Growth hormone transgenic Atlantic salmon: Opportunities, risks, and risk management

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What role might GM fishes play in coldwater marine aquaculture?

- Scope of effort on development of transgenic fishes
- Case study on GH-transgenic Atlantic salmon
- Case study within larger regulatory and societal contexts

A large international effort has developed transgenic aquatic and marine organisms

Fishes:

Zebrafish
Medaka

Grass carp
Common carp
Goldfish
Wuchang fish
Giant loach
Northern pike
Rainbow trout
Coho salmon
Atlantic salmon
Arctic charr
Mummichog
Striped bass
Largemouth bass
Walleye
Nile and hybrid tilapias
Cutthroat trout
Catla
Rohu
Mrigal
Channel catfish
Indian catfish

Crustaceans:

Crayfish
Pacific white shrimp
Brine Shrimp
Tiger shrimp
Giant freshwater prawn
Kuruma prawn

Mollusks:

Pacific oyster
Eastern oyster
Blue mussel
Dwarf surfclam
Red abalone
Japanese abalone
Pearl oyster

A large international effort has developed transgenic aquatic and marine organisms

Transgenes:

Reporter genes

Growth hormone
Antifreeze polypeptide
Interferon
Cecropin
Lactoferrin
Phytase
Carbohydrate metabolism
Vitamin C metabolism
Fatty acid metabolism
GnRH antisense
Aromatase antisense

Human clotting factor VII
Insulin

Reporter genes for contaminants

Countries:

United States
Canada
Cuba
United Kingdom
France
Norway
China
Japan
Korea
India
Israel
Finland

Growth hormone-transgenic fishes developed for potential use in aquaculture:

Esocidae
- Northern pike

Salmonidae
- Atlantic salmon
- Coho salmon
- Chinook salmon
- Rainbow trout
- Cutthroat trout
- Arctic charr

Cyprinidae
- Goldfish
- Common carp
- Catla
- Rohu
- Mrigal

Cobitidae
- Mud loach

Ictaluridae
- Channel catfish

Heteropneustidae
- Indian catfish

Cichlidae
- Nile tilapia
- Hybrid tilapia

Percidae
- Walleye

Case study: AquAdvantage salmon

- Atlantic salmon expressing Chinook salmon growth hormone gene
- 4–6x growth rate enhancement early in life
- 10–20% improvement in feed conversion efficiency
- Prospect of shorter production time, reduced costs, improved efficiency and profitability

Regulatory Context

- No GM animal intended for use as food by humans has received regulatory approval…
- AquaBounty seeks FDA approval for limited production of the product under strict confinement in Panama; full-scale commercial production *not* sought at this time
- December 26, 2012: U.S. Food and Drug Administration announced preliminary finding of no significant impact for specific conditions of use of GH-transgenic Atlantic salmon
- Public comment period open through April 26, 2013
- FONSI potentially opens the way for approval of pilot-scale production under strict confinement in Panama

Regulatory Context

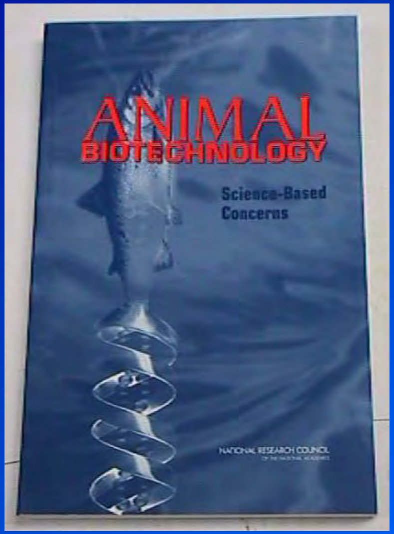
- Approval would be a threshold event in the commercialization of GM animals
- Genetic modification may prove be a useful tool for genetic improvement – what are the implications?
- Food safety
- Environmental safety

Risk assessment framework

- Identify potential *harms* - outcomes
- Identify *hazard* that might lead to harms – the transgenic stock
- Assess *probability of exposure* – likelihood of escape and persistence of transgenics in receiving ecosystem
- Assess *probability of harm given exposure*
- $R = P(E) \times P(H|E)$

