


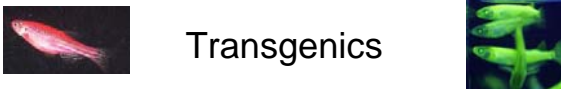
Biotechnology in Aquaculture

Transgenics and Polyploidy
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Overview

- Introduction
- Transgenic Research
 - Major Trends
 - Specific Cases
 - Environmental and Human Health Concerns
- Polyploidy
- Current Regulations
- Discussion Questions




Transgenics

- Transgenics = artificial transfer of foreign DNA
- Advances in biotech have made it possible to produce transgenic organisms.
- Fish are relatively tolerant to artificial gene manipulation.
- Aquaculture industry could exploit transgenics to increase production efficiency
 - Increased growth
 - Cold tolerance
 - Disease resistance
- Selection of desired traits is more effective, direct and selective than with traditional breeding.






Growth Hormone

- Polypeptide that induces synthesis and secretion of insulin-like growth factors
 - Increases appetite, feeding efficiency, and growth rate
- Use of GH in aquaculture is seen as a way to reduce production costs
 - reduce time to market size, exposure to disease, and avoid overwintering
- GH is expressed seasonally and in bursts.
- GH supplements are available, but they have only been somewhat successful in aquaculture and are not cost-effective.



Growth Hormone Transgenics

- Human GH gene first transferred to goldfish in 1985 (Zhu et al. 1985)
- GH has been a major focus of transgenic research
 - Determining optimum balances of GH expression (can cause growth disorders)
 - Switching from human GH to fish-derived GH.
 - GH transgene has been linked to a promoter for an ocean pout gene (AFP) that is expressed year-round.

“All-fish” GH-transgenic Strains

Species	Results
Common carp	20-150% increased growth
Tilapia	2.5-4x increased growth 20% greater feed conversion efficiency
Red sea bream	9% length increase 21% weight increase
Coho salmon	10-30x growth increase
Atlantic salmon	2-13x increased growth, greater feed conversion efficiency, transgene inheritance through 6 generations

GH-Transgenic Chinook Salmon

Age = 11 months



Transgenic salmon
Ave wt = 850 g

Non-transgenic sibling
wt = 280 g

www.oceanatlas.org/.../biotech/genbiotech.htm

GH-Transgenic Tilapia

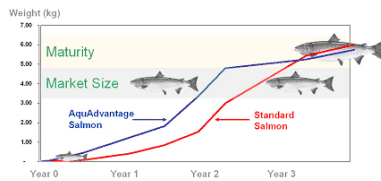


Non-transgenic

Transgenic

<http://www.sbs.soton.ac.uk/staff/nm/nm.php>

AQUA BOUNTY Technologies Health And Productivity



- AquaBounty has created transgenic lines of tilapia, salmon and trout
- Sterile AquAdvantage™ salmon are currently under regulatory review in both the US and Canada
- "Atlantic salmon with a GH gene from Chinook salmon is likely to be the first GM animal on the food market" (WHO)

Cold Tolerance

- Inability to tolerate cold temperatures is a limiting factor in aquaculture production
 - Warm water species (e.g. tilapia and common carp)
 - Cold water species in freezing and subzero coastline conditions (e.g. Atlantic salmon)



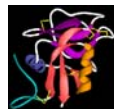
- Along Canada's Atlantic coast, Salmon farming is restricted to a few southern sites.
- Improved cold tolerance could increase the geographic range and production potential for aquaculture.

Antifreeze Proteins

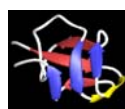
- Marine fish in very cold waters have body fluids with the same freezing point as seawater (-1.7 to -2°C) rather than freshwater (0°C).
- These fish contain proteins that inhibit ice crystal growth and lower the freezing point of bodily fluids – termed antifreeze proteins (AFPs).
- Fish rich in AFPs include winter flounder (type I), sea raven (type II) and Newfoundland ocean pout (type III).



Type I



Type II



Type III

F.D. Sørensen et al. 1993, 1995

AFP-Transgenic Fish

- Ocean pout type III AFP was successfully expressed and inherited in lines of goldfish and Atlantic salmon.



- AFP-transgenic fish show increased cold tolerance but not freeze resistance.
- Current challenge is to increase expression of AFP in the liver and the epithelial tissues.



Disease Resistance



- Disease is a major limitation facing the aquaculture industry.
 - Immune system is weakened due to factors such as high-density culturing and stress.
- Example – Channel catfish
 - Account for >60% of US aquaculture
 - Yearly losses of >\$100 million due to disease
- Antibiotics and vaccines are limited and labor-intensive.
- Several antibacterial peptides have been isolated for genetic research.



Disease Resistance



Cecropins

- Small, antibacterial peptides first identified in the silk moth
- Already being used in transgenic plants, e.g. tobacco and potato
- Cecropin-transgenic fish (channel catfish and Japanese medaka) have shown increased disease resistance against several pathogens

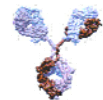


Lysozymes

- Nonspecific antibacterial enzymes
- Shown to increase disease resistance in plants
- Transgenic zebrafish with chicken lysozyme gene showed increased disease resistance



Disease Resistance



- Human lactoferrin (hLF)
 - Nonspecific antimicrobial and immunomodulatory iron-binding protein
 - Already used in agriculture to increase disease resistance of tobacco and potatoes
 - hLF-transgenic carp showed increased resistance to the Grass Carp Hemorrhage virus, a major setback to carp aquaculture
- Immunoglobulin (IgM) proteins
 - Sharks are high in IgMs, which initiate immune responses to bacterial invasions
 - IgM-transgenic fish have shown increased immune activity and growth
 - Excessive doses have resulted in deformed reproductive organs



