



An Overview of Transgenic Fish

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<http://animalscience.ucdavis.edu/animalbiotech/>

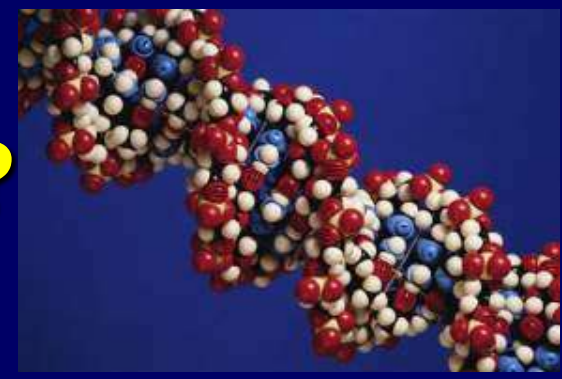


Outline

- What is biotechnology
- Introduction to transgenic fish – products being developed
- Concerns about transgenic fish
- Regulatory structure being developed
- AquAdvantage salmon
- Containment approaches
- Methods for reproductive containment of transgenic fish



What is Biotechnology ?



Biotechnology

Technology based on biology. The application of science and engineering to living organisms.





56% of Americans oppose scientific research into the genetic modification of animals

<http://pewagbiotech.org/research/2005update/2005summary.pdf>





Not breeding ... Just unnatural genetic modification of animals....

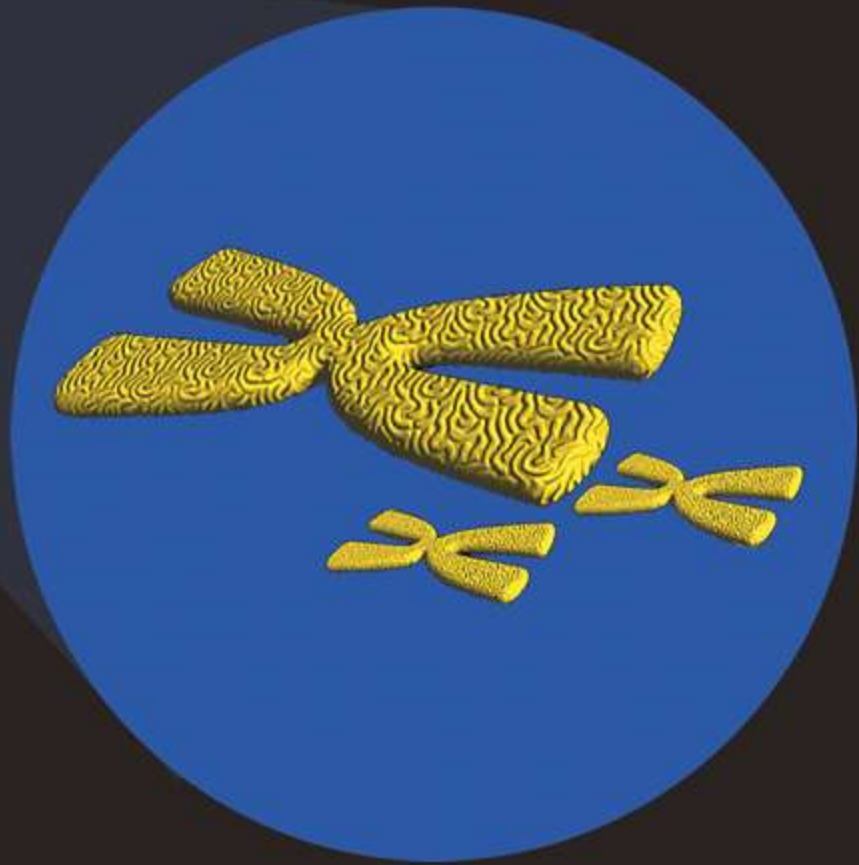
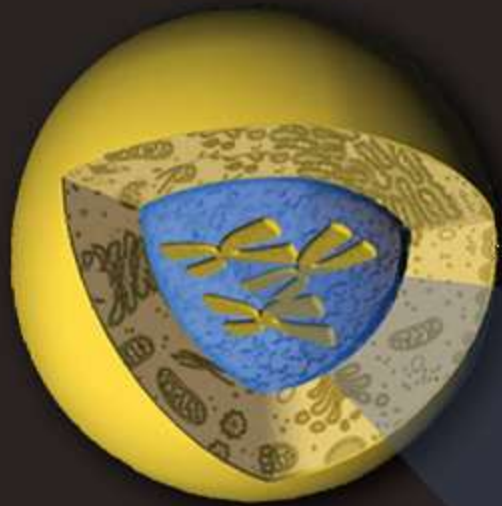


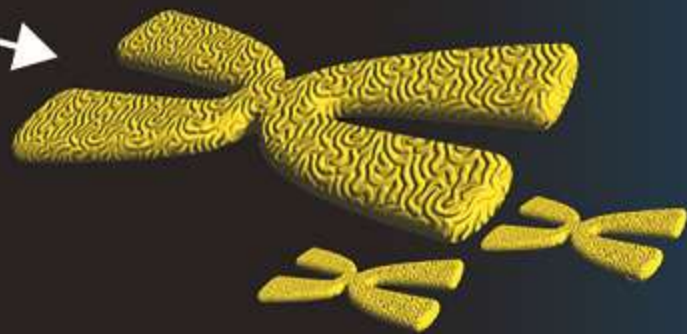
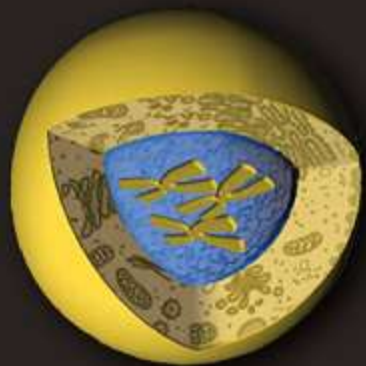


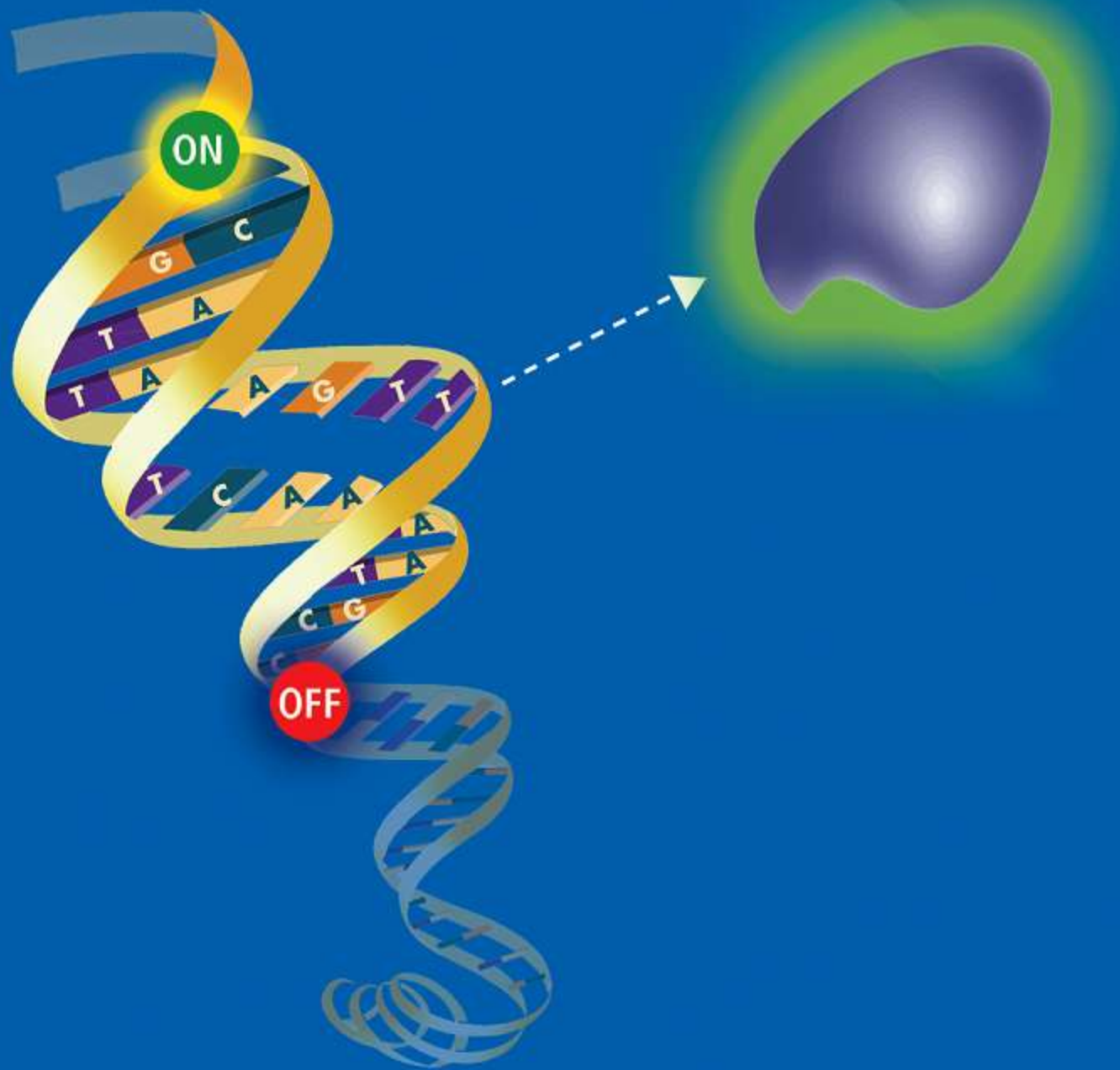
What is a genetically engineered organism ?

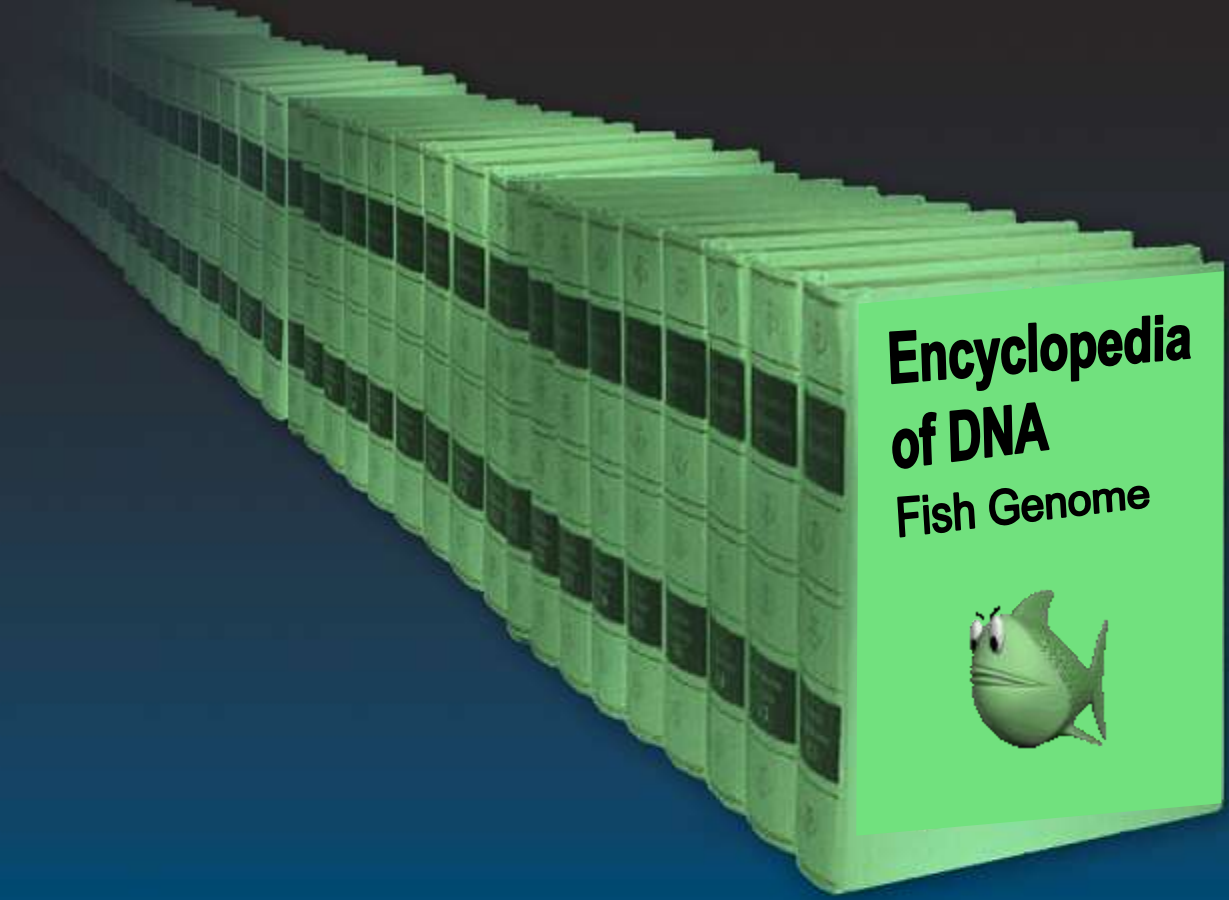
Genetically engineered organisms (GMOs, transgenic, GE) can be defined as those which have received sequences of DNA by artificial means (recombinant DNA techniques), followed by integration of one or more of the novel sequences into their chromosomal DNA





