foraging strategy choice between transgenic and non-transgenic fish, there are obvious behavioural differences between these two groups. Figure 4.6 illustrates the proportion of transgenic and non-transgenic fish that starved or were consumed by a predator under identical environmental conditions. However, under most circumstances, the survival of the two groups is not highly different. This agrees with work done by Mangel and Stamps (2001), suggesting that individual behavioural decisions in trade-offs between growth and mortality can lead to individual variation in growth rates, with equal overall fitness.

One explanation for the enhanced growth of transgenic fish is that they maintain their appetite during winter months when wild salmon may not eat at all. They appear to be detached from seasonal regulation of growth hormone, which controls appetite, as well as a multitude of other physiological properties. This is also a concern with non-transgenic hatchery-reared fish. They have a reduced appetite during the winter, but

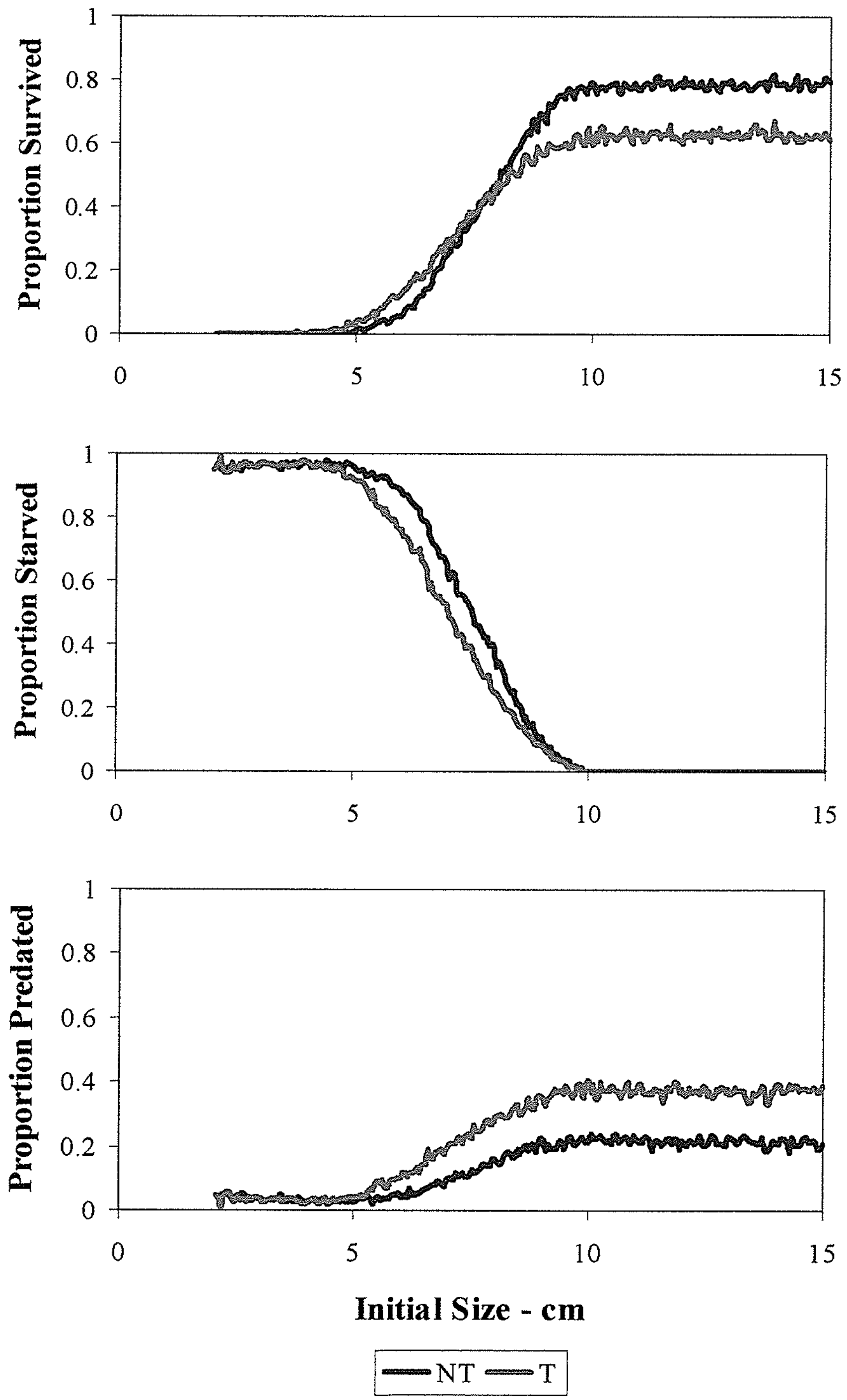

Figure 4.6. Results of simulation.

