

# "Trojan Genes, Frankenfish, and Science"



Alison Van Eenennaam, Ph.D.

Cooperative Extension Specialist  
Animal Biotechnology and Genomics  
Department of Animal Science  
University of California, Davis, USA  
(530) 752-7942  
[alvaneennaam@ucdavis.edu](mailto:alvaneennaam@ucdavis.edu)

With thanks to my colleague  
Bill Muir, Ph.D.  
Professor of Animal Sciences  
Department of Animal Sciences  
Purdue University, Indiana, USA  
(765) 494-8032  
[bmuir@purdue.edu](mailto:bmuir@purdue.edu)





# 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

Risk Assessment in the Federal Government: Managing the Process (1983). Committee on the Institutional Means for Assessment of Risks to Public Health, National Research Council (aka "The Red Book").



- **Harm** = Undesirable Outcome  
Example: Plane accident resulting in death of all passengers  
Species Extinction, Displacement, or Disruption
- **Hazard** = Item that may bring about Harm given exposure  
Example: Not refuel the plane before takeoff  
GM Organism Escapes Into the Environment and Spreads
- **Risk** =  $P(\text{Harm results from Hazard})$   
=  $P(\text{Harm/Exposure}) * P(\text{Exposure})$

Note: Risk management tries to reduce  $P(\text{Exposure})$  to 0  
In Our Context EXPOSURE results from escape and GM spread



$$\text{Risk} = P(\text{Harm/ Exposure}) * P(\text{Exposure})$$

In the context of transgenic animals

P (Exposure)

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

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





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

# 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(Harm/ Exposure) \* P(Escape) x P(Transgene Spread/Escape)**

**2. Prob(Escape)** (Animal Biotechnology, NRC, 2002)



Low



High



High



Low



Low



Moderate

- Can be managed by physical containment (tank, cage)




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

### 3. Prob (Transgene Spread/Escape)

- Ability of transgene to spread will depend upon universal mechanism of natural selection acting on relative fitness.
- If a transgene cannot spread upon escape of fertile fish due to counteracting effects of natural selection then the transgene is contained.
- Can be managed by sterility or biological containment





# Implications For Risk Assessment



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

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

## Methods to minimize risk (risk management):

Prob (Escape): Managed by Physical Containment

P(Spread/Escape): Managed by Biological Containment or Sterility *or may be limited by Natural Selection*



# POSSIBLE ecological risks of transgenic organism release when transgenes affect mating success: Sexual selection and the Trojan gene hypothesis. PNAS (1999).

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

## Possible ecological risks of transgenic organism release when transgenes affect mating success: Sexual selection and the Trojan gene hypothesis

William M. Muir\*<sup>1</sup> and Richard D. Howard<sup>2</sup>

<sup>1</sup>Department of Animal Sciences and <sup>2</sup>Department of Biological Sciences, Purdue University, West Lafayette, IN 47907

Communicated by Richard D. Alexander, University of Michigan, Ann Arbor, MI, September 2, 1999 (received for review May 19, 1999)

Widespread interest in producing transgenic organisms is balanced by concern over ecological hazards, such as species extinction if such organisms were to be released into nature. An ecological risk associated with the introduction of a transgenic organism is that the transgene, though rare, can spread in a natural population. An increase in transgene frequency is often assumed to be unlikely because transgenic organisms typically have some viability disadvantage. Reduced viability is assumed to be common because transgenic individuals are best viewed as macromutants that lack any history of selection that could reduce negative fitness effects. However, these arguments ignore the potential advantageous effects of transgenes on some aspect of fitness such as mating success. Here, we examine the risk to a natural population after release of a few transgenic individuals when the transgene trait simultaneously increases transgenic male mating success and lowers the viability of transgenic offspring. We obtained relevant life history data by using the small cyprinodont fish, Japanese medaka (*Oryzias latipes*) as a model. Our deterministic equations predict that a transgene introduced into a natural population by a small number of transgenic fish will spread as a result of enhanced mating advantage, but the reduced viability of offspring will cause eventual local extinction of both populations. Such risks should be evaluated with each new transgenic animal before release.

Although production of transgenic organisms offers great agricultural potential, introduction of genetically modified organisms into natural populations could result in ecological hazards, such as species extinction (1–3). Such risk has been suggested to pose little environmental threat because transgenic organisms are evolutionary novelties that would have reduced viability (4, 5). However, transgenic organisms may also possess an advantage in some aspect of reproduction that may increase their success in nature. Although a variety of transgene traits have been incorporated into various species (6, 7), a commonly desired characteristic in transgenic fish species (important in aquaculture and sport fishing) is accelerated growth rate and larger adult body size (8). DNA sequences for growth hormone (GH) genes and cDNAs have been well characterized in fish, and transgenic fish of several species have now been produced (9, 10). Growth enhancements of up to several times that of wild type have been obtained, with growth advantages persisting throughout adulthood in some fish species (8, 11). In many animal species, including fish, body size is an important determinant of differential mating success (sexual selection) through advantages in competing for mates against members of the same sex (mate competition) and/or being preferred as a mate by the opposite sex (mate choice) (12). A recent review found that large body size conferred mating advantages in 40% of the 186 animal taxa surveyed (12). The potential for sexual selection to produce a rapid evolution of sexual traits has long been appreciated (12); here we consider its potential to increase transgene frequency and to eliminate populations, specifically when a sexual trait is affected by transgenes.

### Materials and Methods

**Study Organism.** As a model organism, we studied Japanese medaka (*Oryzias latipes*) (13) to explore the ecological consequences of transgene release into natural populations. Medaka were convenient study organisms for obtaining data on fitness components. Individuals were readily bred in the lab, were easily cultured, and attained sexual maturity in about two months. We produced a stock of transgenic medaka by inserting the human growth hormone gene (hGH), with a salmon promoter, sGH (14). We then conducted several experiments to document survival and reproductive differences between transgenic and wild-type medaka (15). We categorized these differences into four fitness components: (i) viability (offspring survival to sexual maturity), (ii) developmental (age at sexual maturation), (iii) fecundity (clutch size), and (iv) sexual selection (mating advantages). We modeled the introduction of a small number of transgenic individuals into a large wild-type population using recurrence equations (described below) to predict the consequences of the model, i.e., of increased male mating success but reduced offspring viability. Elsewhere, we examined the results of model predictions in which GM transgenes influenced developmental and fecundity fitness components as well as offspring viability (unpublished data). Different transgene lines are likely to vary in fitness even when the same transgene construct is used, because of differences in copy number and sites of transgene insertion. To take such variation into account as well as to make our model generally applicable to other organisms and transgene constructs, we used a range of parameter values for mate mating success and offspring viability in our models. The range of values also encompassed the particular fitness component estimates that we obtained.

We conducted a 2 × 2 factorial experiment to assess the early viability of offspring produced from crosses involving transgenic and wild-type medaka parents (15). Each pairing combination consisted of 10 males and 10 females; eggs were obtained from each pair for a period of 10 days, producing a total of 1,910 fertile eggs. Viability was estimated as the percentage of 3-day-old fry that emerged. Results showed that early survival of transgenic young was 70% of that of the wild type (15).

Mating experiments using wild-type medaka were performed to measure the mating advantage that large males obtained over small males (16). We found that, regardless of protocol, large males obtained a 4-fold mating advantage (16). Such size-related mating advantages have been demonstrated in a variety of fish species; they can result from mate competition or mate choice or both (12). We do not expect transgenic male medaka to have a mating advantage over wild-type males, because the hGH transgene we inserted increased only juvenile growth rate, not final

Abbreviation: GM, growth hormone.

\*To whom reprint requests should be addressed. E-mail: muir@ipurdue.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

## Transgenic male mating advantage provides opportunity for Trojan gene effect in a fish

Richard D. Howard\*<sup>1</sup>, J. Andrew DeWoody<sup>2</sup>, and William M. Muir<sup>1</sup>

Departments of <sup>1</sup>Biological Sciences, <sup>2</sup>Forestry and Natural Resources, and <sup>3</sup>Animal Sciences, Purdue University, West Lafayette, IN 47907

Edited by M. T. Clegg, University of California, Riverside, CA, and approved December 8, 2003 (received for review September 29, 2003)

Genetically modified (GM) strains now exist for many organisms, producing significant promise for agricultural production. However, if these organisms have some fitness advantage, they may also pose an environmental harm when released. High mating success of GM males relative to WT males provides such an important fitness advantage. Here, we provide documentation that GM male medaka fish modified with salmon growth hormone possess an overwhelming mating advantage. GM medaka offspring possess a survival disadvantage relative to WT, however. When both of these fitness components are included in our model, the transgene is predicted to spread if GM individuals enter wild populations (because of the mating advantage) and ultimately lead to population extinction (because of the viability disadvantage). Mating trials indicate that WT males use alternative mating tactics in an effort to counter the mating advantage of GM males, and we use genetic markers to ascertain the success of these alternative strategies. Finally, we model the impact of alternative mating tactics by WT males on transgene spread. Such tactics may reduce the rate of transgene spread, but not the outcome.

Genetically modified organism | alternative mating tactics | sperm competition | medaka

The production of genetically modified (GM) organisms (GMOs) continues at a rapid pace, prompting concerns about undesirable ecological consequences if these organisms enter natural communities (1–3). Based on six major components of an organism's fitness (i.e., juvenile viability, adult viability, age at sexual maturity, female fecundity, male fertility, and mate mating advantage), we have recently provided a framework to predict possible risks of ecological harm associated with the spread of transgenes after a GMO release. One risk, extinction, results in the local elimination of conspecific populations (both WT and GM individuals); the other risk, invasion, involves ecosystem disruption as GM individuals replace their WT counterparts (4, 5).