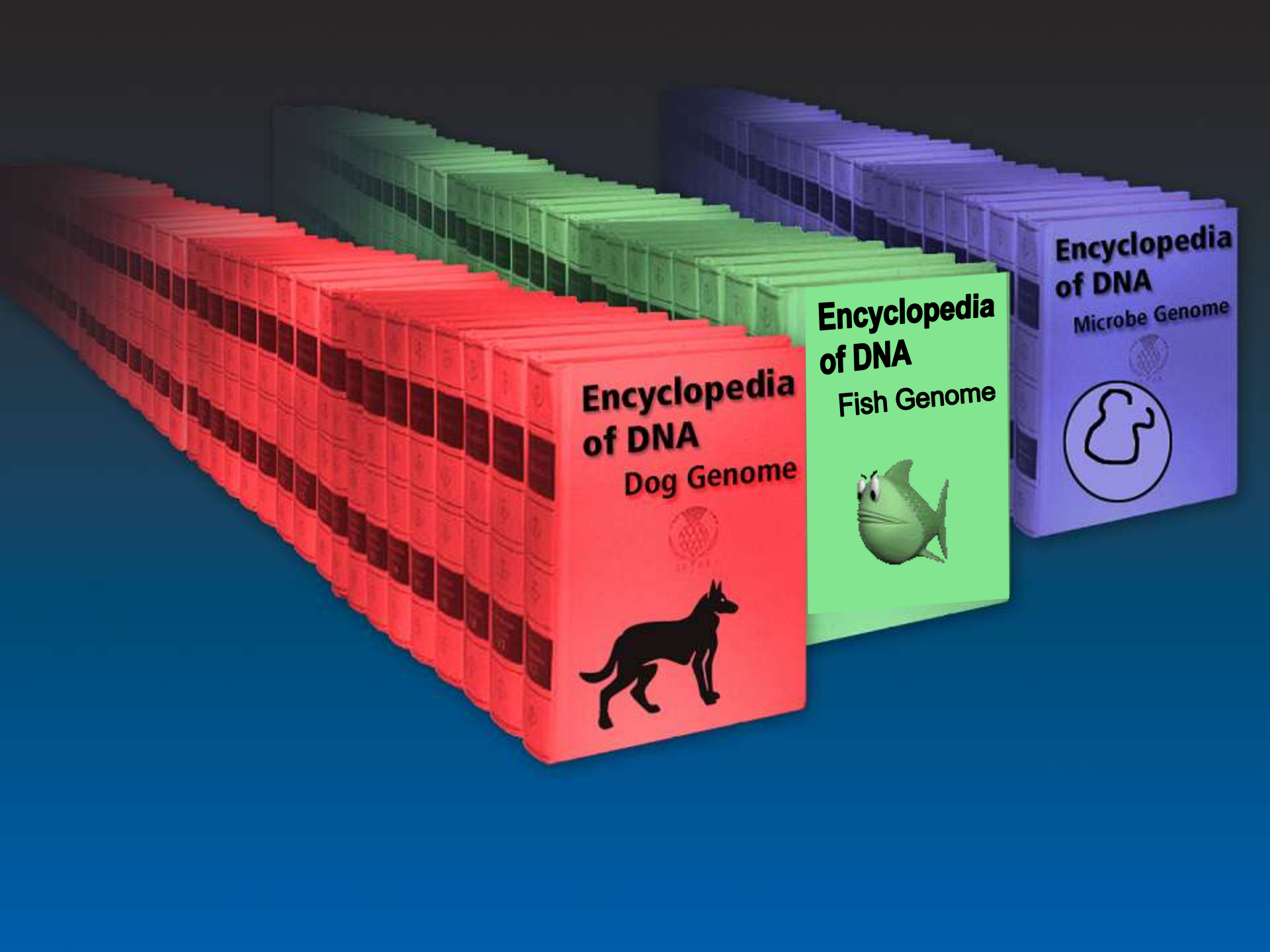






Encyclopedia of DNA Fish Genome





**Encyclopedia
of DNA**
Dog Genome



**Encyclopedia
of DNA**
Fish Genome



**Encyclopedia
of DNA**
Microbe Genome

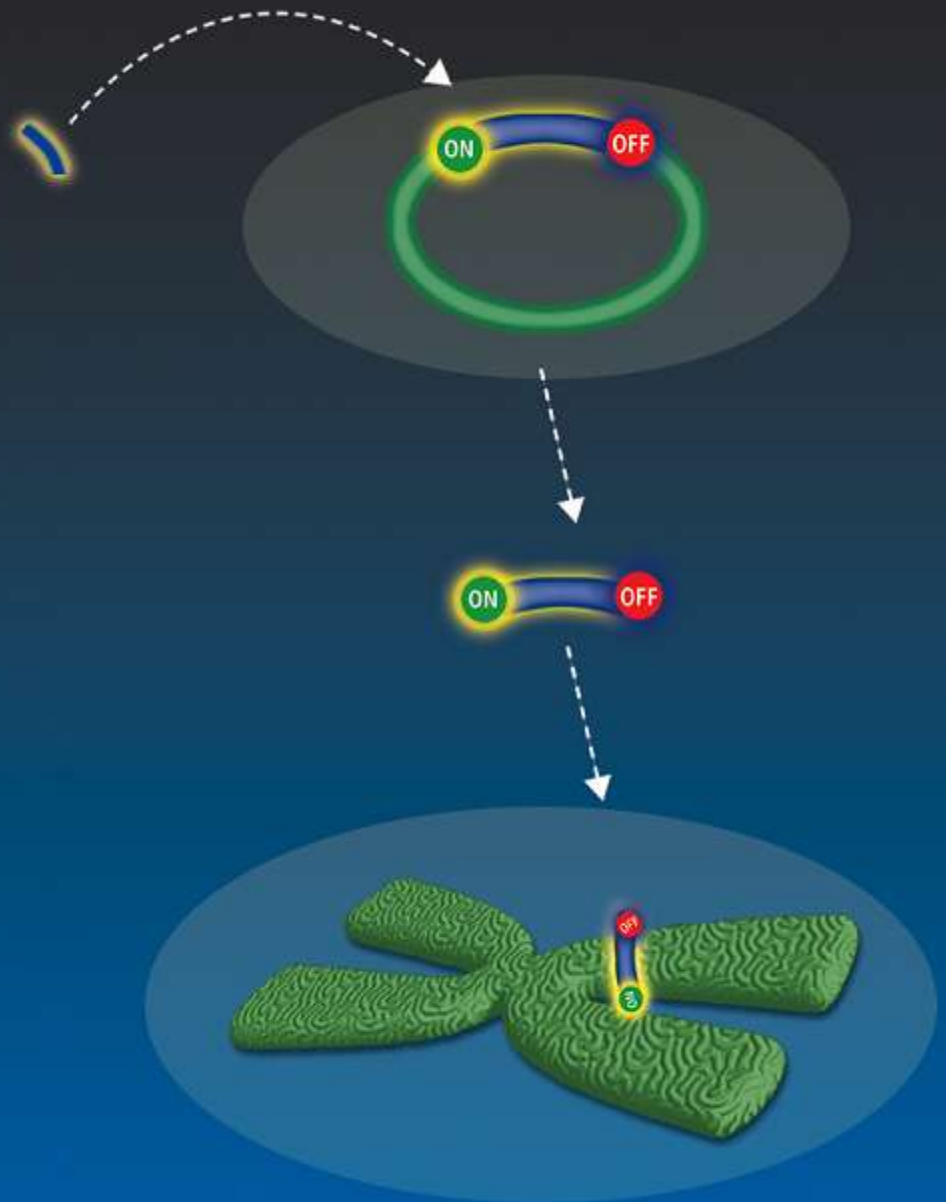


Encyclopedia of DNA Fish Genome

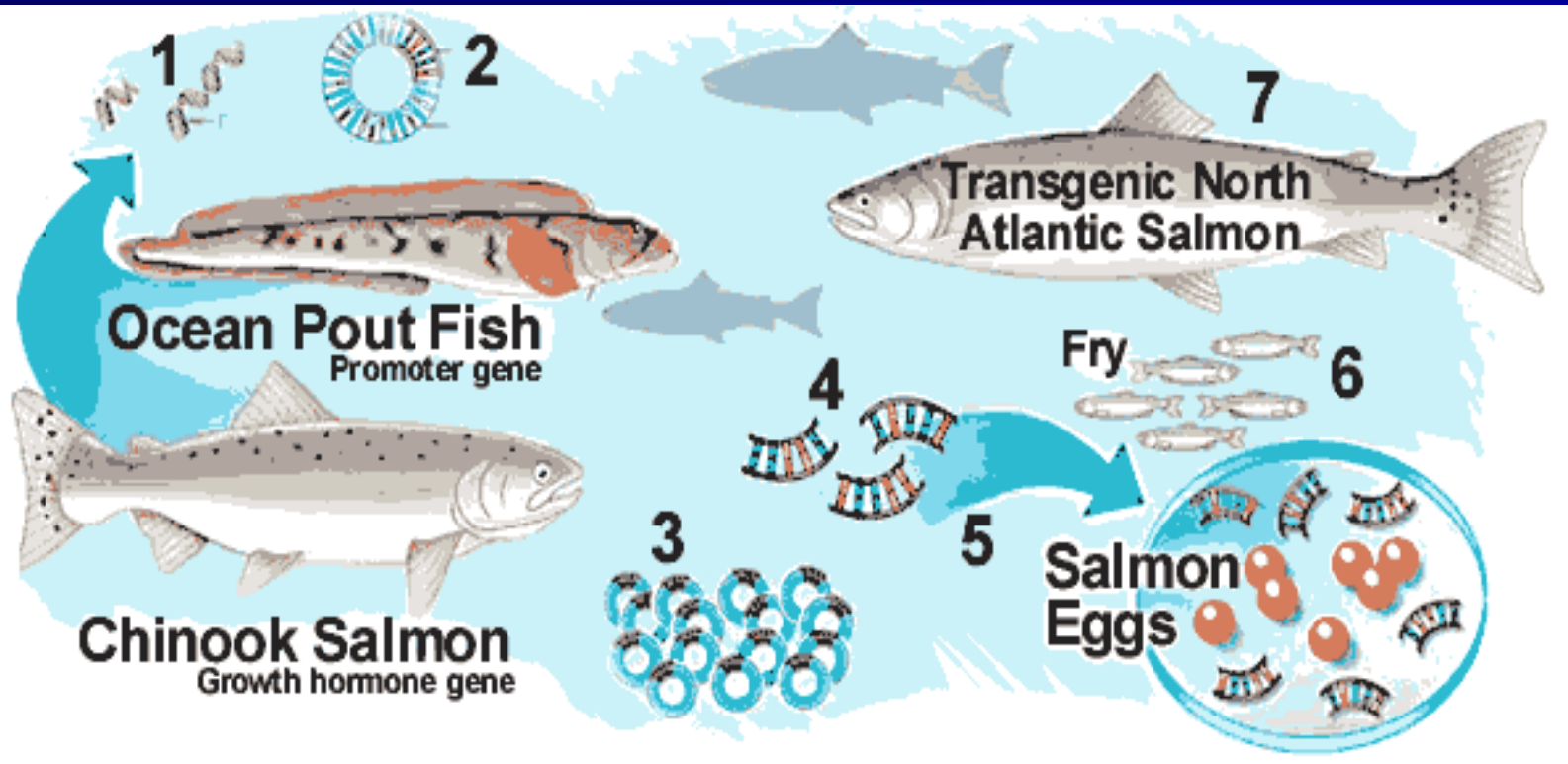




World Record 97 lb 4 oz. chinook salmon
(Les Anderson, Kenai River, AK 1985)