Ecological risk assessment for transgenic organisms

- Considered on a case-by-case basis:
- Host species
- Introduced genetic construct
- Integration event
- Receiving ecosystem



National Research Council (2002)

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. 2000a,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

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 predators

Smoltification (Saunders et al. 1998):

- Transgenics reached smolt size (16cm) sooner
- Not inhibited by high temperature (19C) or constant light

Empirical observations of transgenic Atlantic salmon

<u>Cardiorespiratory function</u> (Deitch et al. 2006)

- 29% larger heart
- 18% greater mass-specific cardiac output
- 14% greater hemoglobin concentration
- 5-1-% higher red muscle and heart aerobic enzyme activities
- 1.7-2-fold higher catecholamines

- Yet, 18% lower metabolic scope
- 9% lower critical swimming speed
- Gill surface area was not enhanced
- Oxygen transfer may have been limiting

What is the *fitness* of transgenic individuals?

- Negative impacts of expression of transgenes led some to suggest that transgenics pose *no* significant environmental risk
- However, empirical observations of *GH* transgenics also show:
- Heightened growth rate
- Heightened food conversion efficiency
- Larger ultimate size – may confer mating advantage
- Increased osmoregulatory ability

What is the *fitness* of transgenic individuals?

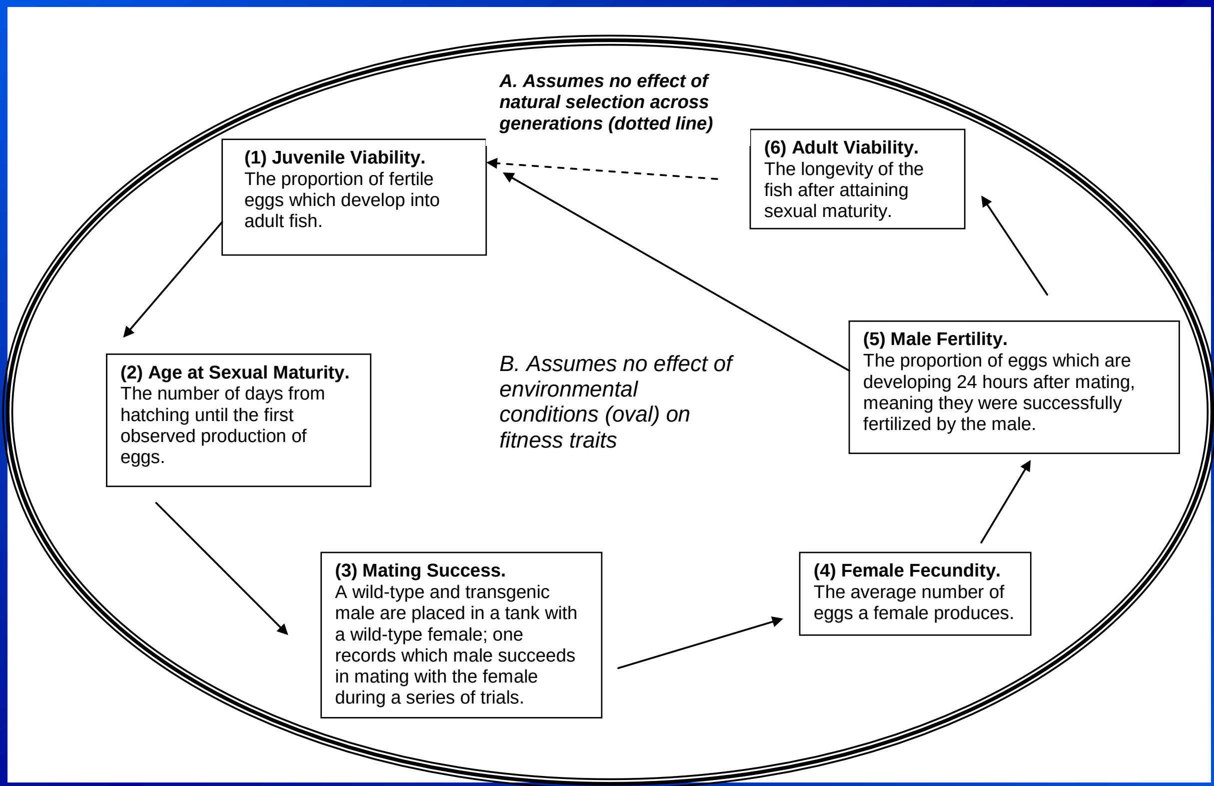
- Trait-by-trait assessments of fitness do *not* address the integrated phenotype of an individual, the "target of selection"
- Especially if there are *tradeoffs* among fitness-related traits, how to predict the fate of the transgene in receiving populations (and hence, likelihood of harm)?
- → Consider effect of transgene expression on *net fitness* of individuals…