Harm to wild populations resulting from either extinction or invasion risk requires that transgenes of GMOs can spread in nature, which in turn requires that GMOs have an advantage over their WT counterparts in at least one fitness component. An extinction risk is predicted when a transgene produces conflicting effects on different fitness components. As a result of an advantage in one component, GMOs replace WT genotypes in a naturally occurring population of conspecifics, whereas a disadvantage in another fitness component reduces population size, ultimately resulting in population extinction. We refer to this scenario as a Trojan gene effect (4, 5).

One scenario in which opposing pleiotropic effects of transgenes are predicted to produce a Trojan gene effect is when GM males have a mating advantage relative to WT males, but the GM offspring they produce have reduced viability relative to WT offspring (4, 5). The predicted extinction outcome resulting from these opposing effects has recently been confirmed with a deterministic model (6). Previous research in our laboratory has shown that GM lines of our study organism have reduced juvenile viability relative to WT controls (4, 7). Here, we

investigate whether the GM male mating advantage required for a Trojan gene effect is also satisfied for one GM line.

To assess the mating success of GM males relative to WT males, we created a GM line of Japanese medaka (*Oryzias latipes*) by inserting a salmon growth hormone gene driven by a metallothionein promoter (8) into eggs just after fertilization (9). Subsequently, we reared both GM and WT medaka under similar conditions in the laboratory and conducted a series of breeding trials designed to assay both mating success (based on direct observations) and reproductive success (based on molecular assays of paternity). We found that GM males possess a significant mating advantage relative to WT males. We also discovered that the competitively disadvantaged WT males combat the mating advantage of GM males by using alternative mating tactics to sire offspring. As a consequence, we extend our original model to explore how, in general, alternative mating tactics might influence the timeline of population extinction.

### Materials and Methods

**Husbandry.** Details of the production of the transgenic line (MtsGH67)<sup>1</sup> and general rearing methods (9) have been reported elsewhere. Populations of both GM and WT lines were maintained in the laboratory by maximizing reproductive productivity and minimizing all external sources of mortality. For the present study, both the GM and WT males used in mating trials were hapazardly chosen from a pool of at least 100 males that originated from 100 different families. All GM and WT males were reared at the same density and were similar in age before mating trials. To accomplish these conditions, we transferred recently hatched fry to 4.5-liter tanks at a density of no more than 35 fry per tank. Each tank was equipped with a sponge filter. We fed fry live *Artemia* once a day and larval AP100 diet (Zeigler Brothers, Gardners, PA) twice a day. At 3 weeks of age, we transferred the fry to 35-liter tanks and fed them flake food until 8–10 weeks of age. At 8 weeks, the number of fry per tank was reduced to six, and fry were fed *Artemia* once a day and flake food twice a day.

**Mating Trials.** We used five GM males and five WT males in the mating trials; all females were WT and were replaced during the course of the trials only if they stopped breeding. Five 4.5-liter tanks were used as breeding observation tanks; each tank contained one GM male, one WT male, and a female. All five tanks were monitored simultaneously at lights on to obtain mating data. After at least five matings were observed in all five tanks, both the GM and WT males were transferred to one of the other five tanks, each with a different competing male of the other genotype. Thus, each of the five GM males eventually competed with each of the five WT males, and vice versa; only females always remained resident in their initial tank.

This paper was submitted directly (Track II) to the PNAS office.

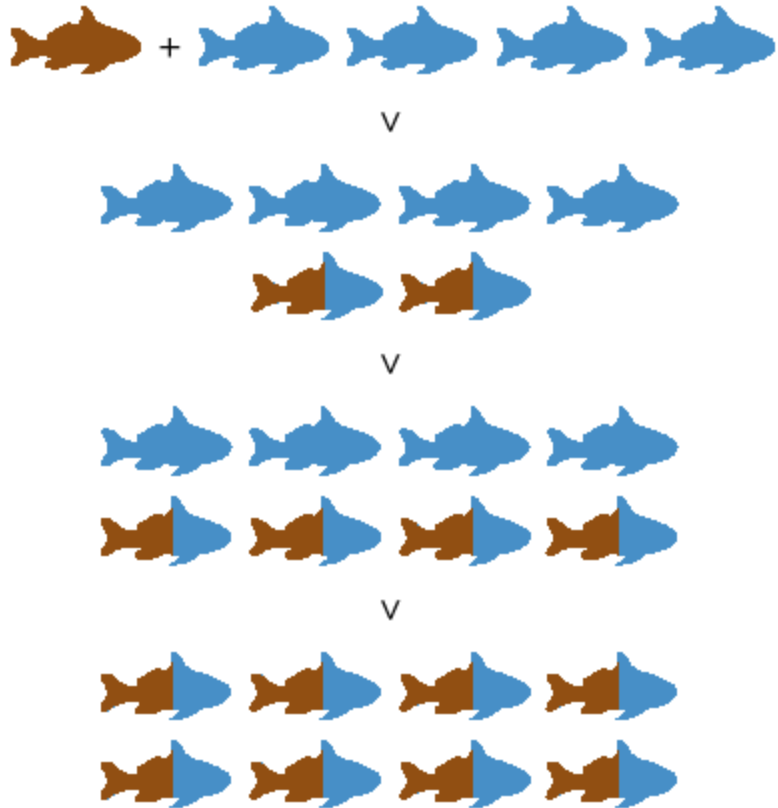
Abbreviations: GM, genetically modified; GMO, GM organism. To whom correspondence should be addressed. E-mail: howard@bio.bio.purdue.edu. \*Muir, R. D., DeWoody, J. A., Hostetler, H. A., Devlin, R. H., & Muir, W. M. (2002) Transgenic fish. 11, 83 (abstr).



© 2004 by The National Academy of Sciences of the USA





# Gene Flow - The Spread Scenario



 fish carrying "wild" or "native" genes  
 fish carrying transgenic DNA

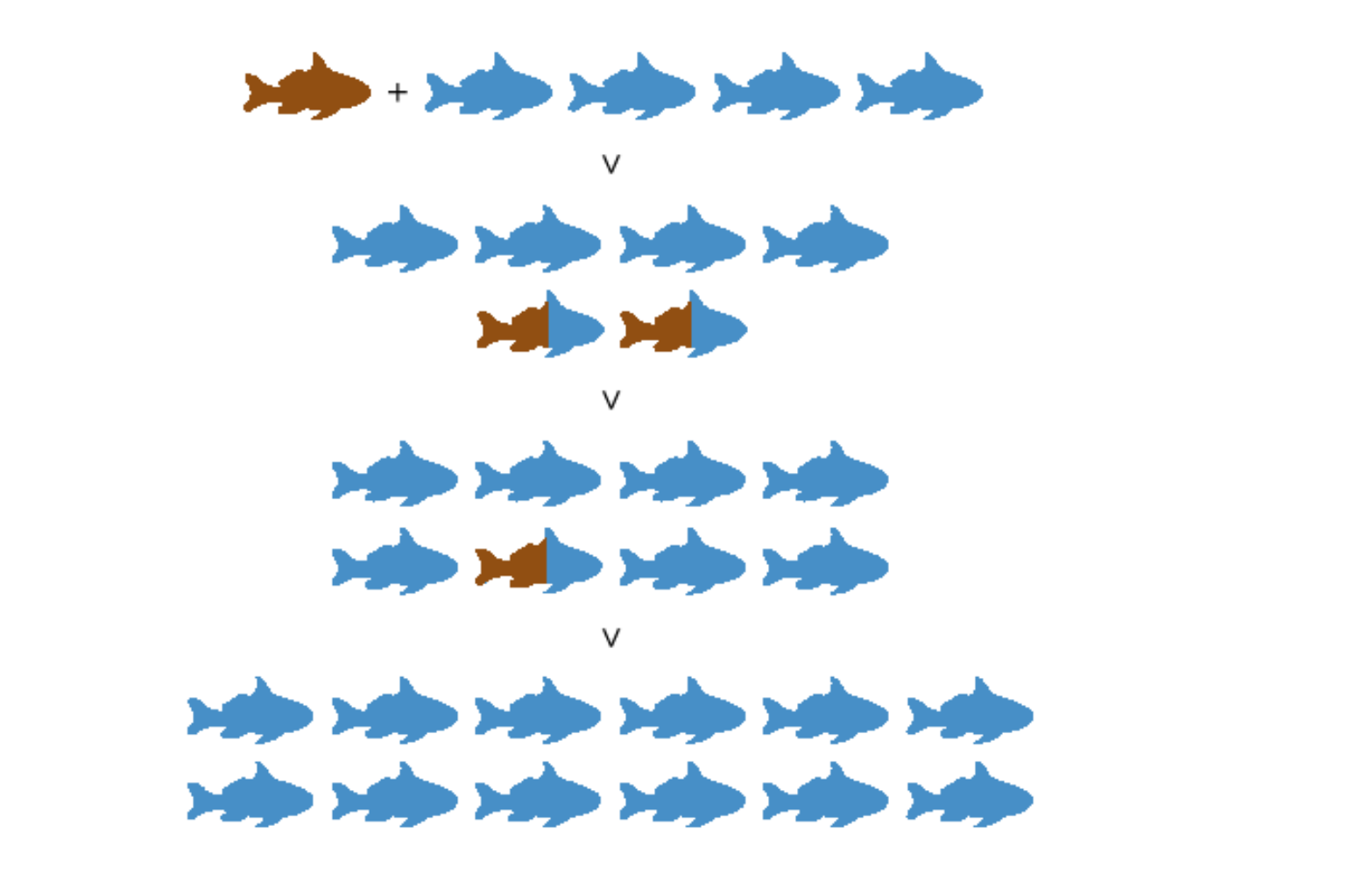
> movement from one generation to the next and the related gene flow