Growth-enhanced Atlantic Salmon





Same-age siblings – one carrying a hemizygous copy of the transgene



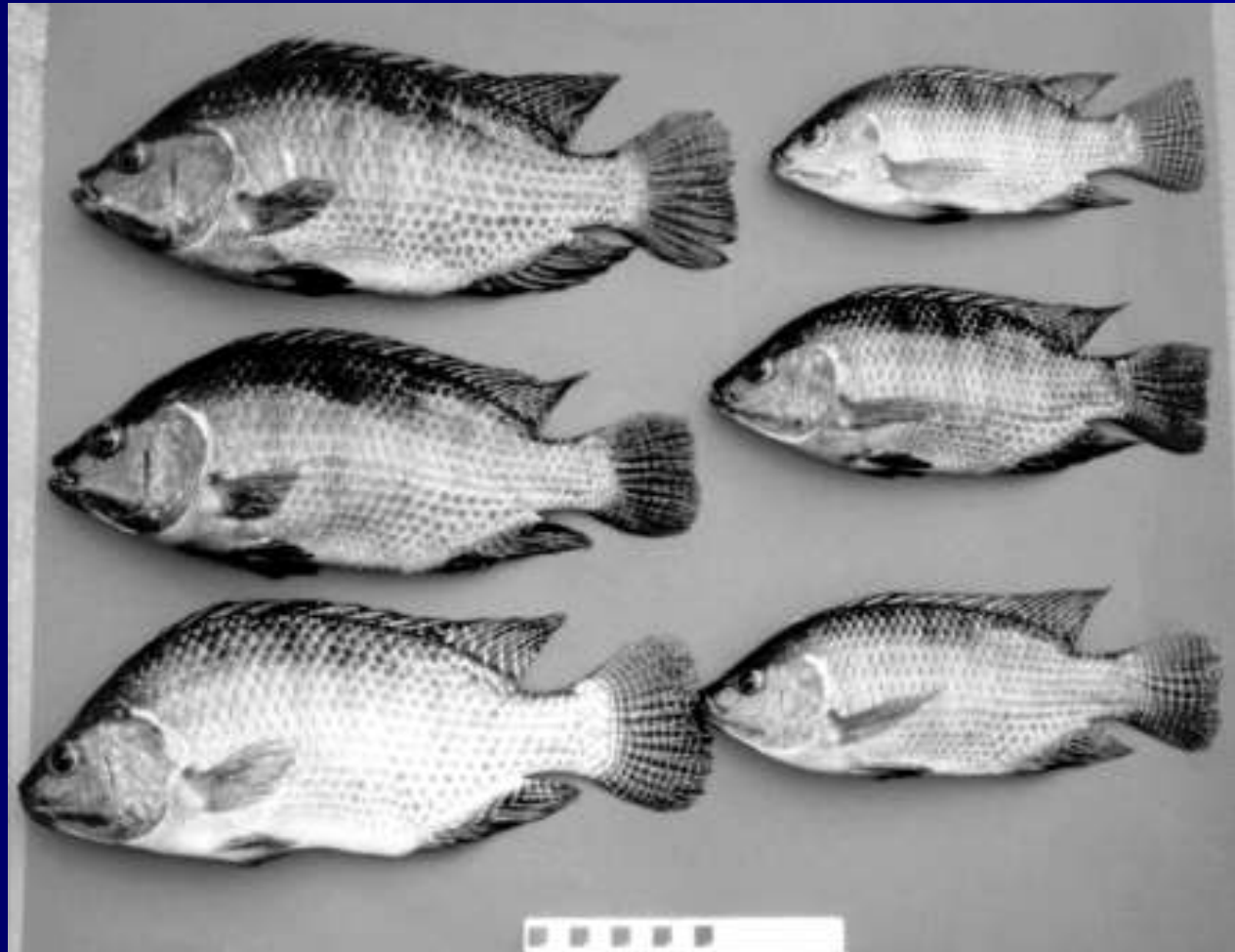


Current and potential applications of transgenic fish

- Growth enhancement
- Freeze resistance and cold tolerance
- Salinity tolerance
- Disease resistance
- Metabolic modification
- Improved product for the consumer
- Sterility
- Fishpharming – production of pharmacological proteins



Transgenic Growth-Enhanced Tilapia





Transgenic Growth-Enhanced Loach



Heterozygote

Homozygote
(androgenetic)

Wild-type



Objections to transgenic fish

- The procedure used to produce the fish is unnatural and therefore undesirable
- Transgenes could confer undesirable as well as desirable new properties on the fish
- Transgene incorporation could lead to other genetic problems (pleiotropic effects)
- Novel proteins could be allergens
- The fish, although not interbreeding, could be viewed as equivalent to an introduced alien species
- Fish might interbreed with the wild native fish and cause ecological harm



Traits that determine net fitness

- Juvenile viability (chances of surviving to sexual maturity)
- Adult viability (chances of surviving to procreate)
- Fecundity (number of eggs produced by a female)
- Fertility (number of eggs successfully fertilized by male sperm)
- Mating success (success at securing mates)
- Age at sexual maturity



