“Risk assessment of GM fish – The Trojan Gene Hypothesis”

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Although the public often say they disapprove of genetically-modified animals – many people live with one!
Outline

Background
- Genetic risk assessment framework
- Definitions
- Trojan Gene Hypothesis
- Net Fitness Methodology

Case Study
- AquAdvantage salmon
Addressing Risk: Definitions


- **Harm** = Undesirable Outcome
  Example: Species Extinction, Displacement, or Disruption

- **Hazard** = Item that may bring about Harm given exposure
  Example: GM Organism Escapes Into the Environment and Spreads

- **Risk** = $P(\text{Harm results from Hazard})$
  = $P(\text{Harm/ Exposure}) \times P(\text{Exposure})$
  Note: In Our Context EXPOSURE results from escape and GM Spread
**Risk** = P(Harm/Exposure) * P(Exposure)

**P (Exposure)**

- Ability of Organism to Escape and Survive In Natural Setting
- Ability of Transgene to Spread

**Risk** = P(Harm/Exposure) * P(Escape) * P(Transgene Spread/Escape)
Risk = \( P(\text{Harm/Exposure}) \times P(\text{Escape}) \times P(\text{Transgene Spread/Escape}) \)

1. **Prob (Harm/Exposure)**
   Very Difficult to Predict
   
   - Biotic Interactions
     - Near Infinite
     - Some Unknown
     - Unknowable
   
   - Time Frame
     - Harm in Evolutionary Time
     - Cannot Measure in Real Time
Risk = \( P(\text{Harm/ Exposure}) \times P(\text{Escape}) \times P(\text{Transgene Spread/Escape}) \)

2. Prob(\text{Escape})

(Animal Biotechnology, NRC, 2002)

Low

High

High

Low

Low

Moderate

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Risk = P(Harm/ Exposure) * P(Escape) * P(Transgene Spread/Escape)

3. Prob (Transgene Spread/Escape)

- Can be controlled by sterility or biological containment
- Natural selection: if a transgene cannot spread upon escape of fertile fish due to counteracting effects of natural selection then the transgene is contained.
- Ability of transgene to spread will depend upon universal mechanism of natural selection acting on relative fitness.
Implications For Risk Assessment

Risk = Prob(Harm/Exposure) \times \text{Prob}(\text{Escape}) \times \text{Prob}(\text{Transgene Spread/Escape})

If the probability of any link in the chain is close to zero, then the product is close to zero.

Methods to minimize risk:

Prob(\text{Escape}): Managed by Physical Containment
P(\text{Spread/Escape}): Managed by Biological Containment or Sterility  
*or may be limited by Natural Selection*

Transgenic male mating advantage provides OPPORTUNITY for Trojan gene effect in a fish. PNAS (2004).

Materials and Methods

Study Organisms. As a model organism, we studied Japanese medaka (Oryzias latipes) (13) to explore the ecological consequences of the release of individuals that were conventionally studied for obtaining data on fitness components. Individuals were mass-bred in the lab, were easily handled, and showed sexual dimorphism. We produced a stock of transgenic medaka by inserting the human growth hormone gene (hGH) with a salmon pituitary GtH (14). We then sequenced several experiments survival and reproductive differences between transgenic and wild-type medaka. We cultured these differences into several models to test the role of the transgenes, (a) on individual (size at sexual maturation), (b) on social (fish tank size), and (c) on sexual selection (mating success).

We modeled the introduction of a small number of transgenic medaka into a large wild-type population using a normal distribution of sex ratios to predict the consequences of the model, i.e., of increased male mating success but reduced offspring viability. However, we estimated the results of model predictions in which GtH transgenes influenced development of male reproductive traits as well as offspring viability (unpublished data). Different transgenic lines are likely to vary in fitness when the same transgenic construct is used, because of differences in copy number and allelic transgenic effects. To make such predictions, we will be asked to make assumptions about the viability of our transgenic medaka. We used a range of parameter values for mate mating success and offspring viability in our models. The range of values encompassed the particular fitness conditions estimated in our experiments.