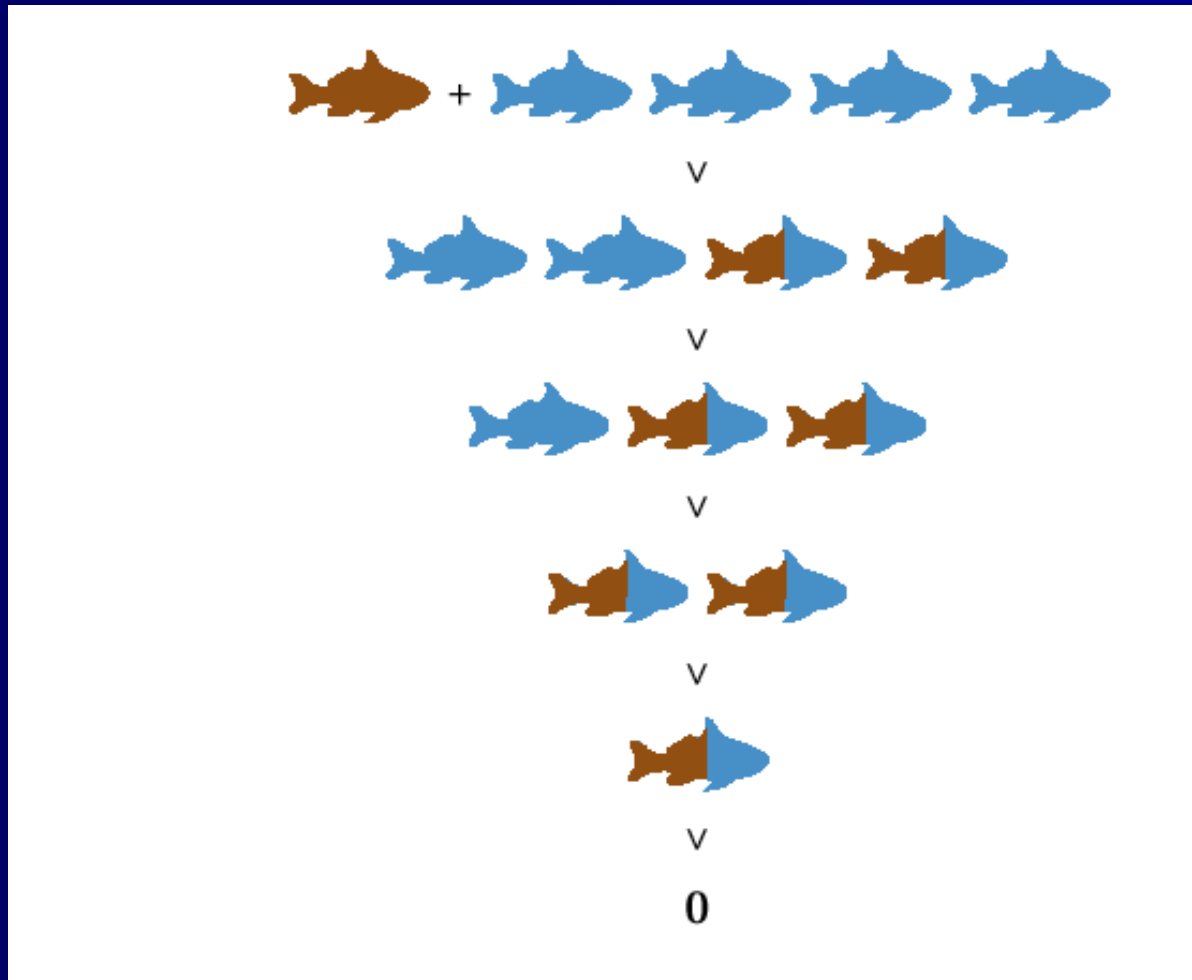


# Gene Flow - The Purge Scenario





# Gene Flow - The Trojan gene effect (Muir and Howard, 1999)



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



# 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

Muir and Howard (2001) Fitness components and ecological risk of transgenic release: a model using Japanese medaka (*Oryzias latipes*) *American Naturalist* 158: 1-16





# Net Fitness Components

Muir Howard (1999); extended (2001)



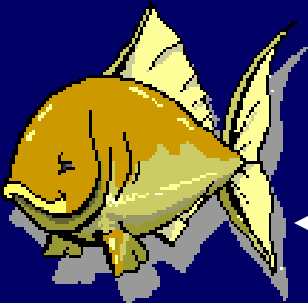
1. **Juvenile viability** (chances of surviving to sexual maturity)
2. **Age** (at sexual maturity)
3. **Mating success** (success at securing mates)
4. **Fecundity** (number of eggs produced by a female)
5. **Fertility** (number of eggs successfully fertilized by male sperm)
6. **Adult viability** (chances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

Muir WM and Howard RD (1999) Possible ecological risks of transgenic organism release when transgenes affect mating success: sexual selection and the Trojan gene hypothesis. *Proc Natl Acad Sci* 96: 13853–13856.

Muir WM and Howard RD (2001) Fitness components and ecological risk of transgenic release: a model using Japanese Medaka (*Oryzias latipes*). *Am Natur* 158: 1–16.



# (1) Juvenile Viability



Genetic improvement or DNA modification

Immune System

Swimming Speed

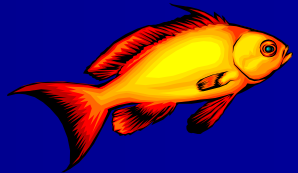
Feeding Motivation

Disease Resistance

Predator Avoidance

Fertile Egg

Adult



**HOW MANY SURVIVE?**



# (2) Age At Sexual Maturity

## (Intrinsic Rate of Increase)

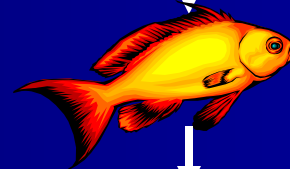


Wild Type

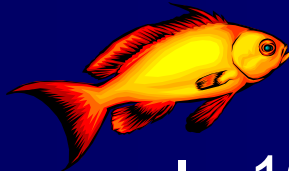


Transgenic

One Year



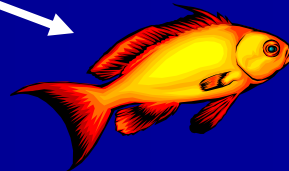
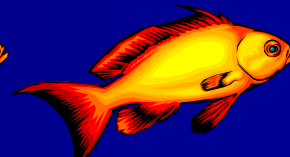
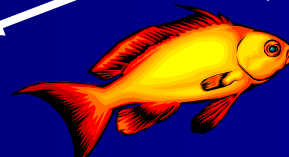
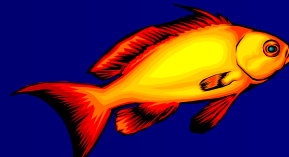
2 Years