Proportion of transgenic and non-transgenic fish that survived, were consumed by a predator, or starved, under the same environmental conditions. $\beta = \beta_{base}$ and $\lambda = 0.5$

due to warm water temperatures and supplied food, they do grow throughout this season. In the spring, they are longer and heavier than wild-type salmon of the same age. This of course could have ecological impacts when the larger salmon are released to compete with the smaller wild salmon. Larsen et al. (2001) found that if hatchery reared salmon were maintained under winter conditions (colder temperatures and no food) they would also stop growth. This did not impair their success at smolting the following spring. It is not known how the transgenic fish would fare if they still maintained a desire to feed under winter conditions in which there is no food. If they follow the results of the previously described study, they may still survive successfully. However, they may not reduce their activity in response to the lower amount of food, and may starve to death.

Ecological significance

The ability of transgenic fish to survive in the wild will be highly influenced by the amount of food available in the environment, in addition to the size of the fish when released, and the level of predation risk. Under high food levels, transgenic fish could experience fitness levels equal to or even slightly greater than the non-transgenic fish, even at high levels of predation. Even if food levels are reduced, a high initial body size or very low level of predation may allow the transgenic salmon to survive successfully.

This model just predicts the relative survival of the two groups. It is not known what level of relative survival would be required for the transgenic salmon to become successfully established within the habitat. Even if they were not capable of successfully reproducing, the fact that they are able to survive in the habitat would be ecologically significant. The transgenic fish would successfully compete for limited space and food resources, perhaps forcing the non-transgenic fish to less profitable feeding locations, or displacing them from the stream altogether.

Another point to consider is that all simulations with this model began with equal numbers of transgenic and non-transgenic fish. If accidental or intentional releases of transgenic fish were larger than the resident population of wild salmon, the relative survival of the transgenic fish would be even larger. Low survival rates for the transgenic fish may not reduce ecological impact if initial releases are much larger than the wild fish population size. Potential impact could increase in proportion to the amount that the initial transgenic population exceeds the wild population.

Limitations of the model

One limitation of this model is that the simulation is run within a set time frame. This limits the ability of assessing the impact of foraging decisions on survival of the individuals within the system, as these fish in the wild would also employ temporal decisions (Ludwig and Rowe 1990), where optimal foraging decisions would depend on time remaining until the end of the time period, and the current size of the individual. Kozlowski and Wiegert (1986) argue that, in addition to mortality, length of the growing season is the key factor in determining optimum body size and age at maturity. Semalparous species, such as the coho salmon, may find it advantageous to shorten their life span under high mortality rates. By doing so, the fish would decrease the amount of time spent in high-risk conditions, and thereby increase the probability of surviving to reproduce. Smolting of coho salmon occurs over a large time frame (February to June), and is not limited to a discrete event as suggested by the model (Weatherly and Gill1995). Correlated with the lack of a dynamic time system is the fact that the simulation does not consider varying environments. For example, this simulation may be very specific in one area, but the results may vary wildly if applied to a warmer or cooler climate. Schindler (1999) found that climate significantly impacts antipredator behaviour of foraging salmon.

Temperature was also not taken into consideration for this model. Juvenile salmon experience very dynamic temperature conditions from hatch until smolt (Beckman et al. 2000), ranging from 18°C in the summer to 0°C in the winter. This temperature range correlates with a range of food availability, which is highest in the summer and lowest in the winter. For this simulation, I have just considered two growth periods: food availability and therefore growth in the summer, and no food correlating to no growth in the winter. I was then able to end the simulation at the end of the growth period, assuming that the fish do not grow during the winter, and the ones that do survive winter will go on to smolt. This simplification has its benefits, but does not consider seasonal variation in food availability. Survival of transgenic fish my be further reduced over-winter if their appetite and metabolism is no longer season-dependent, but remains high during times of low food resources.

Other factors in addition to overall food consumption must be taken into consideration when assessing the potential ability of transgenic fish to survive in the wild. Boisclair and Leggett (1989a) have found that food consumption did not explain a significant proportion of among-population variability in growth. Other factors may come into play that allow or hinder the transgenic fish from successfully surviving in the wild. Boisclair and Leggett (1989b) argue that relative activity levels, and energy costs associated with these activities, were the highest contributors to the variance in growth of actively foraging fish.