We conducted a 2 × 2 factorial experiment to assess the early viability of offspring produced from transgenic transgenic and wild-type medaka parents (15). Each pairing combination consisted of a pair of transgenic males and wild-type females in each pair for a period of 10 days, producing a total of 1,001 fertile eggs. Transgenic male offspring were identified by the presence of transgenic offspring in each family. We estimated the number of transgenic offspring by considering the offspring's viability as well as offspring viability (unpublished data). Different transgenic lines are likely to vary in fitness when the same transgenic construct is used, because of differences in copy number and allelic transgenic effects. To make such predictions, we will be asked to make assumptions about the viability of our transgenic medaka. We used a range of parameter values for mate mating success and offspring viability in our models. The range of values encompassed the particular fitness conditions estimated in our experiments.

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The production of generically modified (GM) organisms (GMOs) continues at a rapid pace, prompting concern about their effects. If these organisms are released into the wild, the effects of their transgenes may not be restricted to the individual organism or the immediate natural community (15–17). Based on our research, we do not know what will happen if GMOs are released into the wild. The effects of GMOs on the environment are not clear, and there is no evidence that GMOs are safe for humans or other animals. In addition, the use of GMOs to produce transgenic organisms could have serious consequences for the environment and human health.
**Gene Flow - The Spread Scenario**

Diagram showing the spread of gene flow from one generation to the next.

- Fish carrying “wild” or “native” genes
- Fish carrying transgenic DNA

> Movement from one generation to the next and related gene flow
Gene Flow - The Purge Scenario
Gene Flow - The Trojan gene effect (Muir and Howard, 1999)

Occurs when there is a conflict of mating success with viability; e.g. increased mating success with decreased juvenile viability.

PEW 2003. Future Fish: Issues in Science and Regulation of Transgenic Fish
Net Fitness Model: An Approach for Predicting the Outcome of Natural Selection

Two Step Process

- Estimation of Net Fitness Components for Alternative Genotypes
- Incorporate Parameters Into A Model that
  - Predicts the Change in Gene Frequency
  - Predicts Change in Population Size

Net Fitness Components
Muir Howard (1999); extended (2001)

1. **Juvenile viability** (chances of surviving to sexual maturity)
2. **Age** (at sexual maturity)
3. **Mating success** (success at securing mates)
4. **Fecundity** (number of eggs produced by a female)
5. **Fertility** (number of eggs successfully fertilized by male sperm)
6. **Adult viability** (chances of surviving to procreate)


(1) Juvenile Viability

Genetic improvement or DNA modification

- Immune System
  - Disease Resistance
- Swimming Speed
- Feeding Motivation
  - Predator Avoidance
- Adult

**Fertile Egg**

**HOW MANY SURVIVE?**
(2) Age At Sexual Maturity
(Intrinsic Rate of Increase)

Wild Type

Transgenic

2 Years

One Year

One Year

1000x

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(3) Mating Success
(4) Fecundity and (5) Fertility

Sperm

Number

Mobility

Survival

Eggs

Size

(4) Egg Number

(5) Percent Eggs Fertilized by Male

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Adult Viability (6)

- Mating
- Eggs
- Cycle Repeats
- Start Own Cycle
- Adults

Start Own Cycle → Mating → Eggs → Cycle Repeats → Start Own Cycle

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Trojan gene effect: conflict of mating success with viability fitness

Occurs if the trait **BOTH**

1. increases mating success
Occurs if the trait **BOTH**

1. Increases mating success **AND**
2. Decreases juvenile viability

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>Transgenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average %</td>
<td>30%</td>
<td>20%</td>
</tr>
<tr>
<td>Surviving to Sexual Maturity</td>
<td>30%</td>
<td>20%</td>
</tr>
</tbody>
</table>

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Trojan gene effect

Mating Success Drives Gene Into Population
Reduced Viability Drives Population to Extinction
When Does the Trojan Gene Effect Occur?