1000x



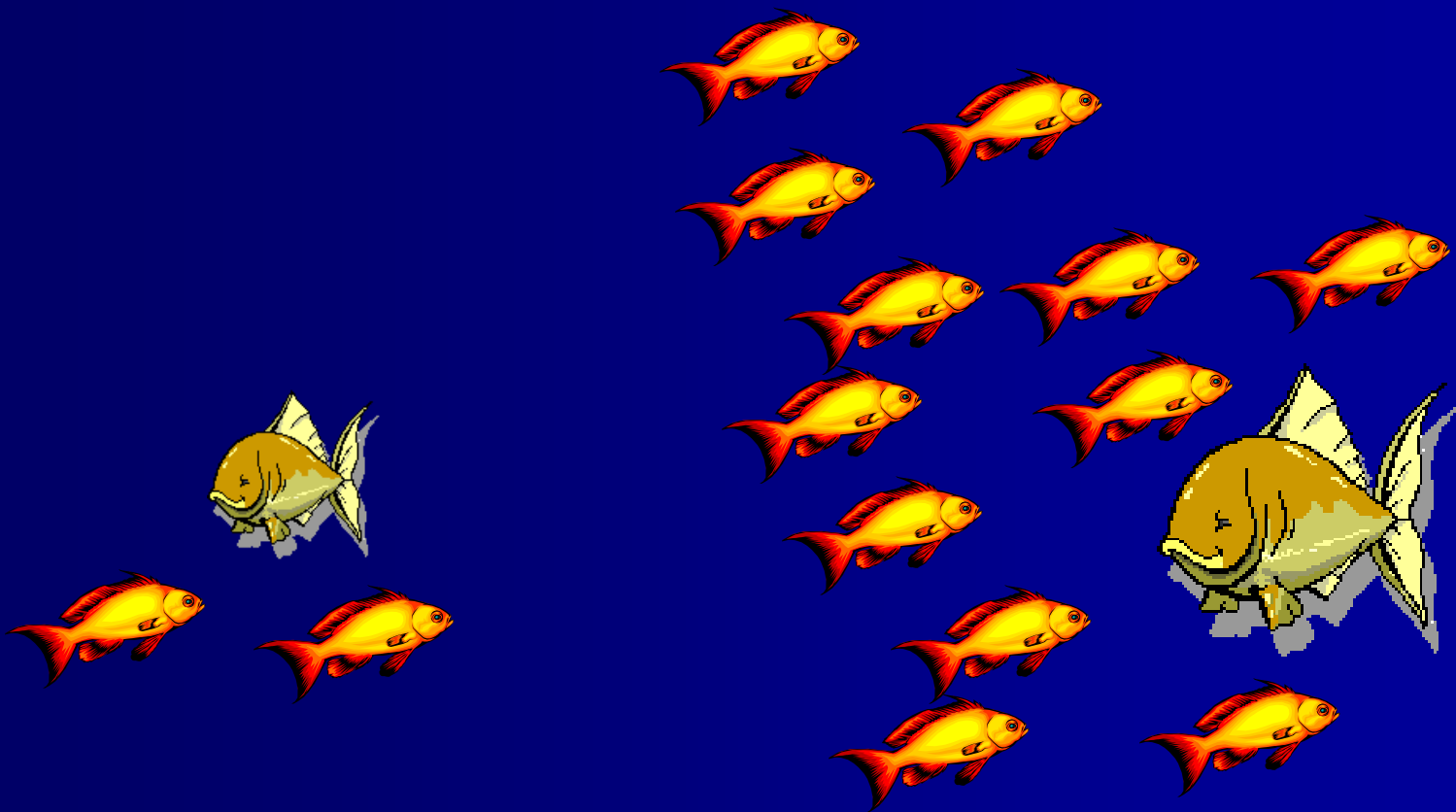
One Year





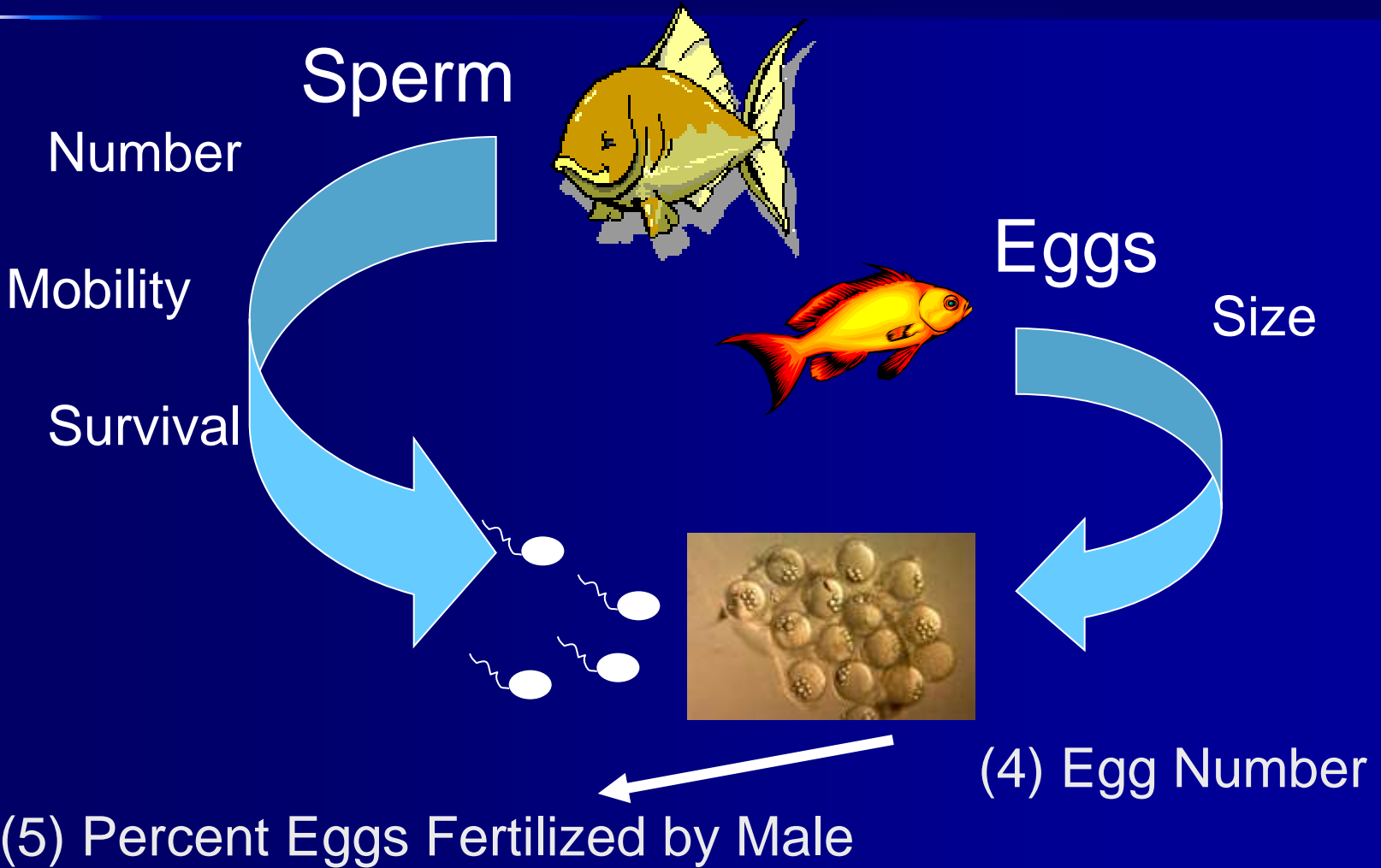


# (3) Mating Success



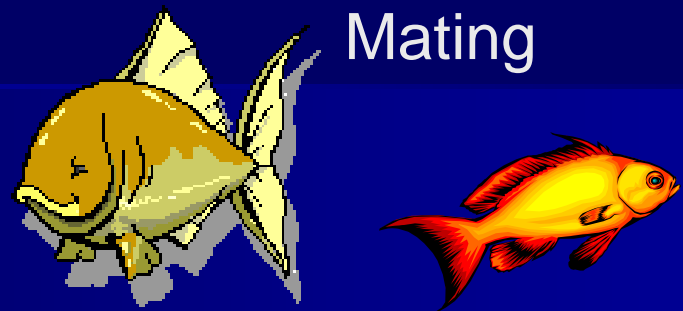


# (4) Fecundity and (5) Fertility



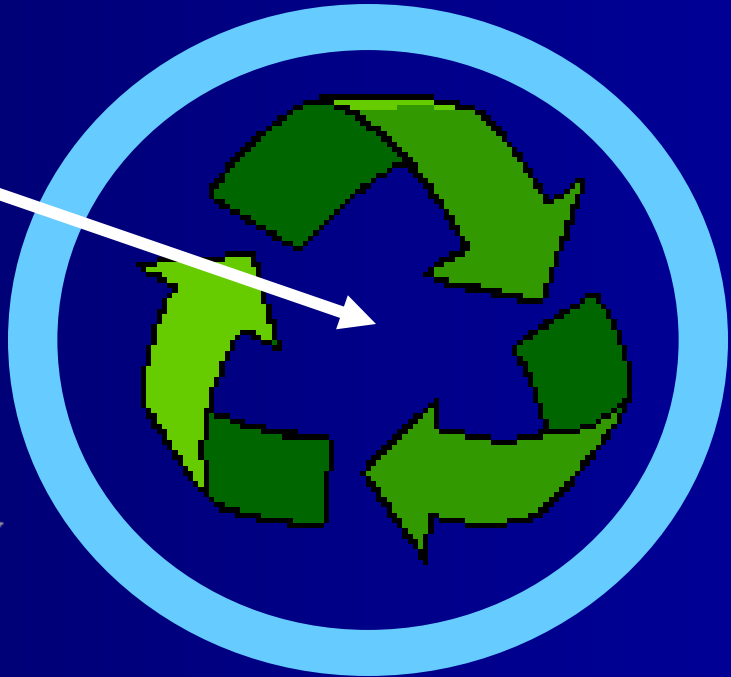


# Adult Viability (6)



Mating

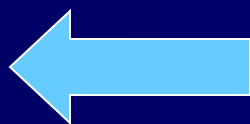
Cycle Repeats



Eggs



Start Own Cycle



Adults



# Trojan gene effect: conflict of mating success with viability fitness

Occurs if the trait BOTH

1. increases mating success



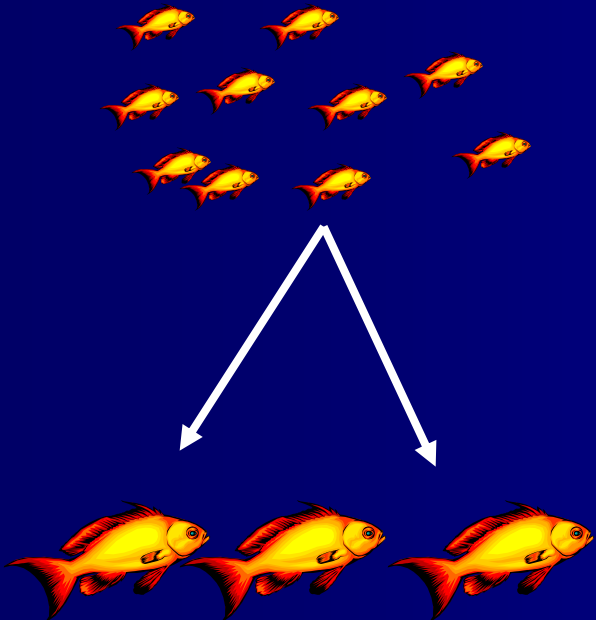




# Occurs if the trait BOTH

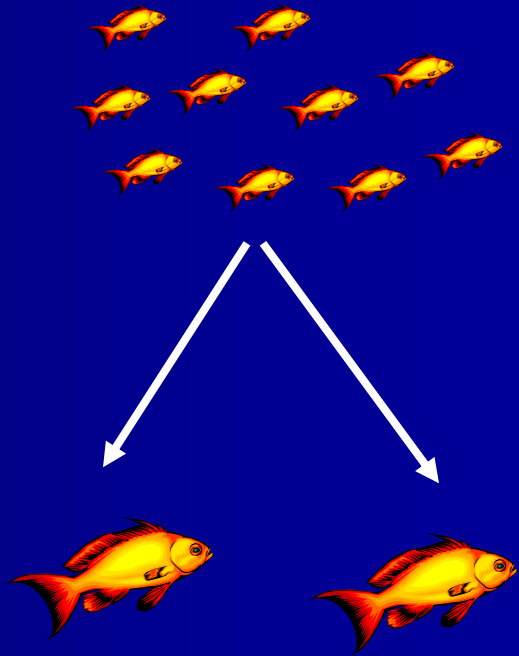
- 1. Increases mating success **AND**
- 2. Decreases juvenile viability

Wild Type



Average %  
Surviving to Sexual  
Maturity  
30%

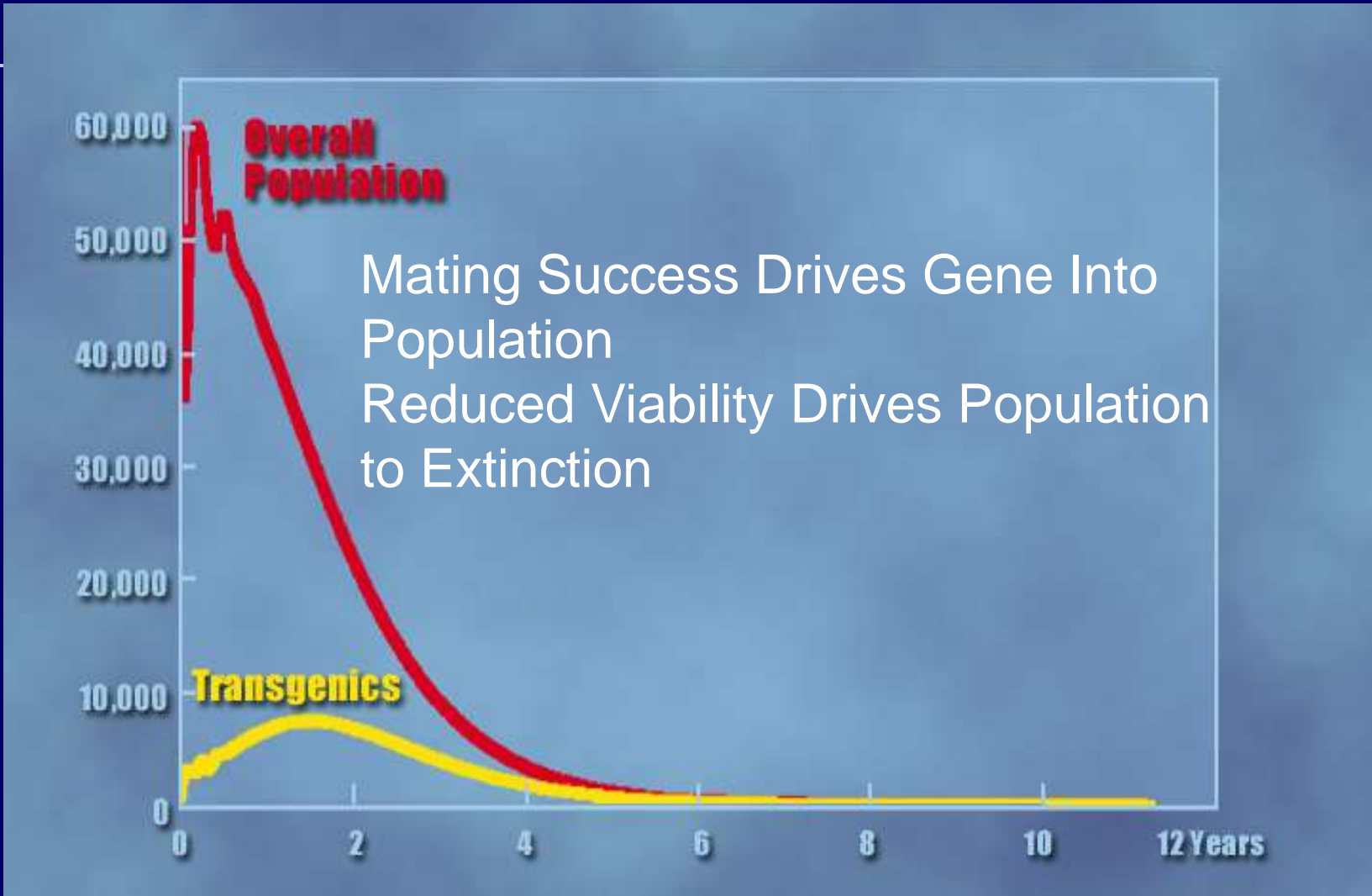
Transgenic



Average %  
Surviving to Sexual  
Maturity  
20%



# Trojan gene effect



# When Does the Trojan Gene Effect Occur?

Requires a specific combination of fitness effects