Gene Flow - The Spread Scenario

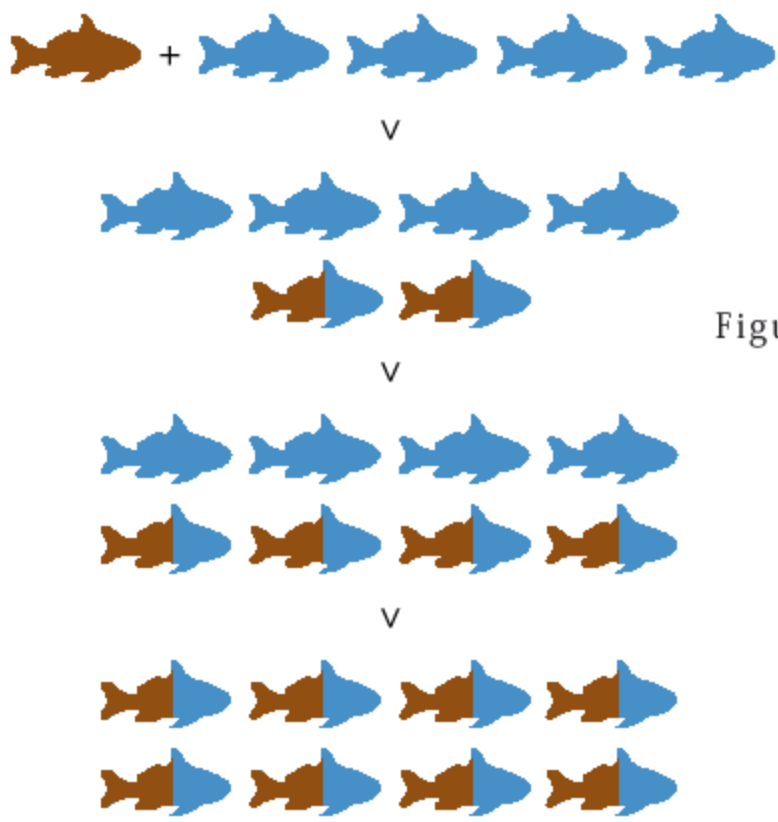




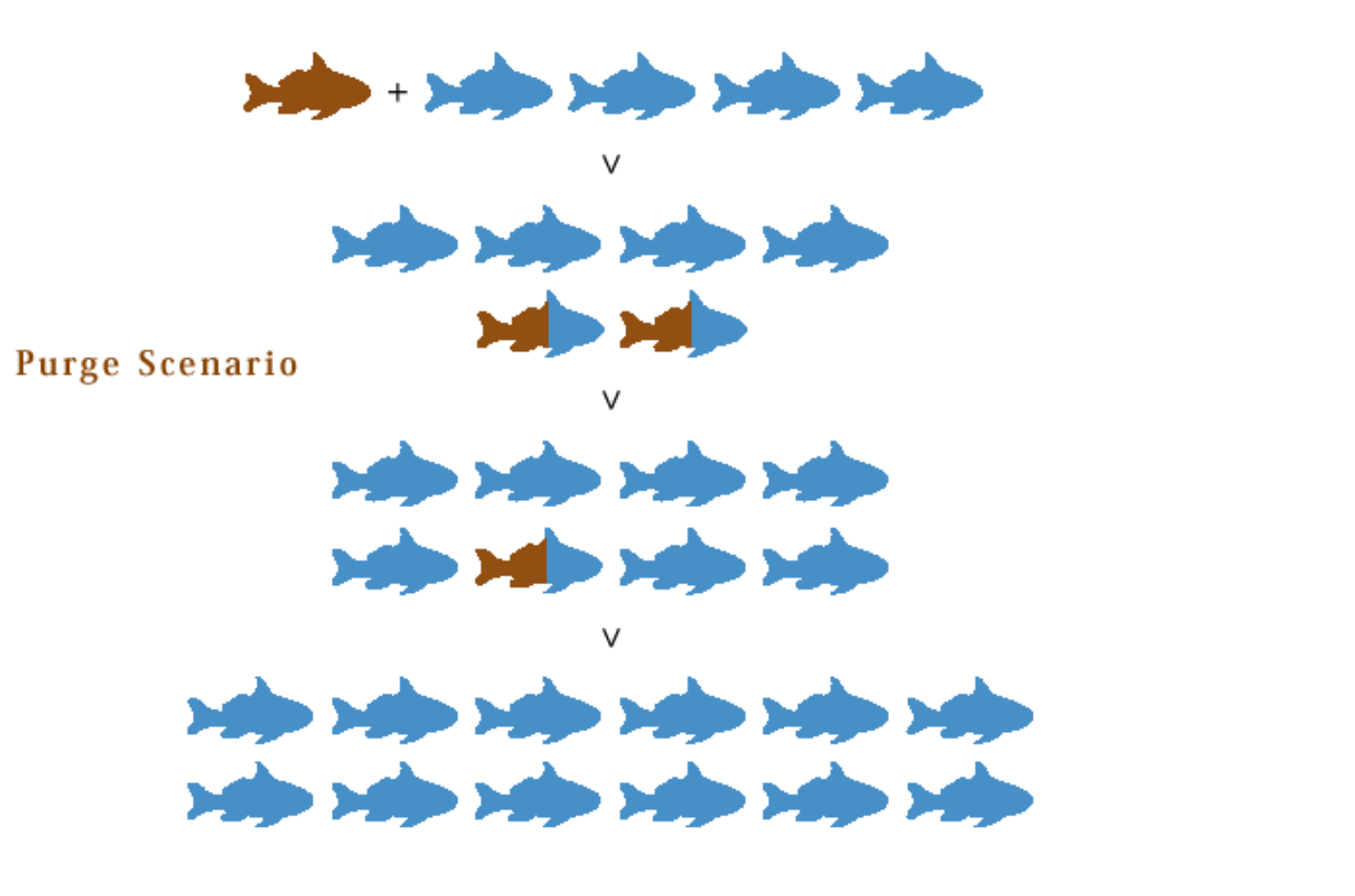
Figure 7: Spread Scenario

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

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

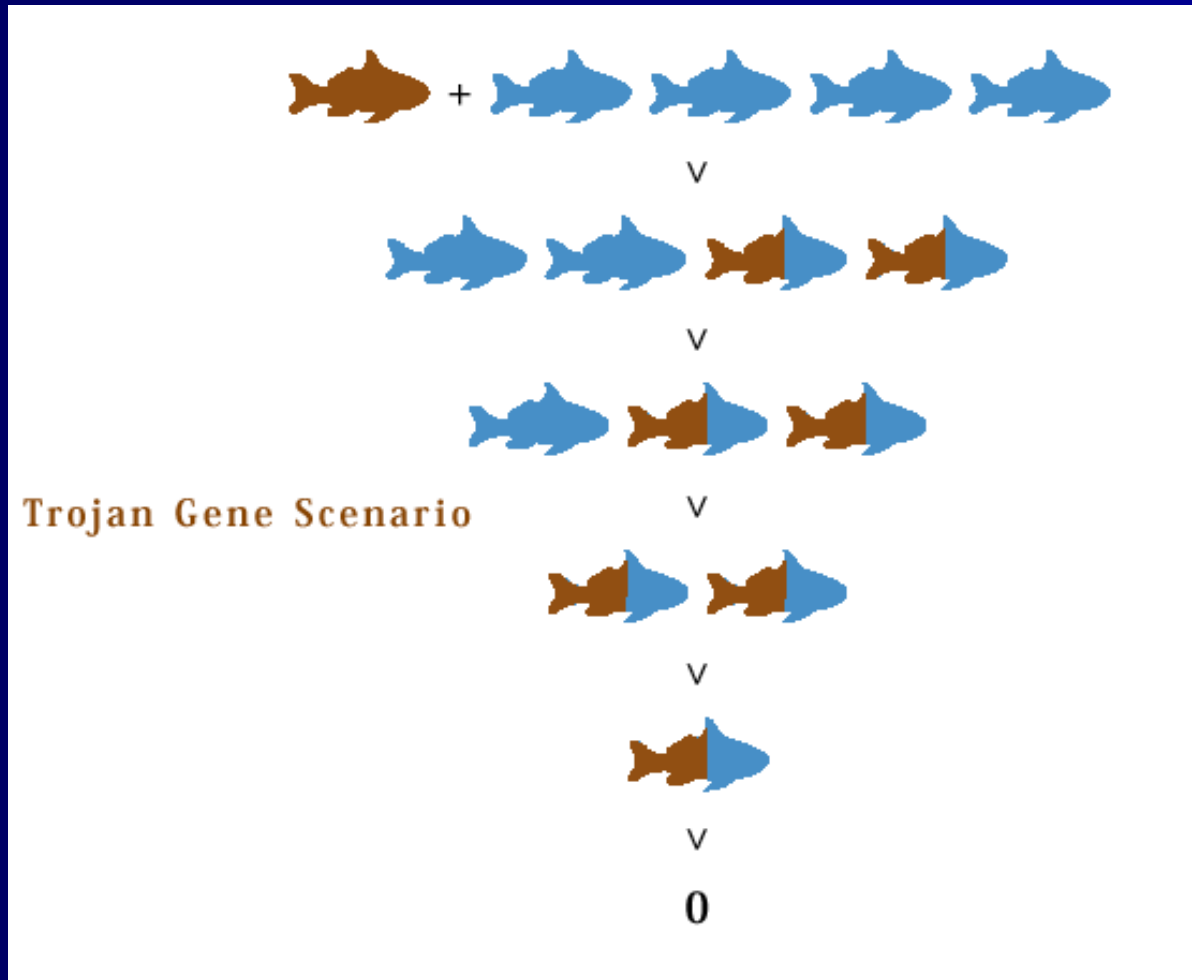


Gene Flow - The Purge Scenario





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



Occurs if the trait both increases male mating success AND lowers the viability of transgenic offspring **OR** Increases juvenile viability but reduces fertility



Gene Flow – Environmental concerns

- Depending on the interaction between the six fitness components, the risk of gene flow can range from none to significant
- Consequences vary case by case depending on the most likely gene flow scenario and the ecological characteristics of the transgenic fish and the fish community that it might affect
- **DIFFICULT (maybe IMPOSSIBLE) TO PREDICT BEFORE THE FACT !!!!**



Harvest of Fear video piece

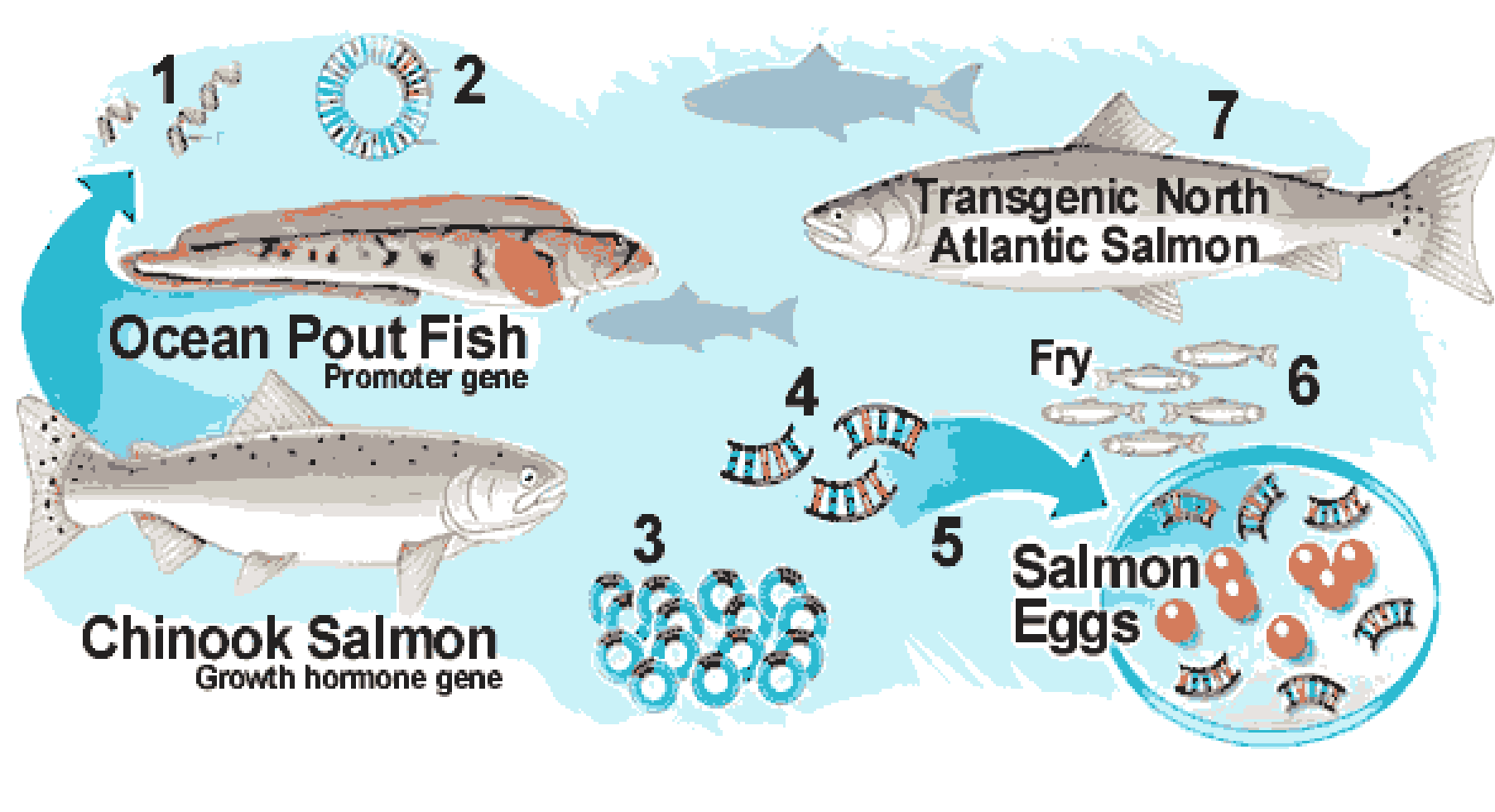
tomato

“Theyre half fish, half-flesh: a plague on them”
Perricles of Tyre 2.1

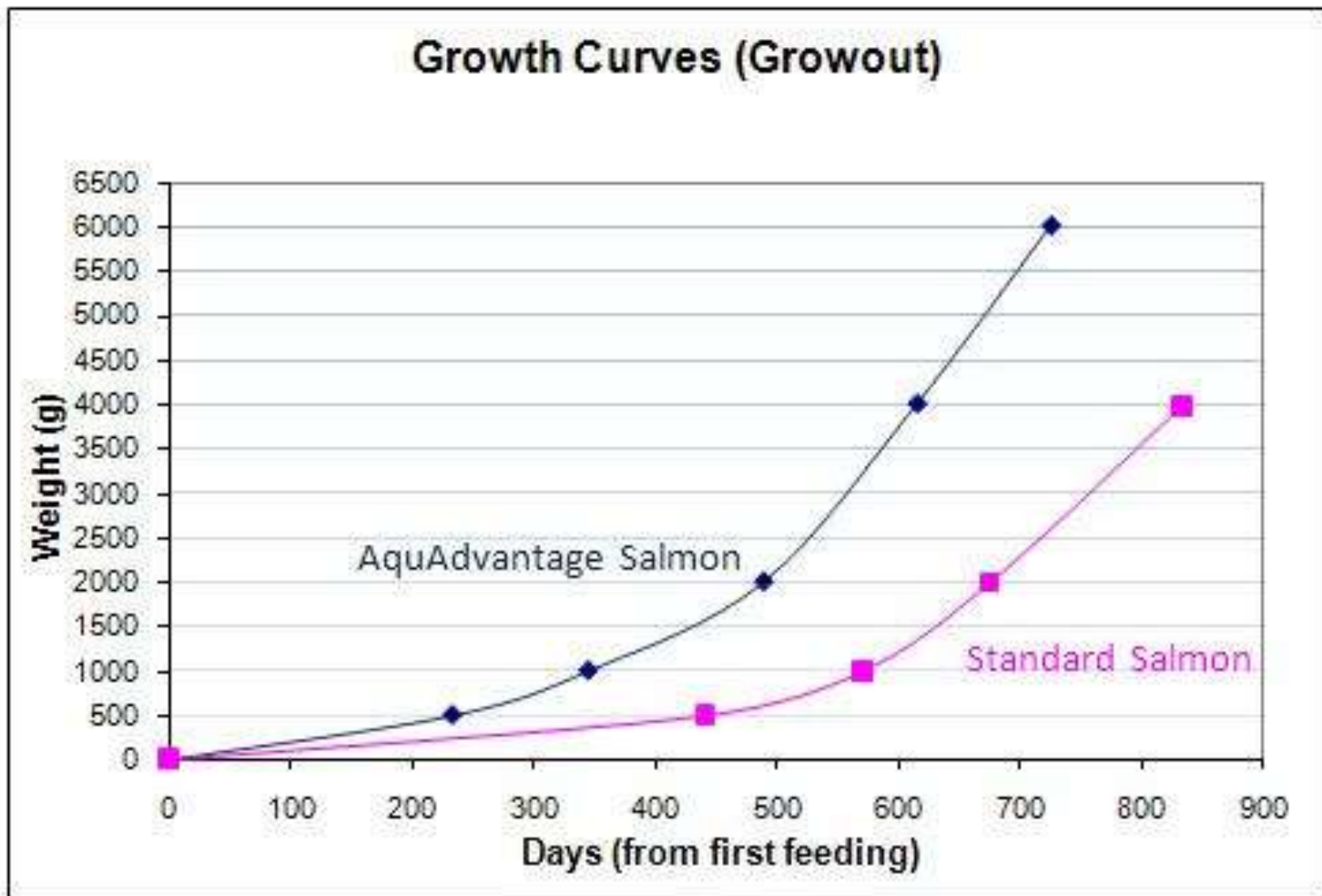




First GE animal for food to market? AquAdvantage salmon



Fish reach adult size in 16 to 18 months instead of 30 months





There was no regulatory paradigm to approve GE animals in 1993....