In this model, foraging speed was linearly related to length of the fish. Foraging speed was then used in calculations for risk of mortality and food consumption. According to Farrell et al. (1997), transgenic coho salmon are inferior swimmers relative to non-transgenic controls. They suggest that the transgenic coho salmon swim at rates comparative to the smaller, but age-matched, non-transgenic controls. If this holds true, the coho salmon may not incur any foraging advantages due to their increased foraging speed. However, their risk of mortality would be concurrently lower, and may balance the reduction in food consumption in the overall fitness equation. Herbert et al. (2001) have found that transgenic Atlantic salmon are 35% more active than non-transgenic controls. Even if the transgenic salmon do not swim faster than the non-transgenic fish, they may spend more time being active, and thereby increase their food consumption.

Conclusion

According to model simulations, there are large ranges of food and predation levels that would allow the transgenic fish to survive in the wild, and experience fitness levels equal to non-transgenic fish in the same environment. Results also indicated that transgenic fish in the simulations were able to maintain a growth advantage over the non-transgenic fish. Given the appropriate conditions, transgenic fish could have the ability to successfully grow, and survive, in the wild. Further research would be required to assess if model conditions relate to environmental conditions, and would be very site-specific. As the model is very simplified relative to a natural environment, other environmental variables will affect the success of the transgenic fish, as discussed above.

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CHAPTER FIVE

General Discussion

CHAPTER FIVE – General Discussion

Although research on the environmental risk of transgenic organisms generates the most controversy and interest, these genetically modified animals also provide a unique opportunity to gain insight into animal design. Transgenic salmon present a means to explore the behavioural, morphological and physiological trade-offs that fish must make during their lifespan. Insertion of a growth hormone transgene endows fish with instantaneous enhanced growth that they are capable of achieving through the more time-consuming process of selective breeding (Devlin et al. 2001). There is also evidence that the fish do not grow larger than the maximum size that wild fish are capable of, but merely get there at an increased rate (Devlin, pers. com.). These findings indicate that the fish have the ability to evolve enhanced growth, but do not, suggesting that there may be limitations of maintaining rapid growth on overall fitness.

In Chapter 2, I demonstrated that transgenic fish are more successful at securing the higher quantity food resources, relative to non-transgenic controls. This outcome provides some insight into the high level of feeding motivation the transgenic fish experience. As the transgenic fish were size-matched with the control fish, size should not have influenced the outcome of the trials. The enhanced competitive ability may be due to the enhanced motivation to feed (Devlin et al. 1999). With an enhanced motivation to feed, it was expected that the transgenic fish should be willing to risk increased predation in order to access food. The results of Chapter 3 did not support this assumption, as there were no observed differences in mortality between the transgenic and control fish.

Competition and predation are often assumed to be the most important processes in determining the success of an invader to become established within a community (Li and Moyle 1981), in addition to environmental factors (Moyle and Light 1996). Although I found transgenic fish to be superior competitors that do not experience differential rates of mortality, these results were obtained in a laboratory setting under one set of environmental conditions that deviate from the variability found in the natural environment. The outcome of the competitive interactions may even be different in an alternate environment (Baltz et al. 1982). Chapter 4 represents an attempt to discern the ability of transgenic fish to survive in varying environmental conditions.

Under appropriate conditions, successful aquatic invasions do occur. These conditions are difficult to define due to high environmental variability, but successful invasions are often related to a high availability of food upon release (Moyle and Light 1996), and a large population of invaders (Knibb 1997; Moyle and Light 1996) relative to the resident population. As my individual-based population model indicated, the ability of transgenic fish to experience survival equal to that of non-transgenic fish is highly dependent on the level of food in the environment. The level of predation in the environment also has an impact on the relative survival of transgenic fish. As the initial size of the fish decreases, the level of predation decreases to a probability of zero. According to this model, fish below a minimum initial size will not survive to smolt. As the initial size of the fish increases, the level of predation required increases until it reaches a maximum probability of one. Obviously, along this continuum, there will be levels of predation that would occur in nature. If the initial size of the released fish is above minimum size, and the level of predation is below the minimum required to reduce survival of transgenic fish to zero, the transgenic fish may be able to survive.

My research indicates that under certain conditions, transgenic fish may pose a threat to wild fish populations. These conditions include a high level of food in the environment to maintain the enhanced growth, a low risk of predation, and a high magnitude of release relative to the wild population. Enhanced growth has been constrained in the natural environment, either due to evolutionary constraints (genetic, developmental or historical constraints; Ridley 1993) or a trade-off between different adaptive needs (Mangel and Stamps 2001; Conover and Schultz 1997, Ridley 1993). Genetic and developmental constraints seem unlikely due to the fact that domesticated trout have growth rates similar to transgenic wild-type trout (Devlin 2001). However, a lack of conclusive evidence demonstrating a real survival disadvantage of enhanced growth should be reason enough to handle this technology with the respect and caution that it deserves.