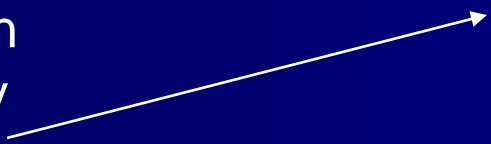
- If viability is reduced too much
  - Transgene is purged
- If viability is enhanced (i.e. increased fitness)
  - Transgene spreads but no Trojan Gene effect
- A Trojan Gene effect is a very rare combination of factors
  - ( $\uparrow$  mating success and  $\downarrow$  juvenile viability; or  $\uparrow$  juvenile viability and  $\downarrow$  fertility)



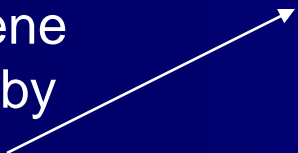
# Interaction of Adult Viability and Mating Success



Population Purged by Natural Selection



Transgene Purged by Natural Selection



Transgene Increased by Natural Selection

Spread Risk

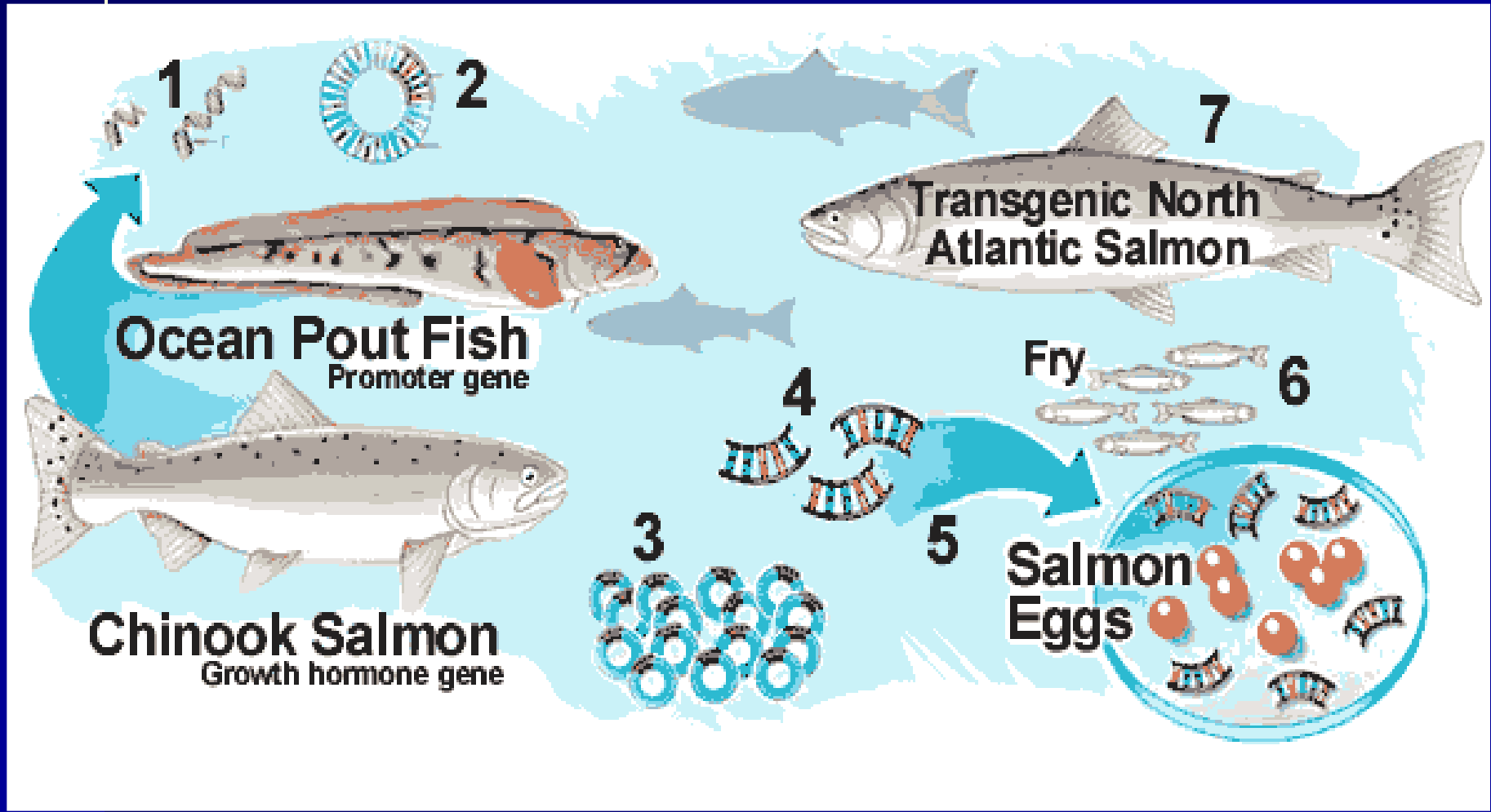


Muir, WM and R.D. Howard. 2002. Assessment of possible ecological risks and hazards of transgenic fish with implications for other sexually reproducing organisms. *Transgenic Research* 11:101-114