“In a letter to the FDA dated April 26, 1993, AquaBounty Technologies (then A/F Protein) initiated discussions with the FDA seeking regulatory guidance for development and approval of a GE Atlantic salmon intended to grow faster than conventionally bred Atlantic salmon (Entis, E. 2011. Personal communication)”

In 2009 FDA issued a final guidance on regulations for GE animals

- In January 2009, the Food and Drug Administration issued a final guidance for industry on the regulation of genetically engineered (GE) animals (had 28,000 public comments on draft guidance – mostly ethical)
- FDA plans to regulate GE animals under the new animal drug provisions of the Federal Food, Drug, and Cosmetic Act (FFDCA), and FDA's regulations for new animal drugs.

Source: <http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf>





"New Animal Drug" approach

- "Drugs are ...articles...intended to affect the structure or function of the body of man or other animals"
- The expression product of the new construct (e.g. growth hormone) is also considered to be the new animal drug
- Application process requires that the developer demonstrate that no harm comes to individuals who use the drug under prescribed conditions





FDA NEWS RELEASE

FOR IMMEDIATE RELEASE

January 15, 2009

Media Inquiries:
Michael Herndon, (301) 796-4673
Consumer Inquiries:
888-INFO-FDA

FDA Issues Final Guidance on Regulating Genetically Engineered Animals

En Español

The U.S. Food and Drug Administration today issued a final guidance for industry on the regulation of genetically engineered (GE) animals under the new animal drug provisions of the Federal Food, Drug and Cosmetic Act (FFDCA). The guidance, titled "The Regulation of Genetically Engineered Animals Containing Heritable rDNA Constructs," clarifies the FDA's statutory and regulatory authority, and provides recommendations to producers of GE animals to help them meet their obligations and responsibilities under the law.

Genetic engineering generally refers to the use of recombinant DNA (rDNA) techniques to introduce new characteristics or traits into an organism. When scientists splice together pieces of DNA and introduce a spliced DNA segment into an organism to give the organism new properties, it is called rDNA technology. The spliced piece of DNA is called the rDNA construct. A GE animal is one that contains an rDNA construct intended to give the animal new characteristics or traits.

"Genetic engineering is a cutting edge technology that holds substantial promise for improving the health and well being of people as well as animals. In this document, the agency has articulated a scientifically robust interpretation of statutory requirements," said Randall Lutter, Ph.D., deputy commissioner for policy. "This guidance will help the FDA efficiently review applications for products from GE animals to ensure their safety and efficacy."

The FDA released the draft guidance in September 2008 with a 60-day public comment period, and received about 28,000 comments. The agency has summarized and responded to these comments on the Web site listed below.

The FDA's Center for Veterinary Medicine (CVM) has been working with developers of GE animals on both early stage and more mature applications.

"At this time, it is our intent to hold public scientific advisory committee meetings prior to making decisions on GE animal-related applications" said Bernadette Dunham, D.V.M., Ph.D., director of CVM.

The FFDCA defines "articles (other than food) intended to affect the structure or any function of the body of man or other animals" as drugs. An rDNA construct that is in a GE animal and is intended to affect the animal's structure or function meets the definition of an animal drug, whether the animal is intended for food, or used to produce another substance. Developers of these animals must demonstrate that the construct and any new products expressed from the inserted construct are safe for the health of the GE animal and, if they are food animals, for food consumption.

The guidance also describes the manufacturer's responsibility in meeting the requirements for environmental review under the National Environmental Policy Act.

For more information:

- [Genetically Engineered Animals](#)





FDA public Veterinary Medicine Advisory Committee (VMAC) Meeting was held September 19-20th, 2010 Labeling meeting was held September 21st, 2010





Elliot Entis, Founder of AquaBounty at the Public Hearing on the Labeling of Food Made from AquaAdvantage Salmon, September 21st, 2010





Product Definition for the AquAdvantage Salmon

Product Identity

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-1a lineage.

Claim

Significantly more of these Atlantic salmon grow to at least 100 g within 2700 deg C days than their comparators.

Limitations for Use

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





Food/Feed Safety: Does food or feed from the GE animal pose any risk to humans or animals consuming edible products from GE animals compared with the appropriate non-transgenic comparators?

Conclusion of food/feed safety evaluations:

*"We therefore conclude the food from AquAdvantage Salmon (the **triploid** ABT salmon) that is the subject of this application is as safe as food from conventional Atlantic salmon, and that there is a reasonably certainty of no harm from the consumption of food from this animal. No animal feed consumption concerns were identified".*

Page 62, AquAdvantage Briefing packet. <http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/UCM224762.pdf>



Environmental Safety: What is the likelihood that AquAdvantage Salmon will escape the conditions of confinement?

Where will the AquAdvantage Salmon be raised?

If approved, the AquAdvantage Salmon will be raised in **inland tanks**. They will not be raised in ocean net pens. Any change would require a new application and approval.

There are multiple and redundant physical and mechanical barriers in place in the water systems at the PEI egg production and Panama grow-out facilities to prevent the accidental release of eggs and/or fish to nearby aquatic environments. These barriers have been designed specifically to prevent the escape of different life stages of AquAdvantage Salmon. Both facilities have a minimum of three to five mechanical barriers in place for all internal flow streams which release water to the environment. Standards and has been verified by an FDA inspection or site visit. **Therefore, the likelihood is considered very low that AquAdvantage Salmon will escape from confinement at these sites.**



Frankenfood, Coming Soon to a Store Near You?

Published September 20, 2010 | FoxNews.com

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Reuters/Barrett & McKay Photo/AquaBounty Technologies

A genetically engineered AquAdvantage Salmon (background) is compared to an Atlantic salmon of the same age (foreground). The U.S. Food and Drug Administration will hold a two-day meeting starting September 19 to discuss whether to approve the altered fish for U.S. consumers to eat.

WASHINGTON – Watch for a new section between "frozen foods" and "organic" in your supermarket: genetically engineered. That is, if the government approves the so-called "frankenfoods" for sale.

The [Food and Drug Administration](#) Monday began a two-day look at the issue Monday, focusing on genetically modified salmon, which would be the first such food approved for human consumption.

The agency has already said the salmon, which grow twice as fast as conventional ones, are safe to eat. But salmon act as a genetic gatekeeper in this case: Approve them and open the door for a variety of other genetically engineered animals, including an environmentally friendly pig that is being developed in Canada or cattle that are resistant to mad cow disease.

"For future applications out there the sky's the limit," said [David Edwards](#) of the [Biotechnology Industry Association](#). "If you can imagine it, scientists can try to do it."

Industry Fights Altered Salmon

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By ALICIA MUNDY And BILL TOMSON

The fishing industry and politicians from commercial-fishing states are mobilizing against a possible Food and Drug Administration approval of genetically modified salmon for the American dinner table.

"Putting unlabeled, genetically altered salmon in the marketplace is simply irresponsible, and the FDA needs to strongly consider what impacts this will have before they approve this Frankenfish," Sen. Lisa Murkowski, a Republican from Alaska, said Thursday.



View Full Image

Associated Press

Icy Bay crewmen remove sockeye salmon from their net in July. Commercial fisheries are fighting the introduction of genetically altered salmon.

The resistance could raise difficulties for the FDA, whose scientists have said the AquAdvantage Atlantic salmon developed by AquaBounty Technologies Inc. is safe for human consumption. AquAdvantage contains a growth-hormone gene from another salmon that helps it grow twice as fast as conventional farmed fish.

A coalition that includes Pacific Coast trollers, Atlantic fishing companies and organic-yogurt maker Stonyfield Farm says the genetically altered salmon might threaten their livelihoods by spreading unease about salmon and other foods.

"This stuff is not healthy for people, and it's not like our fresh fish," said Angela Sanfilippo, president of the Gloucester Fishermen's Wives Association of Massachusetts.

Ms. Sanfilippo's group and others have joined with 39 lawmakers who wrote to the FDA this week asking the agency to stop its approval process for the genetically modified salmon.

They cited concerns about "human health and environmental risks" from the AquAdvantage salmon.



United States Senate

WASHINGTON, DC 20510

September 28, 2010

Margaret A. Hamburg, M.D.
Commissioner of Food and Drugs
U.S. Food and Drug Administration
10903 New Hampshire Ave.
Silver Spring, MD 20993

Dear Commissioner Hamburg:

We the undersigned members of the United States Senate request you halt all proceedings related to the U.S. Food and Drug Administration (FDA) approval of the first genetically engineered (GE) animal for human consumption – a hybrid salmon produced by AquaBounty Technologies. There are a number of serious concerns with the current approval process and many potential human health and environmental risks that are associated with producing GE fish have not been fully or openly reviewed. Critical information has been kept from the public and consequently, only FDA and AquaBounty know important details about the approval process for this GE salmon, or the product itself. Accordingly, we urge you to discontinue the FDA's approval process of the GE salmon at this time to protect consumers, fishing and coastal communities, and the environment.

AquaBounty's GE product is a transgenic Atlantic salmon egg, in which genes from an ocean pout have been inserted into the genes of Chinook salmon, and then inserted into an Atlantic salmon. The egg is meant to produce a fish that grows to full size twice as fast as a normal Atlantic salmon. The eggs are intended for sale to aquaculture companies which will grow them to market-sized fish to be sold for human consumption.

One of the most serious concerns regarding AquaBounty's application is the FDA has no adequate process to review a GE animal intended as a human food product. FDA is considering this GE fish through its process for reviewing a new drug to be used by animals, not for creation of a new animal, especially one intended for human consumption. Clearly, this is inappropriate. Creation of a new genetically engineered species should not be treated as an animal drug issue but undergo formal evaluation by FDA's Center for Food Safety and Applied Nutrition to review the product's potential health effects on humans.

Such a limited review of the first GE animal for human consumption is wholly inadequate to review potential public safety concerns associated and recklessly and needlessly endangers consumer health. A recent *New York Times* article reported, "the engineered salmon have slightly higher levels of insulinlike growth factor," and "some

Letter asking to
**"halt all
proceedings"**
related to GE
salmon signed
by 11 Senators,
and a similar
one signed by
29 members of
Congress sent
to FDA
Commissioner
Hamburg
9/28/10



February 1, 2011

Via Electronic Mail

Commissioner Margaret Hamburg, M.D.
U.S. Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, Maryland 20993

Cc: Secretary Kathleen Sebelius, U.S. Department of Health and Human Services
Dr. Jane Lubchenco, Administrator, National Oceanic and Atmospheric Administration
Rowan W. Gould, Acting Director, U.S. Fish and Wildlife Service

Re: AquaBounty Technologies' Genetically Engineered AquaAdvantage Salmon

Dear Commissioner Hamburg:

We write in further support of our November 8, 2010 letter urging the U.S. Food and Drug Administration (FDA) to fully assess the potential environmental impacts associated with genetically engineered (GE) salmon before taking final action on AquaBounty Technologies' (ABT's) application for the first-ever approval of a GE animal intended for human consumption. In light of continued and considerable concerns surrounding ABT's application, FDA must complete a comprehensive environmental impact statement (EIS) that reaches far beyond the scope of the narrow environmental assessment (EA) submitted by ABT and evaluates the full range of threats that stand to confront wild fish populations if AquaAdvantage Salmon are released into the natural marine environment.