Net fitness model

(Muir, Howard and colleagues)

Quantifies fitness of transgenics relative to that of wild-type

Environmental Risk Assessment of Genetically Modified Organisms: Methodologies for Transgenic Fish
Chapter 5: Approaches to Assessing Gene Flow
DRAFT SUBMISSION FOR PEER REVIEW April 28, 2006



(Kapuscinski et al. 2007)

- Demographic model tracks transgene frequency, population size
- Predicts whether transgene will be lost or become more frequent

Environmental Risk Assessment Parameters for Growth Hormone Transgenic Atlantic Salmon

Eric Hallerman and Ewen McLean
Virginia Polytechnic Institute and State University
Ian Fleming, and Garth Fletcher
Memorial University of Newfoundland

Goal: To develop empirical data useful for quantifying ecological and genetic risks posed by GH transgenic Atlantic salmon in the wild

Could heightened competition,
predation, or other processes
pose ecological harms
to receiving ecosystems?

Survival component of fitness

No difference between transgenics and controls regarding:

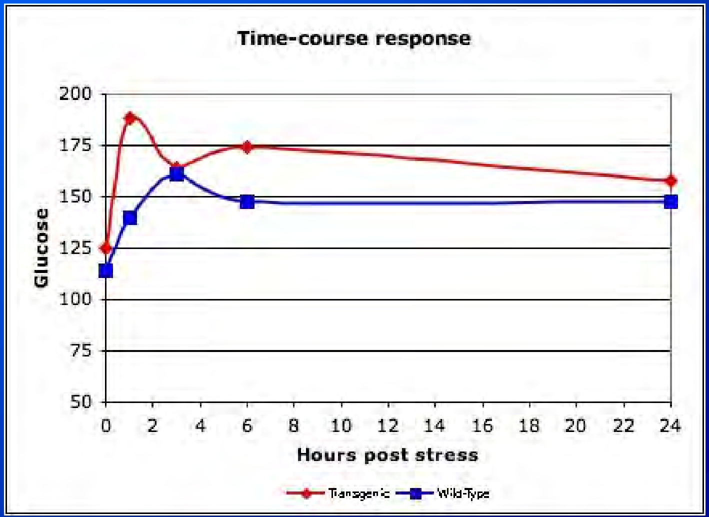
- oxygen consumption rate
- developmental rate
- survival until emergence from gravel
- fry behavior (classic intruder-resident relationships)
- growth and survival in artificial stream
- Moreau et al. 2001a. *Journal of Fish Biology* 78:726-740.

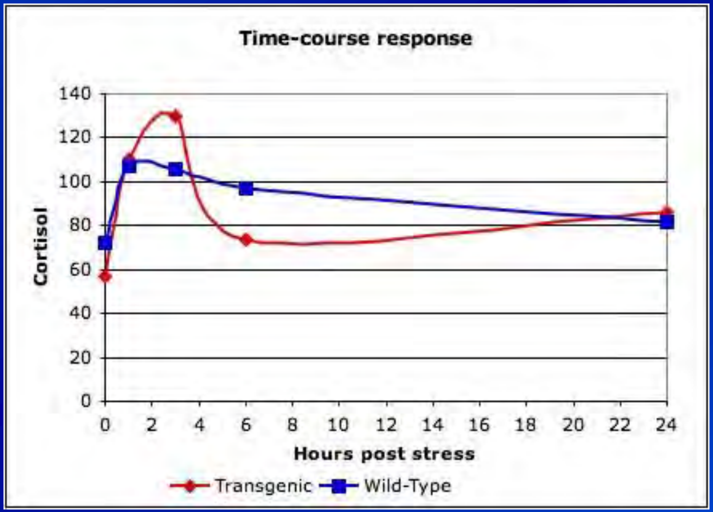
Survival component of fitness

Are transgenics compromised in the face of environmental stress?