# Case Study: AquAdvantage salmon

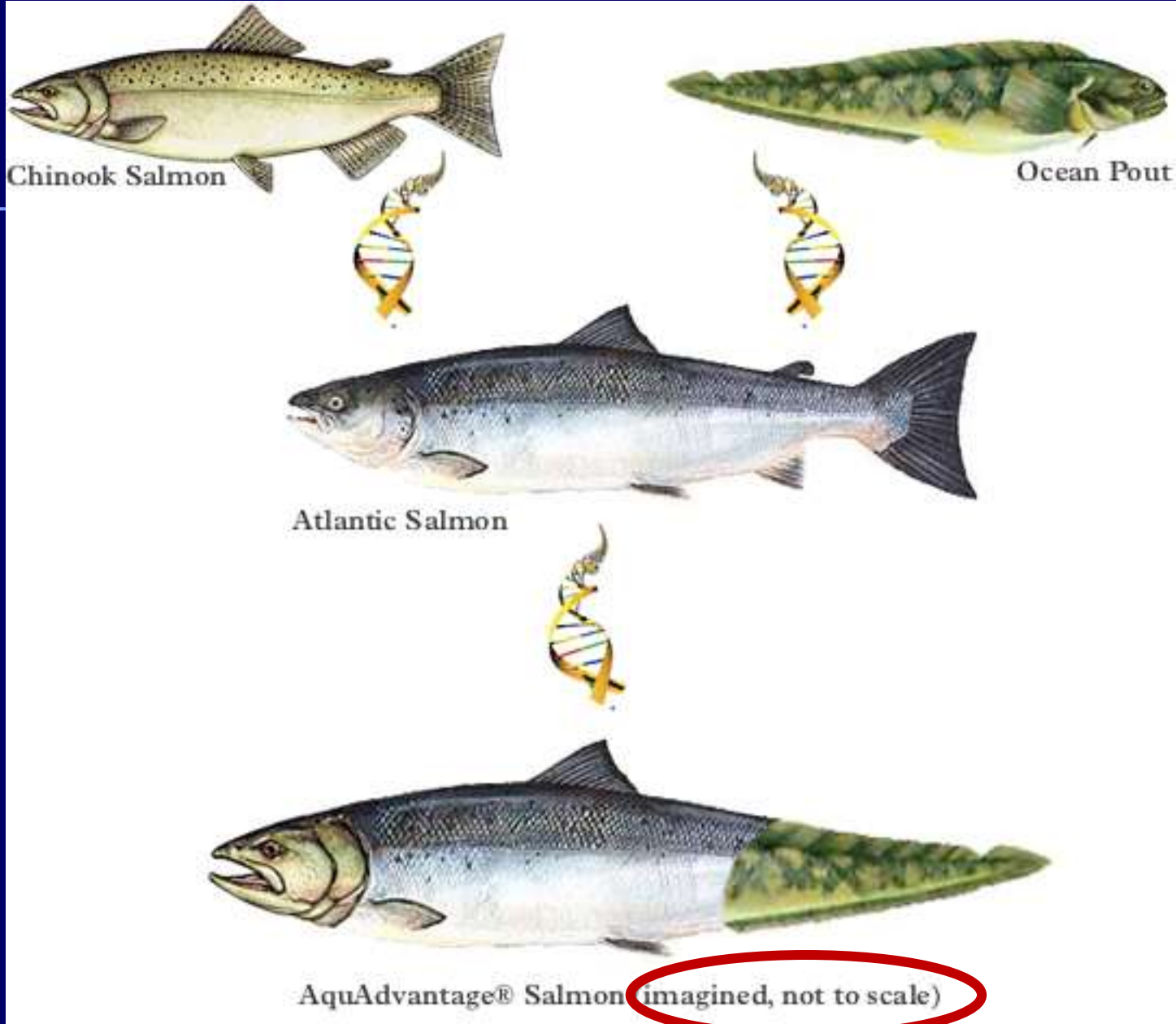


# Salmon reaches market weight in 16-18 months rather than 30 months

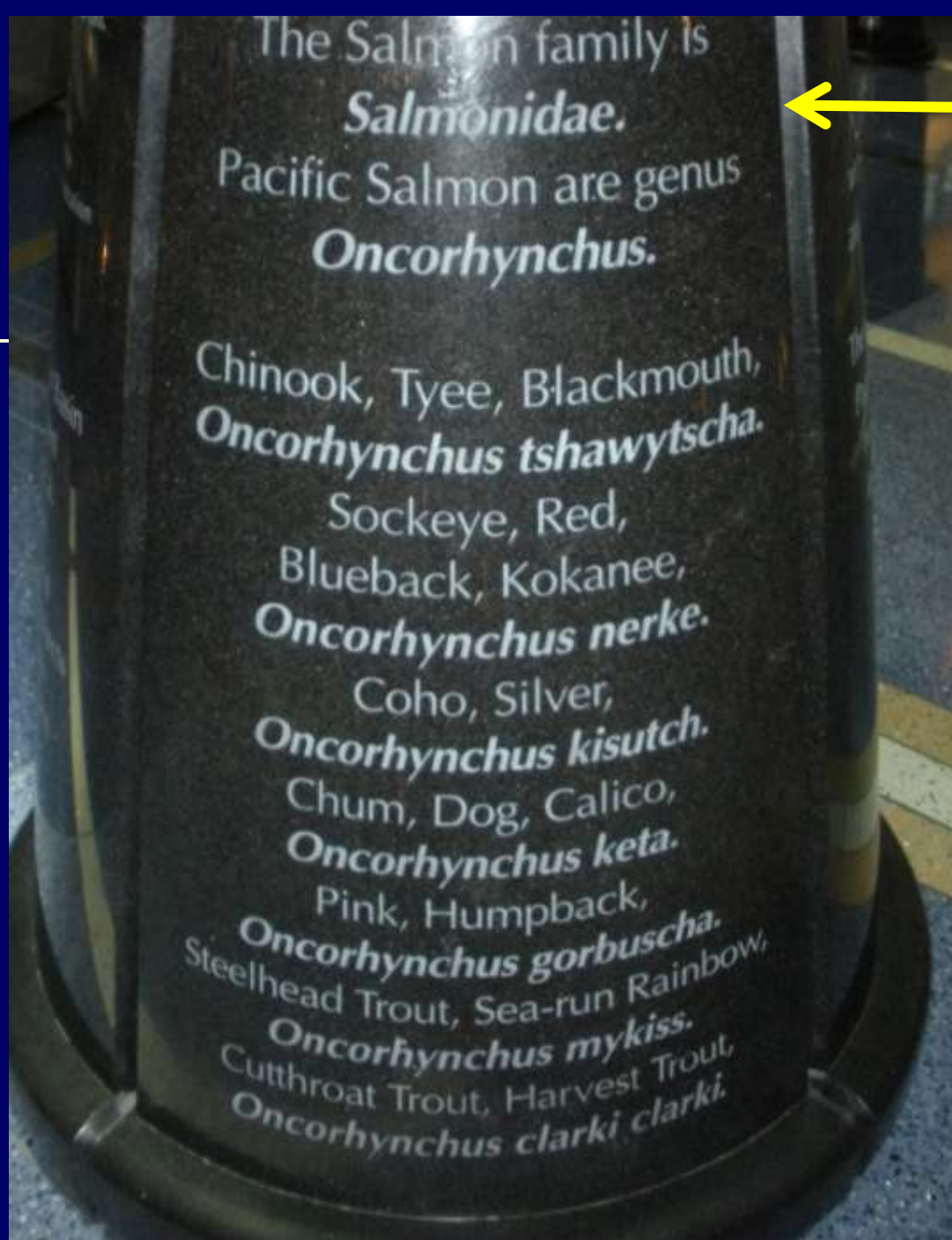




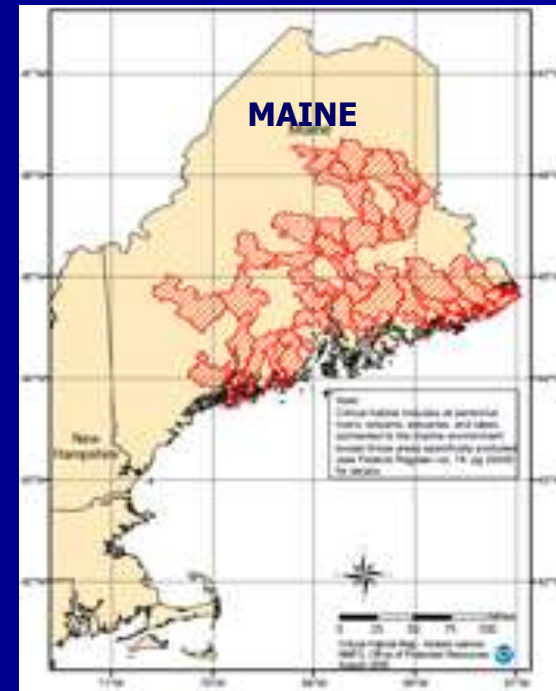
# Retrieved from "AquAdvantage" image search on web