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APPENDIX 1 – BASIC code for the dynamic optimization model

```
Option Explicit

'DESCRIPTION OF VARIABLES - ARRANGED ALPHABETICALLY
'anabolism - coefficient for the conversion of food to length
'beta() - risk of predation associated with each strategy
'c1 - worksheet variable
'c2 - worksheet variable
'c3 - worksheet variable
'c4 - worksheet variable
'c5 - worksheet variable
'c6 - worksheet variable
'catabolism - coefficient for the conversion of length to energy
'ccount - column counter
'cf - condition factor = 100 * (mass / length^3)
'cfopt - optimum condition above which ingested energy will be put towards growth
'choicestrategy() - strategy with the maximum fitness for each day and state
'cost() - cost of foraging associated with each strategy
'costmultiplier - scales relative metabolic costs for T and NT fish
'days - length, in days, of the simulation
'energy() - measure of energy supplied by food for each strategy
'f - worksheet variable
'f0() - F(x, t+1, T)
'f1() - F(x, t, T)
'fitness() - fitness of patch
'food() - chance of finding food for each state and strategy
'foodfit - fitness if food is found
'foragingspeed() - foraging speed at each state for each strategy
'g - worksheet variable
'g1 - excess energy after foraging
'g2 - loss of energy after foraging
'h - worksheet variable
'handlingtime - time required to capture and consume a food item
'i - looping variable
'initiallength - length at the beginning of the growth forward iteration
'intcf - cf as an integer
'intK - K as an integer
'intK1 - K1 as an integer
'intK2 - K2 as an integer
'intlength - length as an integer
'intlength - length as an integer
'intn - n as an integer
'intx - x as an integer
'intx1 - x1 as an integer
'intx2 - x2 as an integer
'iterations - number of times to run throught the forward iteration
'K - looping variable for condition factor
'K1 - new condition factor if food is found
'K2 - new condition factor if food is not found
'Kincrements - discretizes condition state variable
'Kmax - maximum condition
```

```
'Kmin - if K < Kmin, fish dies
'Ksteps - discretized condition state variable
'lambda() - chance of finding food, by drift, for each strategy
'length - length of the fish
'mass - mass of fish as calculated from K and length
'length - state of the fish in the growth forward iteration
'maxfitness() - fitness if best strategy is chosen, for each day and state
'n - looping variable for strategy increments
'nofoodfit - fitness if no food is found
'nosmolt() - the number of fish that are unable to smolt
'p - worksheet variable
'patchmultiplier() - variable used to calculate chance of finding food for each strategy
'pfood - computer generated random number used to determine if a fish finds food
'population - number of fish in the population for the growth forward iteration
'populationloop - variable used for loop in growth forward iteration
'predation() - risk of predation associated with each state and strategy
'predator - computer generated random number, determines if a fish gets consumed by a predator
'preddeath() - number of fish consumed by a predator by end of simulation, for each length
'predvar1 - constant used to calculate risk of predation
'predvar2 - constant used to calculate risk of predation
'rtrack - row counter
'starvedeath() - number of fish that starved to death by the end of simulation, for each length
'strategy - number of strategies available to fish in this simulation
'survivors() - number of survivors at end of simulation for each length
't - looping variable for time increments
'temp - variable used in subroutine to convert number to an integer
'tempint - variable used in subroutine to convert number to an integer
'test - used to determine which strategy has the highest fitness
'testfitness - used to determine which strategy has the highest fitness
'teststrategy - used to determine which strategy has the highest fitness
'tracker - indicates if fish is alive (1) or dead (0) in forward iteration
'tffvar1 - variable for terminal fitness function (tff)
'tffvar2 - variable for tff - slope of relationship between size and survival
'tffvar3 - variable for tff - slope of relationship between K and survival
'x - looping variable for state increments
'x1 - new length if food is found
'x2 - new length if food is not found
'xincrements - discretizes length state variable
'xmax - maximum size
'xmin - minimum size before starvation