Metabolism of Land-Based Plants

- Aquaculture diets: terrestrial feed is generally favored over marine-based feed
 - Lower costs
 - Conserve marine ecosystems
- However, use of terrestrial diets introduces nutritional and metabolic problems
 - Low in certain marine-based nutrients, such as omega-3 fatty acids EPA and DHA
 - Contain indigestible compounds, e.g. phytate



Omega-3 Production



- Long-chain omega-3 fatty acids DHA and EPA are valued for their numerous health benefits.
- Terrestrial plant oils contain the precursor ALA.
- Transgenic plants:
 - Produce up to 19% EPA and up to 3.3% DHA.
- Transgenic fish:
 - Rate-limiting step involves the enzyme n-6-desaturase.
 - Zebrafish with an n-6-desaturase transgene showed increased EPA (1.4x) and increased DHA (2.1x).



Phytate Metabolism



- Phytase breaks down phytate, the major form of phosphorus (P) in plants.
- Nonruminant animals cannot digest phytate and excrete it as waste.
 - Eutrophication
 - Diets must be supplemented with inorganic P
- The phytase gene from *Aspergillus niger* was recently expressed in Japanese medaka.
 - Showed increased survival on diets rich in phytate
- Further research is needed to characterize factors such as phytate excretion levels.

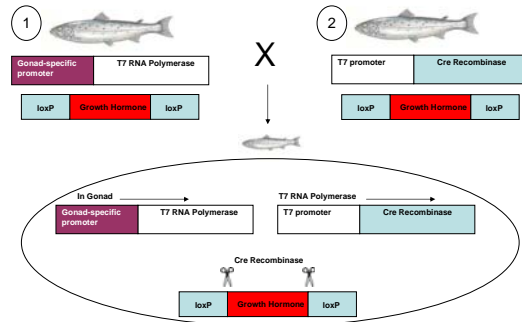




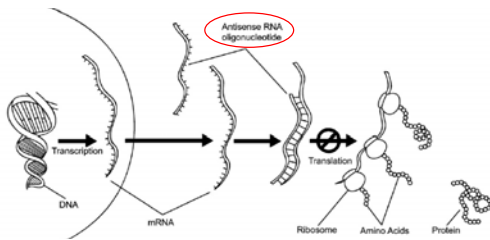
Gene Excision and Sterility

- Escapement and breeding with wild stocks is a major concern in transgenics.
- Gene-excision approach:
 - Gonad-specific transgene excision
- Sterility approaches:
 - Gene manipulation - inhibit expression of reproductive genes with antisense RNA
 - Chromosome manipulation - Polyploidy

Gonad-specific transgene excision



Gene Knockout with Antisense RNA

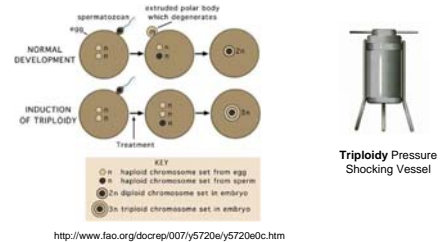


- Antisense RNA binds endogenous mRNA, thus blocking translation of target gene
- Usually targets a gene involved in reproductive development
- Preliminary results show fertility is reduced to ~50% of non-transgenic controls

(Wong and Eenennaam 2008)

Polyploidy

- Manipulation of embryos to induce multiple sets of chromosomes.



<http://www.fao.org/docrep/007/y5720e/y5720e0c.htm>

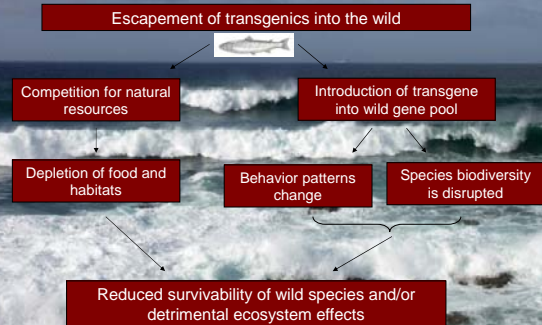
Triploidy

- Results:
 - Lack of gonad development (sterility)
 - More energy for somatic growth (improved food quality)
- Highly effective and widely used in shellfish
- Variable results with fish



The "All Season" Oyster
By Hilton's Coast Seafoods

Environmental Concerns





Human Health Concerns



- Food safety trials were conducted with GH-transgenic tilapia with no adverse effects
 - Human volunteers consumed transgenic tilapia 2x/day for 5 days
- Effects of long-term consumption of GM foods are unknown.
- Insertion of foreign genes into species might result in production of toxins or allergens.
- Increased disease resistance could result in higher levels of pathogens in food supply.
- Bioactive proteins, such as GH or cecropins, could affect consumers.

Regulations on Transgenics

- FDA Center for Veterinary Medicine (CVM) regulates animal biotech products
 - Transgenic animals have not been approved to enter the food supply
 - Transgenic fish are being actively investigated



U.S. Food and Drug Administration



Conclusions



- Transgenics and polyploidy could help to increase aquaculture production and relieve food shortages.
- However, numerous environmental and human health concerns must also be considered.



"This could revolutionize the fast food industry - transgenic fish fingers!"

(Chan and Powers 1990)



Discussion Questions

Scientific

- If you could add a transgene to fish, what would it be?
- What are the scientific boundaries of this technology?
- Is it acceptable to use transgenics to produce medical drugs or beneficial nutrients?

Environmental

- Could GMOs result in improved environmental effects compared to current aquaculture?
- Is escapement a valid issue?

Ethical concerns

- Should transgenic fish be used to increase the protein supply in developing countries?
- What are the animal welfare issues?
- Would you eat a transgenic fish?