Requires a specific combination of fitness effects

– If viability is reduced too much
  ■ Transgene is purged

– If viability is enhanced (i.e. increased fitness)
  ■ Transgene spreads but no Trojan Gene effect

– A Trojan Gene effect is a very rare combination of factors
  ■ (↑ mating success and ↓ juvenile viability; or ↑ juvenile viability and ↓ fertility)
Interaction of Adult Viability and Mating Success


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Case Study: AquAdvantage salmon
Environmental risk assessment parameters for growth hormone Atlantic salmon, *Salmo salar*

- In 2005, Eric Hallerman (PD), E. McLean, J. Brown, I. Fleming, and G. Fletcher were awarded a USDA Biotechnology Risk Assessment Grant (2005-39454-16417)

**OBJECTIVES included:**

- Quantifying key aspects of the survival and reproductive components of fitness of GH transgenic Atlantic salmon in near-natural systems, including survival fitness (early viability, territoriality and anti-predation behavior) and reproductive fitness (age at maturation and mating success)
- To utilize empirical data to predict the net fitness of GH transgenic salmon and transgene fate in near-natural ecosystems.
Life stages: Atlantic salmon


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Early viability: Juvenile fitness testing

- Territorial dominance, growth and survival of first-feeding

  - In stream environments with limited food, the transgene did not influence the growth or survival at high or low fry densities.
  - Transgenic and non-transgenic individuals were equally likely to be dominant.
  - No differences were found between GH-transgenic and non-transgenic *S. salar* fry in any of the fitness-related phenotypic traits measured.

Reproductive Fitness testing

Three stocks:

- **Wild Atlantic salmon:** Exploits River, Newfoundland
- **Transgenic line:** AquaBounty Farms AquAdvantage salmon line – raised in captivity
- **Immature and mature parr** derived from 2004 crosses of wild Exploits River salmon


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Quantify Male Reproductive Fitness: Anadromous (i.e. large, fighter) males

Part 1:
- Anadromous adult transgenic and control males
- Competing for access to breeding females.
- Transgenic males were captive-reared and the control males were wild
- Replicated (n = 11 replicate trials)
  - 2 large anadromous males – 1 transgenic, 1 control
  - 1 breeding female
  - 5 mature male parr - nontransgenic
  - 10 immature parr - nontransgenic

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An illustration of the fully-contained naturalized stream mesocosm (1.25 × 7.8 × 0.25 m per channel)

This was used to compare the reproductive performance of growth hormone transgenic and nontransgenic Atlantic salmon males, both as anadromous fish and precocial parr. Behavioral data - a combination of video observation and passive integrated transponder tag detection - Thick arrows indicate the direction of water flow.

Results: Anadromous Males

- Transgenic anadromous males (i.e. large, fighter males), reared to maturity in captivity, were behaviorally out-competed by the wild control males
  - Nest fidelity
  - Quivering frequency
  - Spawn participation

- Parentage analyses were deemed unnecessary because the behavioral results from the anadromous male experiments “made it unnecessary to assess breeding success at the genetic level”.

Quantify Male Reproductive Fitness: Precocially mature parr (aka: sneaker males)

Part 2:
- Compared early-maturing transgenic and control parr for access to breeding females
- Both transgenic and control mature male parr were captive-reared
- Replicated (n = 11)
  - 1 large anadromous male
  - 1 breeding female
  - 2 mature parr (1 transgenic, 1 control)
  - 4 immature parr
Results: Mature Male Parr

- Large anadromous males sired dominated parr in fertilization success
- Transgenic male parr (i.e. precocially mature, sneaker males) were inferior competitors to wild-type parr in terms of nest fidelity and spawn participation
- Control parr had higher overall fertilization success than transgenic parr
- Offspring fathered by control parr were present in more spawning trials (n=5) than offspring fathered by transgenic parr (n=1).
– **Juvenile viability**: No differences were found between GH-transgenic and non-transgenic *S. salar* fry in any of the fitness-related phenotypic traits measured in first-feeding Atlantic salmon.

– **Mating success**: Transgenic males displayed REDUCED reproductive performance relative to control males.
Collectively these data suggest the purge scenario is the more likely fate for the AquAdvantage growth hormone transgene i.e. it would be purged by natural selection IF FERTILE MALES ESCAPED.

REMEMBER: The trojan gene scenario is predicted to occur if the trait both increases male mating success AND lowers transgenic viability.
The AquAdvantage salmon application to the FDA included proactive risk mitigation/management measures as it was known that fertile diploid males would have “potential to contribute modified genes to wild populations.”