'xsteps - discretized length state increment

Sub sensitivityanalysis1()

Dim predloop, foodloop As Single
Dim filename As Integer

filename = 0

'set up loop for varying levels of beta and lambda

For predloop = 1 To 10 Step 1
For foodloop = 0.05 To 0.2 Step 0.05

'open a new workbook
```

```
Workbooks.Add
Workbooks("DOMstrategies-adv.xls").Activate
filename = filename + 1

Randomize

Dim anabolism As Single
Dim beta() As Single
Dim c1, c2, c3, c4, c5, c6, ccount As Integer
Dim catabolism, cf As Single
Dim choicestrategy(), cost() As Single
Dim costmultiplier As Single
Dim Eacquired As Single
Dim energy() As Single
Dim Eremaining, Erequired, Estored, Etotal As Single
Dim f As Integer
Dim f0(), f1(), fitness(), food() As Single
Dim foodfit As Single
Dim foragingspeed() As Single
Dim g As Integer
Dim g1, g2 As Single
Dim h, i As Integer
Dim initiallength As Single
Dim intcf, intK, intK1, intK2, intlength As Integer
Dim intx, intx1, intx2, iterations As Integer
Dim K, K1, K2, Ksteps As Single
Dim lambda() As Single
Dim length, mass As Single
Dim maxfitness(), mstep As Single
Dim n As Integer
Dim nofoodfit As Single
Dim nosmolt() As Single
Dim p As Integer
Dim patchmultiplier() As Single
Dim pfood As Single
Dim population, populationloop As Integer
Dim predation() As Single
Dim predator As Single
Dim preddeath() As Single
Dim rtrack As Integer
Dim starvedeath() As Single
Dim survivors() As Single
Dim t As Integer
Dim temp As Single
Dim tempint As Integer
Dim test, testfitness As Single
Dim teststrategy, tracker As Integer
Dim x, x1, x2 As Single
Dim xsteps As Single

'***********************************************

'define constants used within the simulation
```

```
Const cfopt = 1
Const days = 100
Const handlingtime = 0.5
Const Kincrements = 20
Const Kmax = 1.5
Const Kmin = 0.3
Const predvar1 = -0.5
Const predvar2 = 100
Const strategy = 3
Const tffvar1 = 0.269
Const tffvar2 = 0.027
Const tffvar3 = 0.25
Const xincrements = 20
Const xmax = 15
Const xmin = 2

'discretize the length and condition variables

Ksteps = (Kmax - Kmin) / Kincrements
xsteps = (xmax - xmin) / xincrements

GoSub programsequence:

'save the new workbook with the simulation data

Workbooks(Workbooks.Count).SaveAs filename
Workbooks(Workbooks.Count).Close

Next foodloop
Next predloop

End

'***********************************************

programsequence:

GoSub definestrategies:
GoSub definecontrol:
GoSub dynamicmodel:
GoSub forwarditeration:
GoSub definestrategies:
GoSub definetransgenic:
GoSub dynamicmodel:
GoSub forwarditeration:

Return

'***********************************************

'variables specific to control fish are defined

definecontrol:

anabolism = 1
```

```
catabolism = 1
costmultiplier = 1

'these variables specify spreadsheet numbers

f = 2
p = 3
g = 7
h = 10

'these variables specify columns for data output

c1 = 1
c2 = 2
c3 = 3
c4 = 4
c5 = 5
c6 = 6

Return

'*************************************************

'variables specific to transgenic fish are defined
'transgenic fish are more efficient at converting food to energy
'transgenic fish have increased metabolic costs

definetransgenic:

anabolism = 2.2
catabolism = 1
costmultiplier = 1.6

f = 4
p = 5
g = 8
h = 11

c1 = 8
c2 = 9
c3 = 10
c4 = 11
c5 = 12
c6 = 13

Return

'*************************************************

'the three strategies available to the fish are defined
'according to cost of foraging, risk of predation,
'chance of finding food, and available energy.

definestrategies:
```

```
Worksheets(1).Select

ReDim cost(Kincrements + 1, xincrements + 1, strategy)
ReDim beta(strategy)
ReDim predation(Kincrements + 1, xincrements + 1, strategy)
ReDim lambda(strategy)
ReDim foragingspeed(Kincrements + 1, xincrements + 1, strategy)
ReDim patchmultiplier(strategy)
ReDim food(Kincrements + 1, xincrements + 1, strategy)
ReDim energy(Kincrements + 1, xincrements + 1, strategy)

For n = 1 To strategy
   rtrack = 3
   beta(n) = predloop / Cells(n + 4, 4)
   lambda(n) = foodloop * Cells(n + 4, 5)
   patchmultiplier(n) = Cells(n + 4, 6)
   For K = Kmin + Ksteps To Kmax + Ksteps Step Ksteps
      temp = K
      GoSub changeKtointeger:
      intK = tempint
      For x = xmin + xsteps To xmax + xsteps Step xsteps
         temp = x
         GoSub changelengthtointeger:
         intx = tempint
         length = x
         cf = K
         cost(intK, intx, n) = Cells(n + 4, 3) * (length ^ 3 / 100)