Pillar at Portland,  
OR airport this AM  
Atlantic salmon  
are genus *Salmo*



**Atlantic Salmon  
(*Salmo salar*)  
Critical Habitat**



# Atlantic salmon can not interbreed with Pacific (pink, coho, chinook etc.) salmon

- There have been some concerns that farmed Atlantic salmon – a species that is not native to the Pacific coast – could escape and establish in the wild, thus harming wild salmon stocks by competing for habitat and food or by producing an unnatural hybrid species.
- During the 20th century, millions of Atlantic salmon were released throughout the west coast of the United States and Canada in an attempt to establish recreational and commercial fisheries. These attempts were not successful.
- Research has shown that Atlantic salmon (farmed or wild) cannot successfully mate with wild Pacific salmon.

**Review of Potential Impacts of Atlantic Salmon Culture on Puget Sound Chinook Salmon and Hood Canal Summer-Run Chum Salmon Evolutionarily Significant Units. 2002. NOAA Technical Memorandum NMFS-NWFSC-53**



# 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



Image from <http://harmon-murals.blogspot.com/2011/01/life-cycle-of-atlantic-salmon-for-new.html>



# 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.

Moreau, D. T. R., I. A. Fleming, G. L. Fletcher, and J. A. Brown. 2011. Growth hormone transgenesis does not influence territorial dominance or growth and survival of first-feeding Atlantic salmon *Salmo salar* L. in food-limited stream microcosms. *Journal of Fish Biology* 78:726–740.





# 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



Moreau, D. T. R., C. Conway, I. A. Fleming. 2011. Reproductive performance of alternative male phenotypes of growth hormone transgenic Atlantic salmon (*Salmo salar*). *Evolutionary Applications*.  
[http://onlinelibrary.wiley.com/journal/10.1111/\(ISSN\)1752-4571/earlyview](http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1752-4571/earlyview)



# 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





# 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”.

Moreau, D. T. R., C. Conway, I. A. Fleming. 2011. Reproductive performance of alternative male phenotypes of growth hormone transgenic Atlantic salmon (*Salmo salar*). *Evolutionary Applications*. [http://onlinelibrary.wiley.com/journal/10.1111/\(ISSN\)1752-4571/earlyview](http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1752-4571/earlyview)



# Quantify Male Reproductive Fitness: Precociously 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).

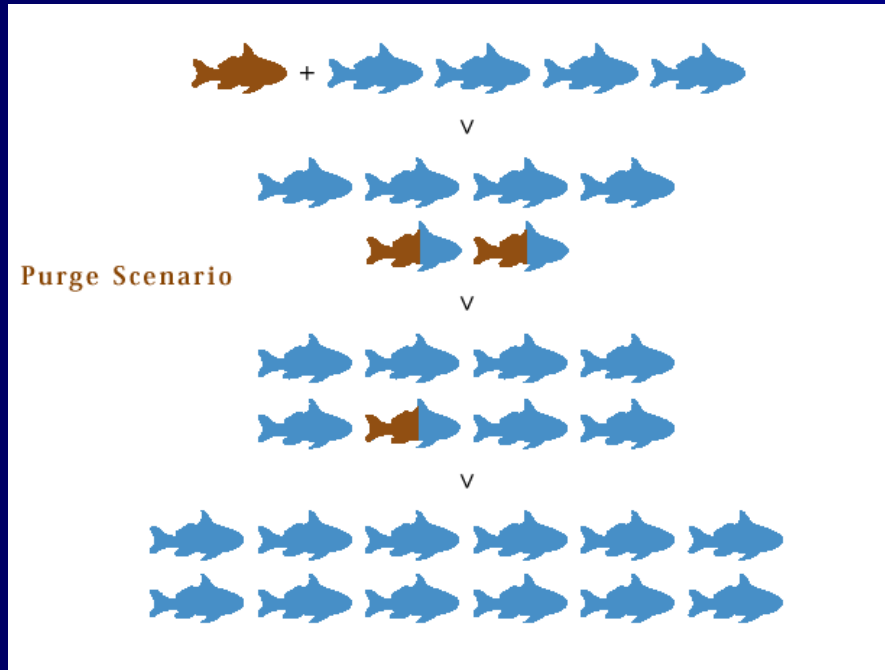
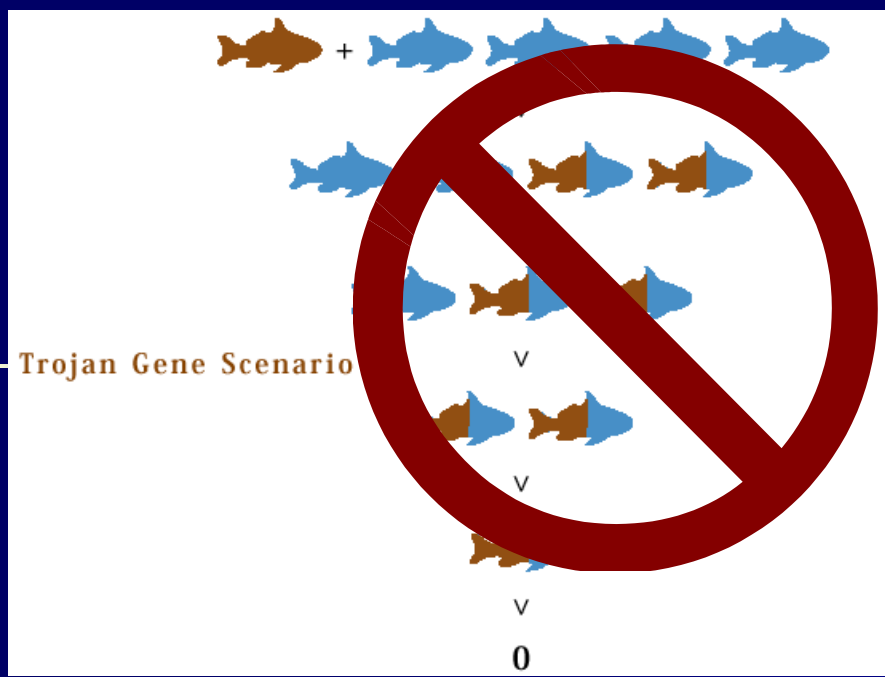




# Summary: Estimates of Components of Net Fitness

- **Juvenile viability:** No differences were found between GH-transgenic and non-transgenic *Salmo 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.





**REMEMBER:** The trojan gene scenario is predicted to occur if the trait both increases male mating success AND lowers transgenic viability

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**





# GM salmon could breed in wild, study shows

Grow GM fish in closed containers on land to minimize risk, researchers urge

By Emily Chung, CBC News | Posted: Jul 15, 2011 5:21 PM ET | Last Updated: Jul 15, 2011 5:21 PM ET

Genes from genetically engineered Atlantic salmon could potentially enter wild populations through natural interbreeding, a new study suggests.



A salmon engineered to contain a certain growth hormone gene (rear) grows twice as fast as its non-transgenic counterpart the same age. AquaBounty Technologies/Associated Press

Male fish carrying a growth hormone gene that causes them to grow twice as quickly as regular salmon can engage in normal breeding behavior and breed with wild females under natural conditions, scientists from Memorial University in St. John's, have found.

The U.S. Food and Drug Administration is currently deciding whether to approve very similar genetically engineered salmon produced by AquaBounty Technologies from eggs at their P.E.I. facility.

Males of the GM or transgenic fish had reduced breeding performance compared to wild fish, said Derek Moreau, lead author of the paper published this week in the *Journal Evolutionary Applications*. But they did show the interest and ability to participate in natural spawning, he said.

"That alone shows that it is possible for the genetic modification to enter wild populations through sexual reproduction," added Moreau, who is completing his Ph.D. in ecology.

### AquaBounty connection

The large adult salmon used in the experiment are descended from the same Newfoundland Atlantic salmon populations and contain the same growth hormone gene as AquaBounty Technologies' AquaAdvantage salmon. The GM parr in the experiment are directly related, as their fathers were all AquaBounty fish. Their mothers were wild fish.



### Related Links

- U.S. Congress votes to block GM salmon
- Genetically modified fish lawsuit threatened
- Genetically modified fish review flawed: economist

### External Links

- Abstract of the article

(Note: CBC does not endorse and is not responsible for the content of external links.)



Researcher Derek Moreau showing a genetically modified salmon. (Photo: Memorial University)

## Transgenic salmon could infect wild populations: study



Researchers have found that genetically modified (GM) Atlantic salmon can reproduce with wild salmon if they escape into the wild. This could allow the genetic modification to enter wild populations, according to Derek Moreau, a researcher in evolutionary ecology at Memorial University in St. John's.