- We applied stressors – starvation, low DO, and handling – and followed stress response variables: hematocrit, pH, pCO_2, Ca^{++}, K^+, Na^+, Cl^-, glucose, cortisol

Stress response





- Wild-type fish maintained hematological homeostasis more effectively than transgenic fish (lower fluctuation in all stress-response parameters measured)
- Transgenic fish were more stressed than wild-type fish (higher and faster stress response)

Predation upon natural populations

- Predation risks have not been assessed for transgenic Atlantic salmon

- Sundstrom et al. (2007) evaluated predation by transgenic and non-transgenic coho salmon upon fry prey in hatchery and naturalized stream environments
- *Under hatchery conditions*, transgenics grew dramatically larger than non-transgenics and exerted stronger predation effects, even after accounting for size difference
- *Under naturalized stream conditions*, trangenics grew only 20% larger than non-transgenics, and magnitude of difference in predation effects much reduced

Inferences:
- Environment influences predation intensity
- Laboratory studies may overestimate predation risk
- Use of naturalized environments will be critical for obtaining reliable risk assessment data

Could heighted predation, competition, or other processes pose ecological harms to receiving ecosystems?

My assessment: Transgenics *less* fit than wild type. Yet, under a range of ecological conditions, there *would* be considerable risk of ecological harm becoming realized

Could interbreeding of transgenic fish with wild populations pose genetic and evolutionary harms to receiving populations?

- Introgression is a risk *pathway*, but not a risk *endpoint* (i.e., not a harm in and of itself)
- Possible *harms*:
- Loss of (local) adaptation
- Reduced genetically effective population size (→ loss of genetic variation)
- In the extreme case, extinction of receiving population

Reproductive component of fitness

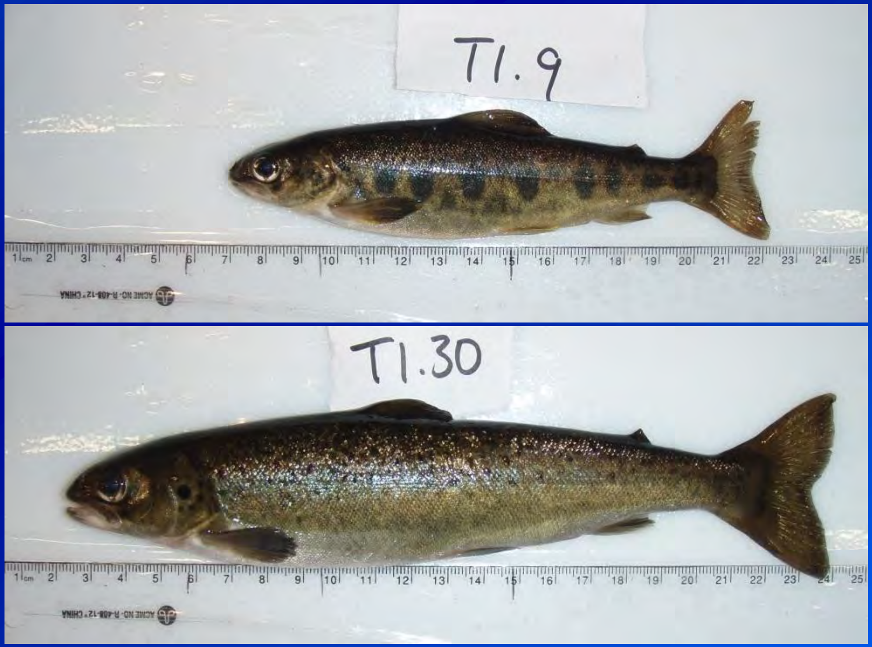
Background: Atlantic salmon males exhibit alternative reproductive tactics:

- *Precocious parr* do not migrate, mature young and small, and sneak fertilizations, sometimes with great success (11-65% of fertilizations in some populations)
- *Grilse* migrate to sea for one year, mature medium-sized, and sneak fertilizations
- *Parental males* migrate to sea, mature large, defend territory, and court females