The existing EA and FDA's analysis of ABT's application raise serious questions concerning the efficacy of ABT's proposed "containment" measures, which are intended to mitigate the risk that AquaAdvantage Salmon will escape confinement, become established in the environment, and spread to other areas. In particular, ABT suggests that the risks associated with potential escape of AquaAdvantage Salmon are dramatically lessened by a process of triploidy induction which is used to sterilize the eggs before they are transported to Panama for grow-out. In our November 8 letter to you we stated that data from ABT show that its proposed triploidy sterilization technique is "not effective in up to 5% of all eggs treated." Our statement confused two points: (1) the potential effectiveness of the ABT technique as suggested by ABT's data, and (2) the potential net result of the application of that technique. We regret this confusion and now clarify our concern. Contrary to our previous statement, ABT's data show that its process induced triploidy in 98.9% to 100% of the egg batches treated in its study. However, information contained in ABT's EA (and FDA's conclusions based on analysis of that information) indicates that up to 5% of the eyed-eggs taken from the ABT facility on Prince Edwards Island to the ABT facility in Panama may not be sterile.

This letter
dated 2/1/11
was signed by

- Friends of the Earth,
- Earthjustice,
- Greenpeace,
- Oceana,
- Ocean Conservancy,
- Pew Environmental Group,
- Union of Concern Scientists



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SUSTAINABILITY

Senators Introduce Bill to Ban GE Salmon

BY HELENA BOTTEMILLER | FEB 02, 2011

Senators Mark Begich (D-AK) and Lisa Murkowski (R-AK) reintroduced a bill Tuesday that would ban genetically engineered (GE) salmon, a fast-growing fish that the U.S. Food and Drug Administration is likely to approve in the near future.

If the FDA gives its stamp of approval, and many expect the agency will, the GE salmon would be the first genetically engineered food animal to be approved for human consumption.



Developed by Massachusetts-based AquaBounty Technologies, the modified fish, formally known as AquaAdvantage salmon, are essentially Atlantic salmon with an inserted growth gene from a Chinook salmon and an antifreeze gene from an ocean pout. They grow twice as fast as typical Atlantic salmon and require approximately 10 percent less feed to achieve the same weight.

Sens. Begich and Murkowski--a bipartisan team from a salmon-rich state--are fighting tooth-and-nail to keep the AquaAdvantage off the market.

"Frankenfish threatens our wild stocks, their habitat, our food safety, and would bring economic harm to Alaska's wild salmon fishermen," said Begich Tuesday, adding that he believes the modified fish are "risky, unprecedented and unnecessary."

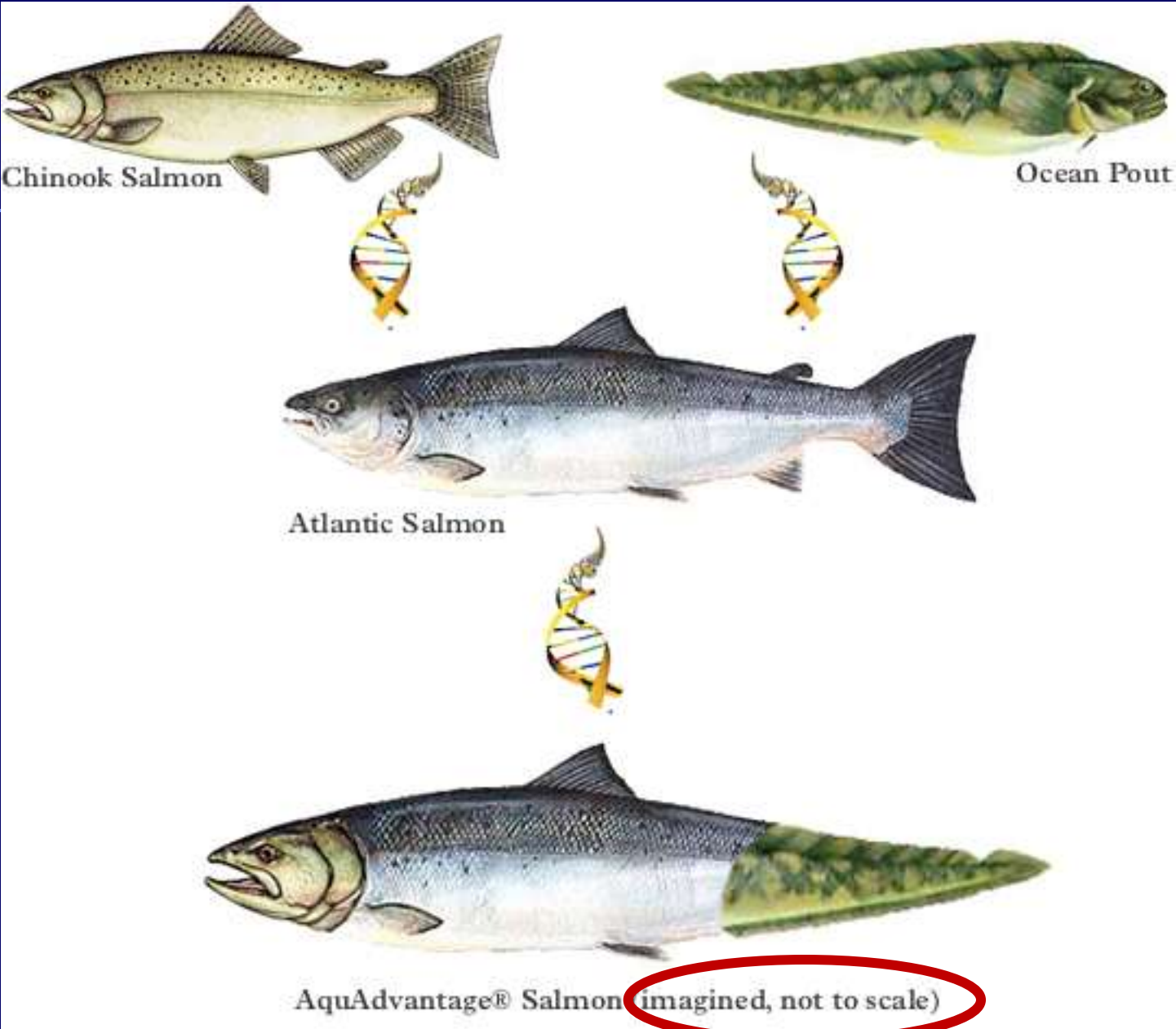
Murkowski said it was "completely irresponsible" for the agency to consider the fish without first considering the impacts to Alaska's wild salmon fisheries. "The FDA has not studied the environmental effects, let alone the economic impacts on the salmon and seafood markets that would result from approval," she added.

2/2/11 bill to ban GE salmon was introduced by:

Senator Mark Begich, D-AK
Senator Lisa Murkowski, R-AK



Retrieved from "AquAdvantage" image search on web



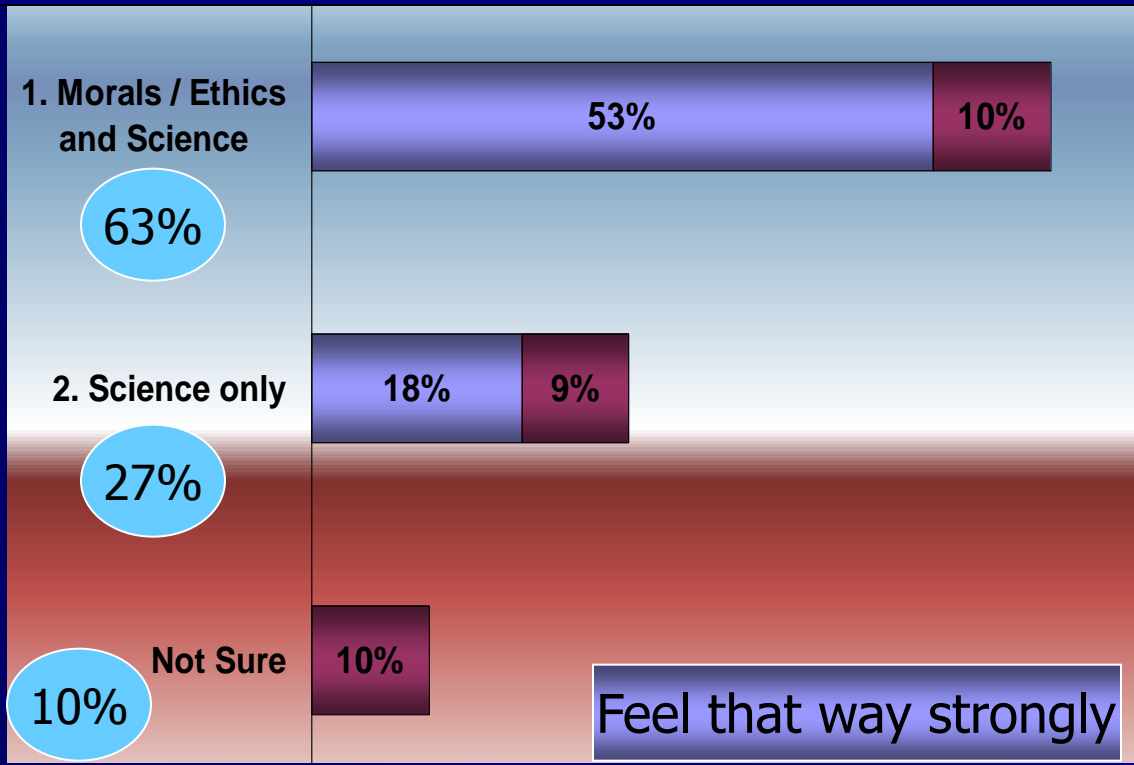


Retrieved from "AquAdvantage" image search on web Frankenfish

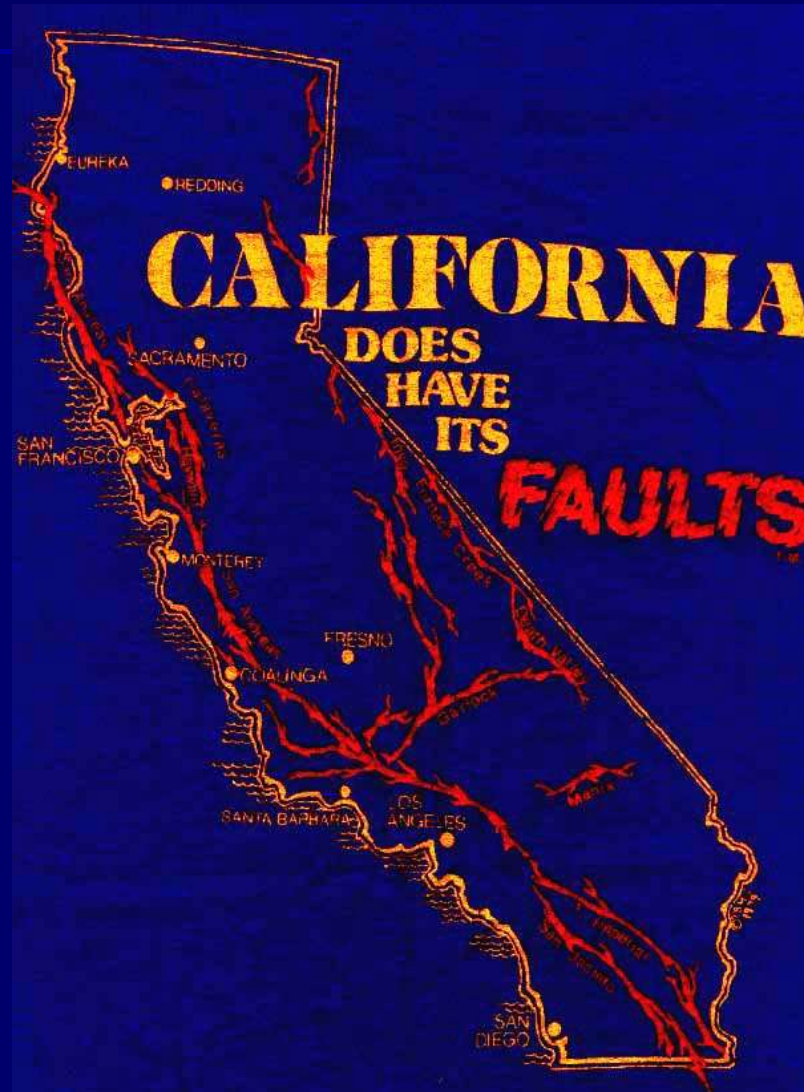




1. Government regulators **should include ethical and moral considerations**, in addition to scientific evaluation of risks and benefits, when making regulatory decisions about cloning or genetically modifying animals.
2. Though ethical and moral considerations are important, government regulators **should consider only scientific evaluation of risks and benefits** when making regulatory decisions about cloning and genetically modifying animals.