The findings appear in the July online edition of the journal *Evolutionary Applications*.

For two years, Moreau and colleagues have looked at the breeding behaviour of wild and GM male Atlantic salmon in a lab setting to gauge the ability of transgenic males to complete with wild males during the reproductive season. The researchers observed that, while wild male salmon were more successful at breeding, the GM males managed to reproduce naturally, although they were usually less interested in female salmon and bred less frequently.

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."







**The AquAdvantage salmon application to the FDA included proactive risk mitigation/management measures as it was assumed 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*) carrying a single copy of the transgene

### **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.

<http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/UCM224762.pdf>

- **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.

FAST COMPANY The LED L Prize Lamp. Good news for all of us. LEARN MORE

TECHNOLOGY DESIGN ETHNOMICS LEADERSHIP MAGAZINE | NEWSLETTER

## Genetically Modified Salmon Will Kill Regular Salmon

BY ABIEEL SCHWARTZ Thu Jul 14, 2011

Escaped GM salmon could breed and pass on their genes in the wild—and those genes could cause weak salmon that eventually die off. The GM salmon companies say they have a solution to keep their fish sterile, but remember: Nature finds a way.



# GM Fish Study Raises Red Flags

by Cathryn Wellner | July 16, 2011 | 10:40 am

123 comments



Like 297

Send FACEBOOK

Tweet

+1 2

REDDIT

STUMBLEUPON

DIGG

E-MAIL

TEXT SIZE: A | A | A | A

One look at a wild salmon against the background of a same-aged [AquaAdvantage® Salmon](#) explains why [AquaBounty](#) is at the front of the pack in the race to put genetically modified salmon on our plates. The transgenic fish is ready to harvest in 16 to 18 months, while the wild species requires twice that time. With human population on a steady increase and fish stocks on a decline, that's a promise raising a lot of interest.

Insert a Chinook salmon gene into Atlantic salmon, and the result is this fast-growing fish. It is easy to make a business case for fish that require a lot less feed and all the other inputs that go into aquaculture. As to its safety, [AquaBounty](#) advises, "Fish grown from AquaAdvantage® eggs are all female and sterile, making it impossible for them to breed amongst themselves. In addition, FDA approval requires them to be grown in physically contained systems, further reducing any potential impact on 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."***



# How much data is/will be enough? Should additional data on net fitness in natural environments be required for approval when multiple physical and biological containment measures are in place i.e. Probability (exposure) $\sim 0$ ?



"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.**"

Moreau, D. T. R., C. Conway, I. A. Fleming. 2011. Reproductive performance of alternative male phenotypes of growth hormone transgenic Atlantic salmon (*Salmo salar*). *Evolutionary Applications*. [http://onlinelibrary.wiley.com/journal/10.1111/\(ISSN\)1752-4571/earlyview](http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1752-4571/earlyview)



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

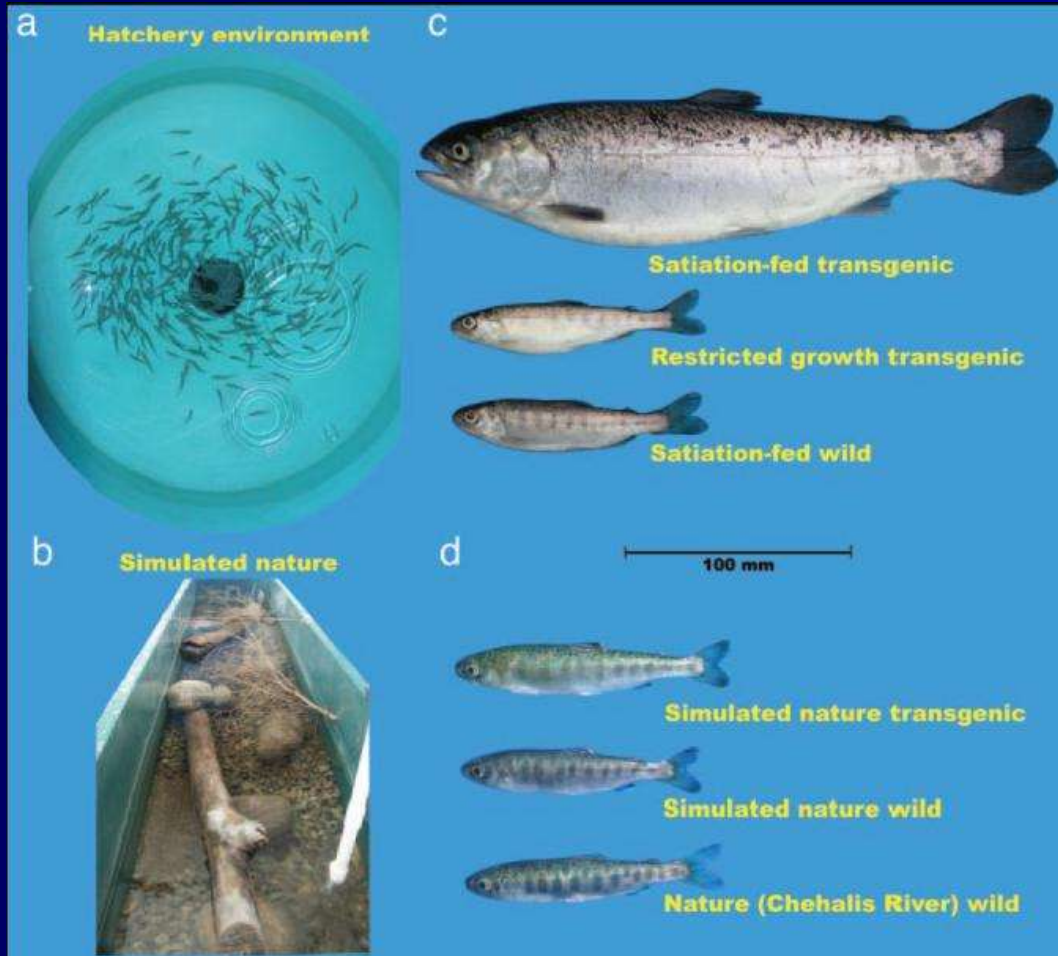
- Laboratory Environments Are Benign
- Nature tends to be less hospitable (predators, disease, food availability, competition, etc.)
- Net Fitness Model is conservative
  - If GM Fish is Found To Be A Risk In The Lab: May Not Be Real Risk In Nature
  - If Found Not To Be A Risk In The Lab, Then Unlikely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







# Genotype x Environment (GxE) 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.



# Approach 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

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

- The net fitness methodology provides an approach to predict transgene fate resulting from natural selection following an escape i.e. Prob (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 GM; it depends upon the relative net fitness of the transgenic and receiving population(s).







HARDIN

"We've considered every potential risk except the risks of avoiding all risks."



# Empirical Observations of Transgenic Atlantic Salmon



- **Oxygen metabolism** (Stevens et al. 1998):
  - Oxygen uptake 1.7x controls
  - Higher critical oxygen level, 6 mg/l vs. 4 mg/l
- **Energy metabolism** (Cook et al. 2000 a,b,c):
  - Feed consumption 2.1-2.6 x controls
  - Under starvation, transgenics depleted body protein, dry matter, lipids and energy more quickly than controls, and had lower initial energy reserves

- Cook, J.T., McNiven, M.A., Richardson, G.F. and Sutterlin, A.M., 2000a. Growth rate, feed digestibility and body composition of growth enhanced transgenic Atlantic salmon (*Salmo salar*). *Aquaculture*. 188, 15-32.
- Cook, J.T., McNiven, M.A., and Sutterlin, A.M., 2000b. Metabolic rate of pre-smolt growth-enhanced transgenic Atlantic salmon (*Salmo salar*), *Aquaculture*, 188, 33-45.
- Cook, J.T., Sutterlin, A.M. and McNiven, M.A., 2000c. Effect of food deprivation on oxygen consumption and body composition in growth enhanced transgenic Atlantic salmon (*Salmo salar*). *Aquaculture*. 188, 47-63.
- Stevens, E. D., Sutterlin, A. M. and Cook, T. (1998). Respiratory metabolism and swimming performance in growth hormone transgenic Atlantic salmon. *Can. J. Fish. Aquat. Sci.* 55, 2028-2035.



# Empirical observations of transgenic Atlantic salmon



- **Feeding behavior** (Abrahams and Sutterlin 1999):
    - Transgenics consumption 5x controls
    - Rate of movement 2x controls
    - Spent time feeding in presence of predator
  - **Territorial dominance, growth and survival of first-feeding** (Moreau et al 2011):
    - No differences were found between GH-transgenic and non-transgenic *S. salar* fry in any of the fitness-related phenotypic traits measured.
    - Transgenic and non-transgenic individuals were equally likely to be dominant.
    - In stream environments with limited food, the transgene did not influence the growth in mass or survival at high or low fry densities.
- Abrahams, M. V., and A. M. Sutterlin. 1999. The foraging and antipredator behaviour of growth-enhanced transgenic Atlantic salmon. *Animal Behaviour* 58:933–952.
- Moreau, D. T. R., I. A. Fleming, G. L. Fletcher, and J. A. Brown. 2011. Growth hormone transgenesis does not influence territorial dominance or growth and survival of first-feeding Atlantic salmon *Salmo salar* L. in food-limited stream microcosms. *Journal of Fish Biology* 78:726–740.