- Expression of early maturation is related to growth rate - precocious parr may pose potent route for introgression of GH transgene

Maturation as parr

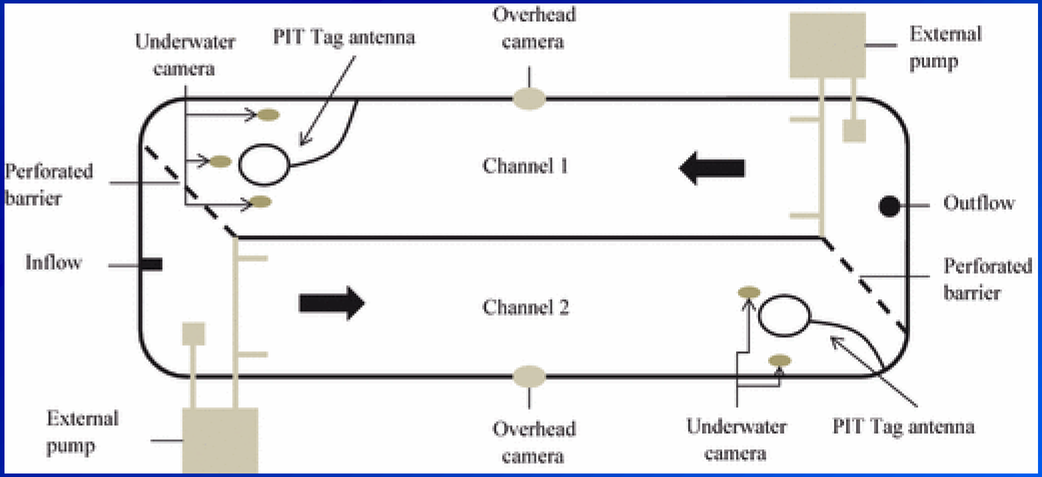
- Transgenics matured as parr less frequently than non-transgenics *(Moreau and Fleming 2012. Functional Ecology 26:399-405)*
- Opportunity to introgress is likely *reduced* for transgenic parr
- Transgenesis diverts resources toward growth and smoltification



Reproductive success

Experimental design:

- Compare behavior and reproductive success of transgenic and control males
- Concrete raceway (2.5m wide, 7.8m long, 2m deep) modified into circular stream, water depth 25-40 cm, current 12-15 cm/s, gravel substrate



Reproductive success

Anadromous male experiments:

- Stocked: Single wild female, 1 or 2 anadromous males, 15 parr (5 mature and 10 immature)
- Competitive phase – transgenic and non-transgenic anadromous males competed for breeding opportunities
- *Non*competitive phase – transgenic or non-transgenic anadromous male had sole access to female
- Each phase had 1-5 spawning events

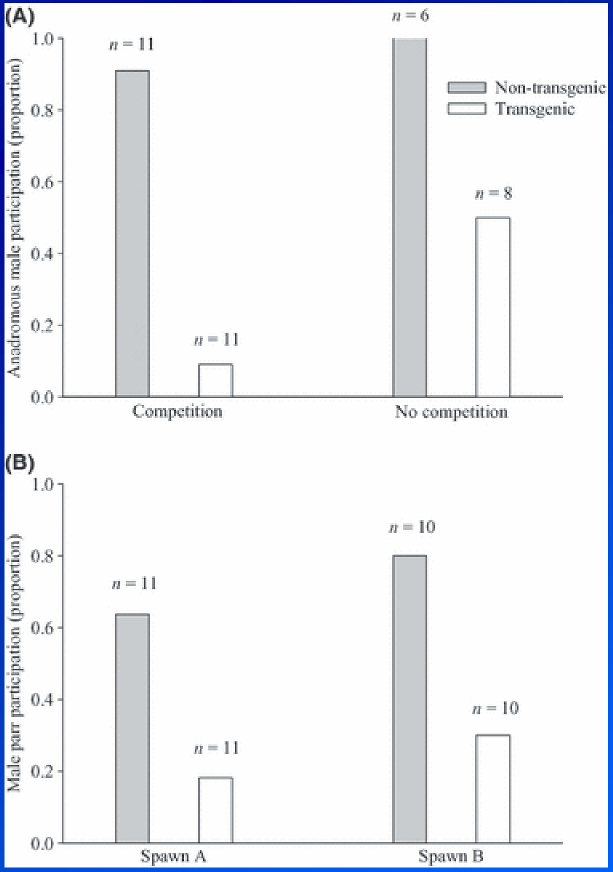
Reproductive success

Precocious parr experiments:

- Stocked: 2 wild anadromous females, 1 wild anadromous male, 6-8 transgenic parr, 6-8 control parr
- Each breeding trial had 1-4 spawning events
- Parr PIT-tagged, behavior videotaped, eggs suctioned from gravel, fry PCR-typed to determine parentage

Reproductive fitness of GH-transgenic Atlantic salmon

- Transgenic anadromous males were outcompeted in terms of nest fidelity, quivering frequency, and spawn participation (A)
- Transgenic parr were inferior competitors relative to wild-type parr in terms of nest fidelity, spawn participation (B), and fertilization success
- Transgenic males exhibiting either reproductive strategy exhibited *low*, but *non-zero* reproductive fitness



Moreau et al. 2011b. *Evolutionary Applications* 4(4):736-405.

Could interbreeding with wild populations pose genetic and evolutionary harms to receiving populations?

My assessment: Our knowledge of reproductive fitness of GH-transgenic Atlantic salmon is still limited – risk may generally be low, but it is non-zero.

Predicting net fitness of GH transgenic Atlantic salmon and transgene fate in near-natural ecosystems

To summarize…

- Survival fitness equal or less than wild type
- Reproductive fitness decreased relative to wild type
- Net fitness is reduced → transgene will be purged from population following a *single* episode of introduction
- *(What if introductions are recurring?)*

Risk assessment and risk management for GM Atlantic salmon

- Ecological and genetic risks are small, but non-zero
- Because of GxE interactions, we can *never* know and quantify risks for all potential receiving ecosystems

Risk management → Risk assessment

- Recognizing that $R = P(E) \times P(H|E)$, R may be minimized by minimizing $P(E)$
- Ecological risk may be minimized by culturing transgenic fish under strict confinement:

Assessment of AquaBounty proposal

- The AquaBounty proposal before US-FDA is for pilot-scale production of GH-transgenic Atlantic salmon:
- In Panama
- In indoor recirculating aquaculture systems with redundant physical confinements
- With reproductive confinement – all-female triploid fish