What about California ?





Existing regulations governing genetically-engineered aquatic organisms in CA

CA Senate Bill 245 "In the waters of the Pacific Ocean that are regulated by this state, it is unlawful to spawn, incubate, or cultivate any species of finfish belonging to the family Salmonidae, transgenic fish species, or any exotic species of finfish."

[Approved by Governor October 12, 2003. Filed with Secretary of State October 12, 2003.]



Existing regulations governing genetically-engineered aquatic organisms in CA

Additionally, California Fish and Game department regulations require the possession of a permit to raise GE fish in contained onshore systems in California. This regulation applies to all transgenic fish based on the assumption that they “pose a threat to native wildlife, the agricultural interests of the state or to public health.”

February 2003



To date several permits have been granted to medical & scientific research laboratories

- (9) Transgenic Aquatic Animals. The department may issue permits for importation, possession, transportation or rearing of, or research on, transgenic aquatic animals pursuant to the following terms and conditions:
- (A) All transgenic aquatic animals shall be held, raised, and transported in a closed-water system or in a system which treats effluent discharge from the facility with a disinfection system adequate to ensure against the inadvertent release of live animals. A closed-water system means that there is no discharge to waters of the state. Municipal treated sewage systems are not considered waters of the state. The Commission may grant an exception to this regulation if it is determined that doing so shall not pose a significant risk to the waters or wildlife of the state.
 - (B) Access to facilities containing transgenic aquatic animals must be restricted through means determined to be adequate by the Department to assure against unauthorized removal of animals.
 - (C) Movement of live transgenic aquatic animals from facilities is prohibited unless specifically permitted by the Department.
 - (D) Release of transgenic aquatic animals or their progeny into waters of the state is prohibited.
 - (E) If transgenic aquatic animals are held with non-transgenic animals of the same species, all such animals that commingle with transgenic animals shall be treated as transgenic for the purposes of regulation and may not be introduced into waters of the state. Nontransgenic individuals that can be individually identified as nontransgenic may be exempt from this provision with prior department approval.
 - (F) In addition to any other penalty provided by law, any unauthorized release of transgenic aquatic animals or their progeny into the waters of the state may be subject to the penalties provided for under Fish and Game Code Sections 2125, 12007, and/or 12023
 - (G) A university, college, governmental research agency or other bona fide scientific institution, as determined by the department, may apply for an expedited permit review under 671(b)6 of this section by demonstrating that they meet or exceed the requirements stipulated in subsections (A) through (F) as part of a federal program or permit, for example, National Institute of Health guidelines administered by an Institutional Animal Care and Use Committee (IACUC). Such institutions shall have 120 days from the date of adoption of these regulations to submit supporting documentation for an initial permit.



GloFish™





FDA statement on Glofish

“Because tropical aquarium fish are not used for food purposes, they pose no threat to the food supply. There is no evidence that these genetically engineered zebra danio fish pose any more threat to the environment than their unmodified counterparts which have long been widely sold in the United States. In the absence of a clear risk to the public health, the FDA finds no reason to regulate these particular fish.”

December 9, 2003



GloFish™ – barred on ethical grounds in California - “just a pet”





“just a floral arrangement”





GloFish sparks off classroom study in US

Genetically modified pet fish from Singapore used to help undergraduates understand transgenic technology

By [Chang Ai-Lien](#)

THE world's first genetically engineered pet fish is doing more than lighting up fish tanks in the United States.

The made-in-Singapore fluorescent fish is beginning to help some university students there understand transgenic technology better.

To its creator, Associate Professor Gong Zhiyuan of the National University of Singapore, the mutant zebrafish is a mascot for the potential benefits of genetic modification.





The "Precautionary" principle

Although there is no consensus definition of what is termed the precautionary principle, one oft-mentioned statement sums it up: **"When an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically."**

In other words, actions taken to protect the environment and human health take precedence. Therefore, some advocates say, governments should immediately ban the planting of genetically modified crops, even though science can't yet say definitively whether they are a danger to the environment or to consumers.



Possible future advantages

- What if biotechnology could be used to reduce the amount of mercury or other toxic compounds that fish accumulate from their environment ?
- What if you could remove the allergens that make shellfish intolerable for some consumers ?
- What if you could use transgenic fish to detect environmental contaminants ?

"BE CAREFUL NOT TO OBSCURE THE BENEFITS NOT READILY APPARENT IN THE FIRST GENERATION OF PRODUCTS"

Who gets to decide ?





Need for biological or genetic containment of GE fish

It is becoming clear that unless total physical containment is available, the benefits of transgenic manipulation in fish will be dependent upon the development of methods to reproductively isolate transgenic fish

What can science do ?



Approach taken by Aqua Bounty has been to produce monosex (female), triploid lines



Induced triploidy ?

- Effectiveness varies greatly (10-95%)
- Difficult to screen every fish individually and cannot check eggs
- Release of triploid fish into the environment presents certain hazards as they still have enough hormones to enter into normal courtship and courting behavior and so could interfere with reproduction of wild fish
- Sterile fish survive longer so could increase population pressure on wild fisheries

DEVELOPMENT OF METHODS FOR THE BIOLOGICAL CONTAINMENT OF TRANSGENIC FISH

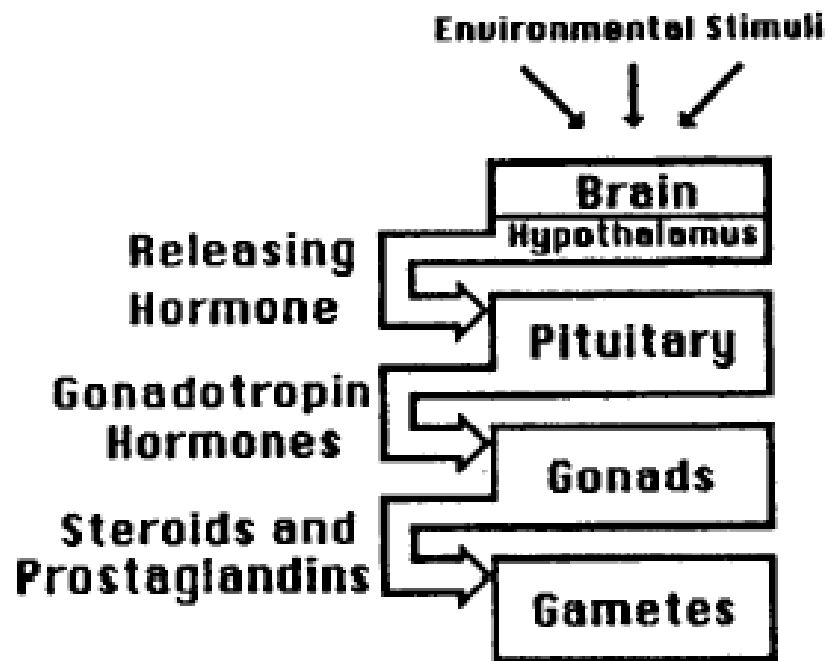


Figure 1. Mechanism that regulates reproduction in fishes.



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Review article

Transgenic approaches for the reproductive containment of genetically engineered fish

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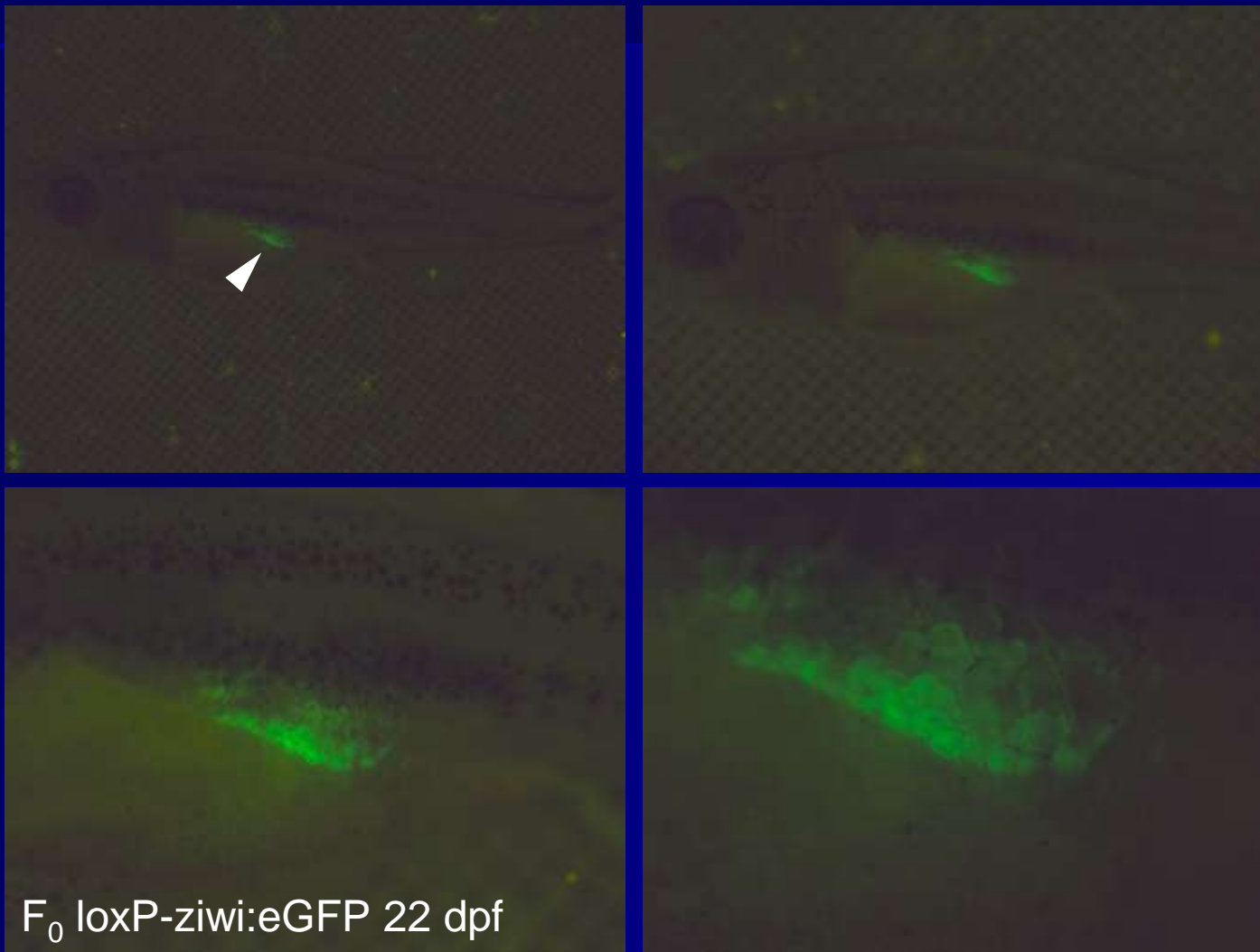
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Abstract

Aquacultural applications of transgenic technologies have the potential to supply the ever growing demand for food products derived from aquatic resources. However, before any benefits of genetically engineered fish can be realized, methods for the containment of transgenic fish must be developed to help prevent their interbreeding with native populations should they be accidentally released or escape. In this review, the current methods of physical and biological containment in use by aquaculturists for confinement of domestic farmed fish are outlined and discussed with regard to their applicability and effectiveness for the containment of transgenic fish. New and developing transgenic approaches for the confinement of genetically engineered fish, including transgenic sterilization, disruption of embryonic development, and gonad-specific transgene excision are then discussed in detail. Although some preliminary studies allude to ongoing experiments, there are few peer-reviewed scientific publications that describe the implementation of these techniques. The current dearth of publications demonstrating the success of these technically complex methods, even in model fish species, suggests that difficulties may be prevalent when trying to implement such transgenic