         'calculate foraging speed, as determined by length
         'from Weatherly and Gill (1987)
         foragingspeed(intK, intx, n) = patchmultiplier(n) * (2 * x)
         'calculate chance of finding food, as determined by foraging speed
         'from Werner & Anholt (1993)
         food(intK, intx, n) = ((foragingspeed(intK, intx, n) * lambda(n)) / (1 + (foragingspeed(intK, intx, n)
* handlingtime * lambda(n))))
         If food(intK, intx, n) > 1 Then food(intK, intx, n) = 1
         'calculate risk of predation
         'risk of predation inversely proportional to length
         'from NerkaSim, predation(x, n) = beta(n) * x ^ m
         'positive linear correlation between foraging speed and risk of predation
         'from Werner & Anholt (1993)
         predation(intK, intx, n) = (beta(n) * (length ^ 3 / 100) ^ predvar1) + ((beta(n) / predvar2) *
foragingspeed(intK, intx, n))
         If predation(intK, intx, n) > 1 Then predation(intK, intx, n) = 1
         energy(intK, intx, n) = Cells(n + 4, 7) * (length ^ 3 / 100)
      Next x
   Next K
Next n

Return

'*************************************************

dynamicmodel:

GoSub terminalfitness:
```

```
ReDim maxfitness(days, Kincrements + 1, xincrements + 1)
ReDim choicestrategy(days, Kincrements + 1, xincrements + 1)

'cycle backwards through time
For t = days - 1 To 1 Step -1
  ccount = 1
  'cycle through all possible condition factors
  For K = Kmin To Kmax + Ksteps Step Ksteps
    temp = K
    GoSub changeKtointeger:
    intK = tempint
    'cycle through all possible lengths
    For x = xmin To xmax + xsteps Step xsteps
      temp = x
      GoSub changelengthtointeger:
      intx = tempint
      'cycle through all available strategies
      For n = 1 To strategy
        length = x
        cf = K
        intlength = intx
        intcf = intK
        GoSub findfoodgrowth:
        K1 = cf
        temp = K1
        GoSub changeKtointeger:
        intK1 = tempint
        x1 = length
        temp = x1
        Select Case temp
          Case Is > 0
            GoSub changelengthtointeger:
            intx1 = tempint
          Case Is <= 0
            intx1 = 0
        End Select
        length = x
        cf = K
        GoSub nofoodgrowth:
        K2 = cf
        temp = K2
        GoSub changeKtointeger:
        intK2 = tempint
        x2 = length
        temp = x2
        Select Case temp
          Case Is > 0
            GoSub changelengthtointeger:
            intx2 = tempint
          Case Is <= 0
            intx2 = 0
        End Select
        'fitness if food is found
        foodfit = food(intK, intx, n) * f1(intK1, intx1)
```

```
            'fitness if no food is found
            nofoodfit = (1 - food(intK, intx, n)) * f1(intK2, intx2)
            'total fitness of the strategy
            fitness(n) = (1 - predation(intK, intx, n)) * (foodfit + nofoodfit)
        Next n
        'pick the best strategy
        'the default strategy is 0, where the fish cannot smolt
        testfitness = 0
        teststrategy = 0
        For n = 1 To strategy
            test = fitness(n)
            Select Case test
                Case Is > testfitness
                    testfitness = test: teststrategy = n
            End Select
        Next n
        'store the best strategy in an array
        choicestrategy(t, intK, intx) = teststrategy
        maxfitness(t, intK, intx) = testfitness
        f0(intK, intx) = testfitness
        ccount = ccount + 1
    Next x
  Next K
  'update the fitness function
  For K = Kmin To Kmax + Ksteps Step Ksteps
    temp = K
    GoSub changeKtointeger:
    intK = tempint
    For x = xmin To xmax + xsteps Step xsteps
        temp = x
        GoSub changelengthtointeger:
        intx = tempint
        f1(intK, intx) = f0(intK, intx)
    Next x
  Next K
Next t

Return

'************************************************

forwarditeration:

Worksheets(1).Select

ReDim survivors(Kincrements + 1, xincrements + 1)
ReDim starvedeath(Kincrements + 1, xincrements + 1)
ReDim preddeath(Kincrements + 1, xincrements + 1)
ReDim nosmolt(Kincrements + 1, xincrements + 1)

x = 0
t = 0
iterations = 1000

For K = Kmin + Ksteps To Kmax Step Ksteps
```

```
temp = K
GoSub changeKtointeger:
intK = tempint
For x = xmin + xsteps To xmax Step xsteps
   temp = x
   GoSub changelengthtointeger:
   intx = tempint
   survivors(intK, intx) = 0
   starvedeath(intK, intx) = 0
   preddeath(intK, intx) = 0