**Product Definition for AquAdvantage Salmon**

Triploid hemizygous, all-female Atlantic salmon (Salmo salar) bearing a single copy of the α-form of the opAFP-GHc2 rDNA construct at the α-locus in the EO-1 α lineage.

**Limitations for Use**

These Atlantic salmon are produced as eyed-eggs for grow-out only in the FDA-approved physically-contained fresh water culture facility.  


- **Prob (Spread/Escape):** Managed by Biological Containment including the production of 100% female fish and triploidy induction with an average success rate of 99.8% (98.9–100%). All-female fish are unable to interbreed with each other, and triploidy results in sterility.

- **Prob (Escape):** managed by Physical Containment. Facilities were inspected by FDA and featured simultaneous, multiple and redundant physical and geographical containment measures, to preclude concerns about the possibility of transgenic fish escape.
Canadian researchers at Memorial University of Newfoundland inserted the same salmon gene into farmed Atlantic salmon to provide an independent assessment of the potential impact on wild stocks. They have just released results that raise a red flag. They warn that although wild males outperformed their more aggressive transgenic cousins under naturalized laboratory conditions, the transgenic males were able to fertilize eggs. The study’s lead author, Derek Moreau, said that showed the genetically engineered fish “have the potential to contribute modified genes to wild populations.”
A study conducted by Canadian researchers found that transgenic Atlantic salmon can pass their genes on to wild salmon if they escape into the wild. Echoing the concerns raised by members of Congress and the public over the past year, lead author Darek Moreau from the Memorial University of Newfoundland, Canada said, “little is known about the potential impact on wild salmon populations if the GM species were to escape captivity.”

Limitations: Net fitness model does not account for Genotype x Environment (G x E) Interactions

“In the present study, extrapolation of data from hatchery-reared fish would lead to an overestimation of predation effects posed by transgenic fish reared in nature”

Sundström et al. 2007. Gene–environment interactions influence ecological consequences of transgenic animals. PNAS.
How much data is/will be enough? Is this data on net fitness in natural environments required when multiple physical and biological containment measures are in place?

“There are limitations and difficulties associated with collecting the breadth of empirical data required to accurately represent the full range of genotype by environment interactions affecting fitness-related life history traits in the wild. The findings of this study are valuable with respect to a first-generation invasion scenario; but beyond that, reproductive performance is difficult to predict and is, therefore, an unavoidable source of epistemic uncertainty for both quantitative and qualitative invasion models. Further work is thus required to compare the breeding performance of transgenic and nontransgenic salmon in a range of ecologically relevant scenarios.”

Genotype x Environment (GxE) Interactions

- Laboratory Environments Are Benign
- Nature tends to be less hospitable (predators, disease, food availability, competition, etc.)
- Net Fitness Model is conservative
  - If GM Fish is Found To Be A Risk In The Lab: May Not Be Real Risk In Nature
  - If Found Not To Be A Risk In The Lab, Then Unlikely to Be A Risk In Nature
Solution to Genotype x Environment Interactions

- Always measure fitness components in environment most favorable to the GE animal
- That will tend to over estimate risk or provide a worst-case scenario
- Alternative is measuring net fitness in an infinite number of G x E scenarios which would effectively prevent the commercialization of GE fish (and animals?) as can never test all environments
Final Comments

- No model can ever capture the infinite number of possible G x E scenarios
- The Net Fitness Model captures the important key parameters
- Refinements and expansions can be added
- However there needs to be biological support for added additional layers of complexity
- Scientific Principle of Parsimony: Use the simplest model that explains the data
Conclusions

**Risk** = \( \text{Prob} (\text{Harm/Exposure}) \times \text{Prob} (\text{Escape}) \times \text{Prob} (\text{Transgene Spreads/Escape}) \)

- The net fitness methodology provides an approach to predict transgene fate resulting from natural selection following an escape i.e. \( \text{Prob} (\text{Transgene Spreads/Escape}) \)
- If the transgene is likely to spread then biological (e.g. sterility) and/or physical (e.g. on-land tanks) containment measures can be used to manage risk
- The Trojan gene effect is not an unconditional consequence of GE; it depends upon the relative net fitness of the transgenic and receiving population(s).
"We’ve considered every potential risk except the risks of avoiding all risks."