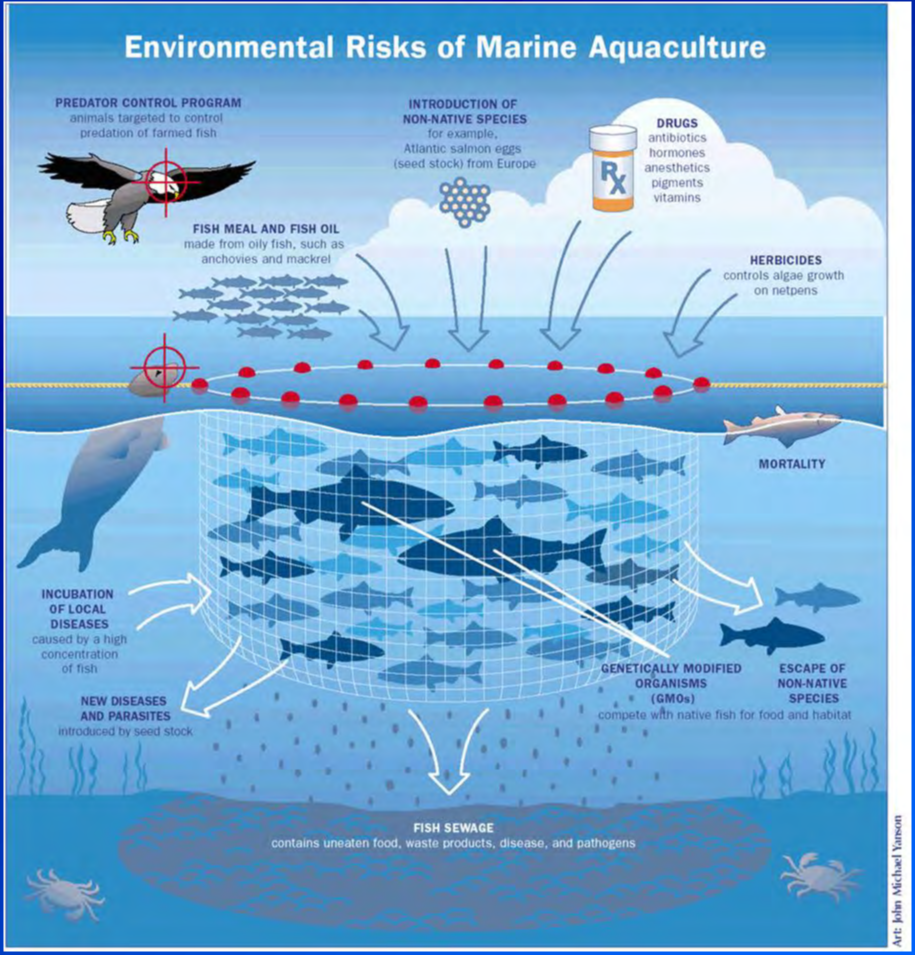
→ Evaluate production economics

→ Demonstrate effective confinement

- *Should outcomes prove positive, AquaBounty would have to seek further approval for expanded production.*
- We are still years away from possible commercial-scale production of GM Atlantic salmon

Risk placed into perspective

- Risk should be assessed relative to *appropriate* comparators
- Non-zero risk is *not* an option.
- Consider the status quo… conventional Atlantic salmon production itself poses environmental impacts:
- Local eutrophication
- Transmission of parasites and pathogens
- Escape and interbreeding



Biotechnology can be applied to reduce the environmental impacts of conventional aquaculture

- Produce only sterile triploid selectively bred salmon in netpens → addresses genetic impacts (incompletely)

- Produce (transgenic) salmon onshore in biosecure systems → addresses genetic and (most) ecological impacts

- Commercial production of Atlantic salmon in recirculating systems is being evaluated in Maine and Nova Scotia

- The FDA decision on the AquaBounty salmon could be a threshold event in the commercialization of transgenic fishes and animals more generally…

Other transgenic fishes in the pipeline

- GH-transgenic fishes: Tilapia in Cuba, common carp in China, other species, several countries
- Disease resistant lines: Cecropin in channel catfish, Lactoferrin in grass carp
- Use of plant-derived feeds: Phytase in tilapia
- Sterility: Antisense gonadotropin-releasing hormone in rainbow trout, other genes aimed at transgenic sterilization in channel catfish

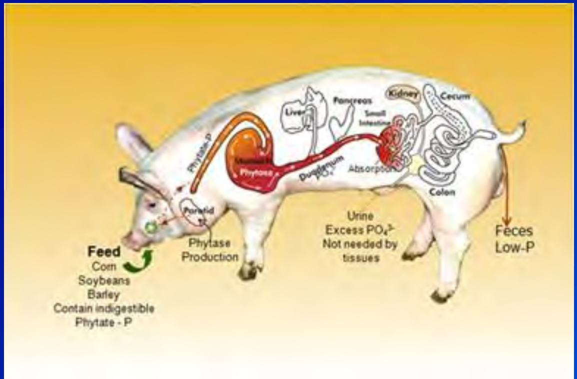
Other transgenic animals in development

- Goats expressing lysozyme – reduces milk bacterial loads
- RNAi → Cattle producing β-lactoglobulin-free milk
- Pigs expressing α-lactalbumin to increase pre-weaning growth

- Biopharm cattle – cost-effective production of somatropin, bGH, insulin, monoclonal antibodies…

Other transgenic animals in development

- Enviropig – phytase expression → better utilization of phosphorous, lower excretion of P…but recently euthanized because of ongoing cost and regulatory uncertainty
- Porcine models of disease hung up in review because they "could" be eaten
- Regulatory uncertainty is stifling development of all GM animals



Take-home messages

- Genetic modification of aquaculture species poses both economic benefits and environmental risks
- Risks are likely to be non-zero; hence, risk management must be part of how we consider GM fishes
- Benefit and risk issues must be considered with regard to appropriate comparators; biotechnology can be applied to manage risk
- Regulatory uncertainty is stifling development of GM animals

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