   nosmolt(intK, intx) = 0
   For i = 1 To iterations
      length = x
      cf = K
      tracker = 1
      For t = 1 To days - 1
         temp = length
         GoSub changelengthtointeger:
         intlength = tempint
         temp = cf
         GoSub changeKtointeger:
         intcf = tempint
         n = choicestrategy(t, intcf, intlength)
         'if strategy = 0, the fish cannot smolt
         Select Case n
            Case Is = 0
               nosmolt(intK, intx) = nosmolt(intK, intx) + 1
               tracker = 0
               Exit For
         End Select
         'generate a random number for chance of predation
         predator = Rnd
         'select the predation rate of the current strategy
         Select Case predation(intcf, intlength, n)
            'individual is not consumed by a predator
            Case Is < predator
            'generate random number for chance of finding food
            pfood = Rnd
            Select Case food(intcf, intlength, n)
               'food is found
               Case Is > pfood
                  GoSub findfoodgrowth:
                  Select Case cf
                     Case Is = Kmin
                        starvedeath(intK, intx) = starvedeath(intK, intx) + 1
                        tracker = 0
                        Exit For
                  End Select
               'no food is found
               Case Is <= pfood
                  GoSub nofoodgrowth:
                  Select Case cf
                     'if condition is too low, fish dies
                     Case Is = Kmin
                        starvedeath(intK, intx) = starvedeath(intK, intx) + 1
```

```
                    tracker = 0
                    Exit For
                End Select
            End Select
          'consumed by a predator
          Case Is >= predator
            tracker = 0
            preddeath(intK, intx) = preddeath(intK, intx) + 1
            Exit For
        End Select
        Next t
        If tracker = 1 Then survivors(intK, intx) = survivors(intK, intx) + 1
      Next i
    Next x
Next K

GoSub outputFI:

Return

'***********************************************

outputFI:

Workbooks(Workbooks.Count).Activate

Cells(1, 1) = "BETA"
Cells(2, 1) = "LAMBDA"
Cells(1, 2) = predloop
Cells(2, 2) = foodloop

rtrack = 3
For K = Kmin + Ksteps To Kmax Step Ksteps
    temp = K
    GoSub changeKtointeger:
    intK = tempint
    For x = xmin + xsteps To xmax Step xsteps
        temp = x
        GoSub changelengthtointeger:
        intx = tempint
        Worksheets(1).Select
        Cells(rtrack, c1) = K
        Cells(rtrack, c2) = x
        Cells(rtrack, c3) = survivors(intK, intx) / iterations
        Cells(rtrack, c4) = starvedeath(intK, intx) / iterations
        Cells(rtrack, c5) = preddeath(intK, intx) / iterations
        Cells(rtrack, c6) = nosmolt(intK, intx) / iterations
        rtrack = rtrack + 1
    Next x
Next K

Workbooks("DOMstrategies-adv.xls").Activate

Return
```

```
'**************************************************

'individual fitness is measured as the probability of survival at smolt
'from Mangel, 1996 and Holtby, 1990
'survival is a linear function of length
'survival = -0.269 + (0.027 * length)
'I also added a condition factor (K) component
'survival is directly proportional to K
'survival = var * K
'where "var" was adjusted so that maximum survival corresponds with literature values

terminalfitness:

ReDim fitness(strategy)
ReDim f1(Kincrements + 2, xincrements + 1)
ReDim f0(Kincrements + 2, xincrements + 1)

'set all values of (K, xmin) = 0
For K = Kmin To Kmax Step Ksteps
   temp = K
   GoSub changeKtointeger
   intK = tempint
   f1(intK, 0) = 0
   f0(intK, 0) = 0
Next K

'set all values of (Kmin, x) = 0
For x = xmin To xmax Step xsteps
   temp = x
   GoSub changelengthtointeger
   intx = tempint
   f1(0, intx) = 0
   f0(0, intx) = 0
Next x

For K = Kmin + Ksteps To Kmax + Ksteps Step Ksteps
   temp = K
   GoSub changeKtointeger:
   intK = tempint
   For x = xmin + xsteps To xmax + xsteps Step xsteps
      temp = x
      GoSub changelengthtointeger:
      intx = tempint
      f1(intK, intx) = (-tffvar1 + (tffvar2 * x)) + (tffvar3 * K)
      If f1(intK, intx) < 0 Then f1(intK, intx) = 0
   Next x
Next K


Return

'**************************************************

findfoodgrowth:
```

```
'determine the energy available for growth
g1 = energy(intcf, intlength, n) - (cost(intcf, intlength, n) * costmultiplier)
If g1 < 0 Then g1 = 0
g2 = (cost(intcf, intlength, n) * costmultiplier) - energy(intcf, intlength, n)
If g2 < 0 Then g2 = 0
Select Case g2
   Case Is = 0
      Select Case cf
         'if K > optimum, all excess energy is directed to growth
         Case Is > cfopt
            GoSub energyrequired_l:
            Estored = ((cf * length ^ 3) / 100 - (cfopt * length ^ 3) / 100) * 0.01
            cf = cfopt
            Eacquired = g1
            Etotal = Estored + Eacquired
            GoSub increaselength:
            GoSub energyrequired_K:
            GoSub increaseK:
         'if K = optimum, energy obtained is directed to growth
         Case Is = cfopt
            Eacquired = g1
            Etotal = Eacquired
            GoSub energyrequired_l:
            GoSub increaselength:
            GoSub energyrequired_K:
            GoSub increaseK:
         'if K < optimum, energy obtained is directed to increasing condition
         Case Is < cfopt
            Erequired = ((cfopt * length ^ 3) / 100 - (cf * length ^ 3) / 100) * 0.01
            Eacquired = g1
            Eremaining = g1
            Select Case Eacquired
               Case Is < Erequired
                  GoSub energyrequired_K:
                  GoSub increaseK:
               Case Is >= Erequired
                  Etotal = Eacquired - Erequired
                  cf = cfopt
                  GoSub energyrequired_l:
                  GoSub increaselength:
                  GoSub energyrequired_K:
                  GoSub increaseK:
            End Select
      End Select
   Case Is > 0
      Estored = ((cf * length ^ 3) / 100) * 0.01
      Etotal = g2
      GoSub decreaseK:
End Select
Select Case length
   Case Is > xmax
      length = xmax
   Case Is < xmin
      length = xmin
End Select
```

```
Select Case cf
   Case Is > Kmax
      cf = Kmax
   Case Is < Kmin
      cf = Kmin
End Select

Return

'*************************************************

nofoodgrowth:

'determine the amount of energy stored
Estored = ((cf * length ^ 3) / 100) * 0.01
Etotal = catabolism * (cost(intcf, intlength, n) * costmultiplier)
'decreased condition in order to pay energy costs
GoSub decreaseK:
Select Case cf
   Case Is > Kmax
      cf = Kmax
   Case Is < Kmin
      cf = Kmin
End Select
Select Case length
   Case Is > xmax
      length = xmax
   Case Is < xmin
      length = xmin
End Select

Return

'*************************************************

'this subroutine calculates the energy required to increase one length increment

energyrequired_l:

mstep = (((cf * (length + xsteps) ^ 3)) / 100 - (cf * length ^ 3) / 100)
Erequired = mstep / anabolism

Return

'*************************************************

'this subroutine calculates the energy required to increase one K increment

energyrequired_K:

mstep = (((cf + Ksteps) * length ^ 3) / 100 - (cf * length ^ 3) / 100) * 0.01
Erequired = mstep / anabolism

Return
```

```
'************************************************

increaselength:

Do While Etotal >= Erequired
   length = length + xsteps
   If length >= xmax Then Exit Do
   Eremaining = Etotal - Erequired
   Etotal = Eremaining
   GoSub energyrequired_l:
Loop

Return

'************************************************

increaseK:

Do While Etotal >= Erequired
   cf = cf + Ksteps
   If cf >= Kmax Then Exit Do
   Eremaining = Etotal - Erequired
   Etotal = Eremaining
   GoSub energyrequired_K:
Loop

Return

'************************************************

decreaseK:

Do While Etotal > 0
   GoSub energyrequired_K:
   Eremaining = Etotal - Erequired
   Etotal = Eremaining
   cf = cf - Ksteps
   If cf <= Kmin Then Exit Do
Loop

Return

'************************************************

'this subroutine accepts a number and changes it to an integer
'this integer is then ready to be used to call an array

changeKtointeger:

temp = Kincrements / (Kmax - Kmin) * (temp - Kmin)
tempint = CInt(temp)

Return

'************************************************
```

```
'this subroutine accepts a number and changes it to an integer
'this integer is then ready to be used to call an array

changelengthtointeger:

temp = xincrements / (xmax - xmin) * (temp - xmin)
tempint = CInt(temp)

Return

'***********************************************************

'calculates mass from K and length

calculatemass:

mass = (cf * length ^ 3) / 100


Return

'***********************************************************

End Sub
```