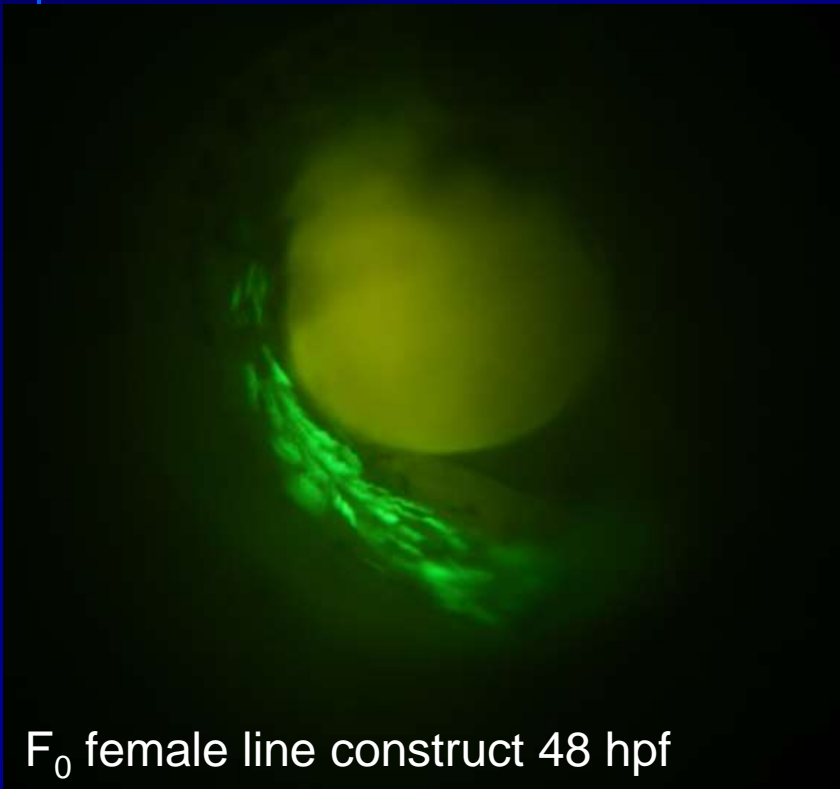


Use genetic engineering as a method to remove the transgene from the germ cells, but leave it expressing in the somatic cells.

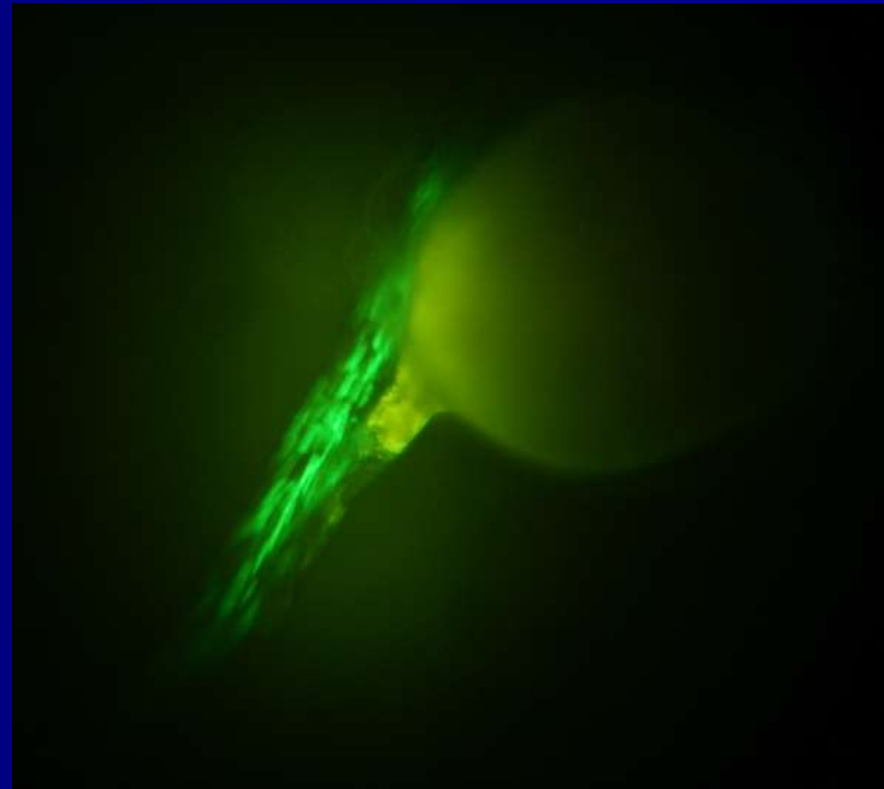


F₀ loxP-ziwi:eGFP 22 dpf

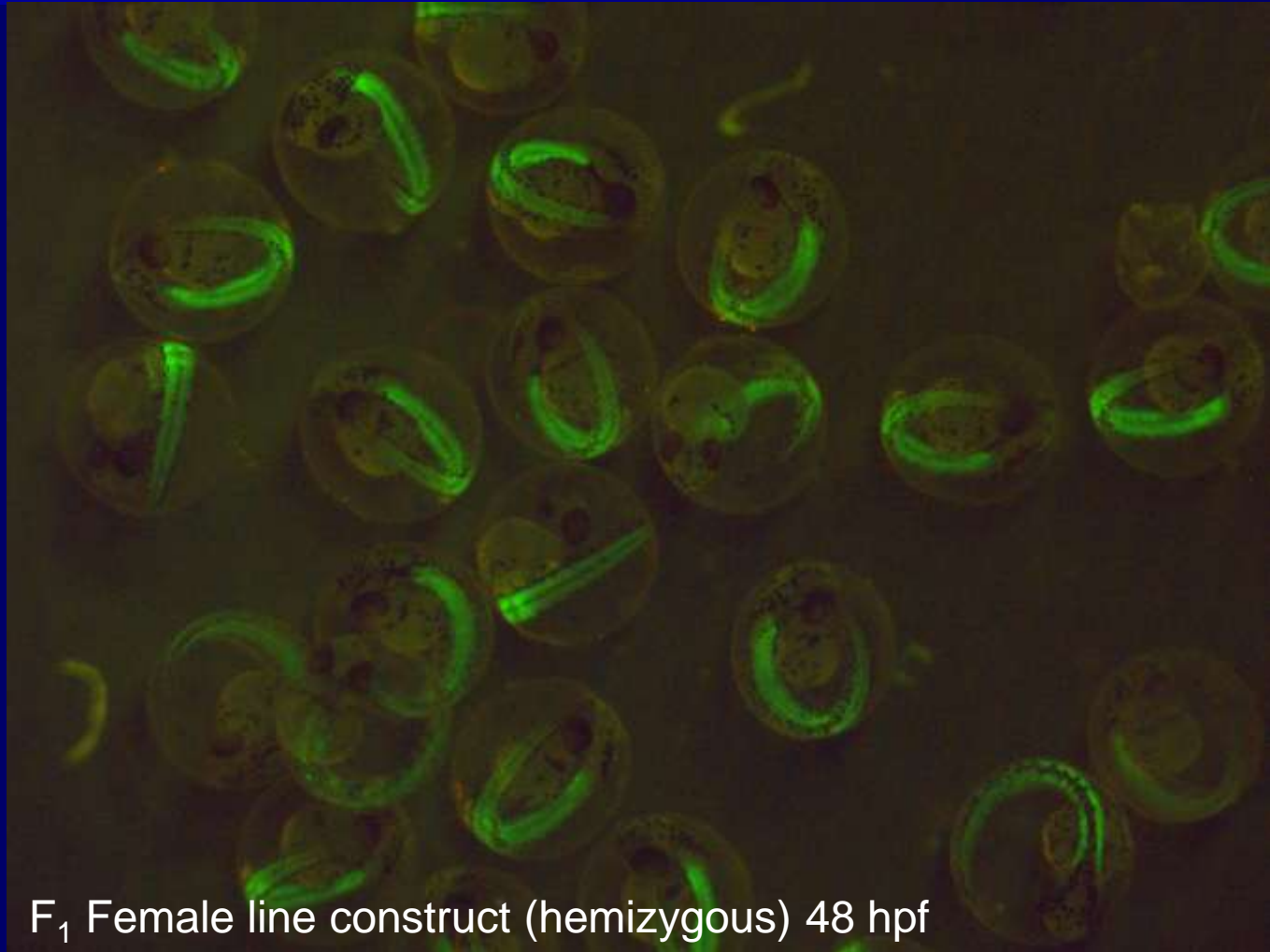
Mosaic expression in founder fish



F₀ female line construct 48 hpf



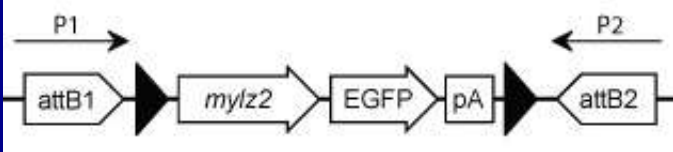
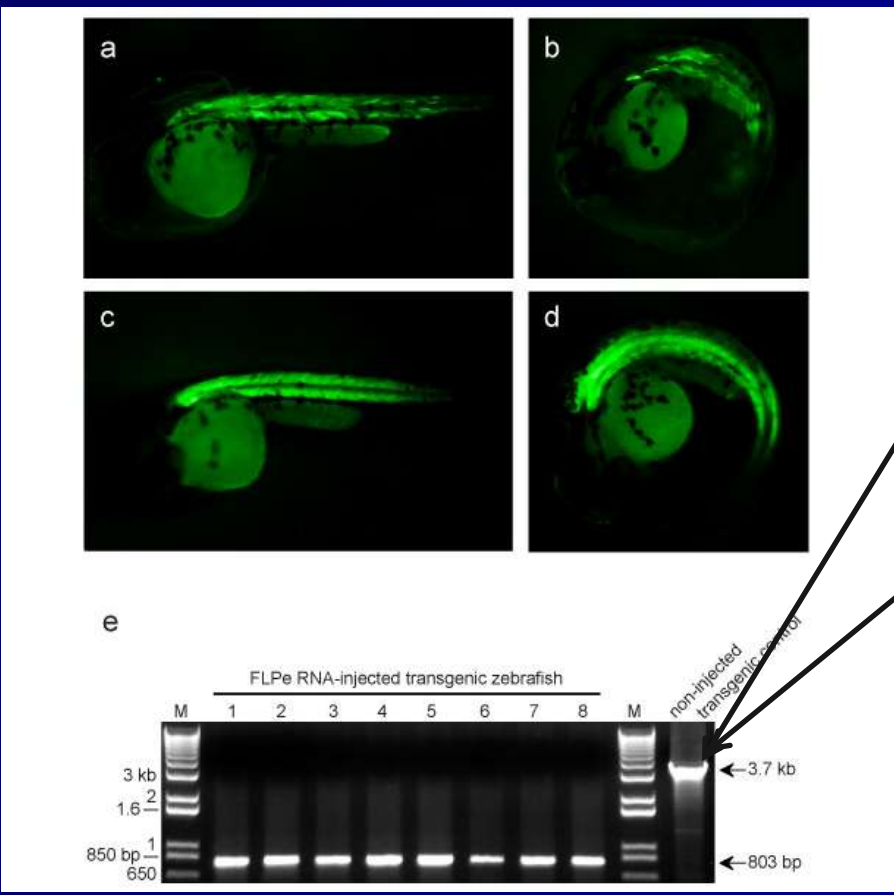
Uniform expression in F₁ fish



F₁ Female line construct (hemizygous) 48 hpf



Mosaic expression in FLPe RNA injected fish





Final thoughts.....



- The environmentally-responsible utilization of transgenic techniques in aquaculture will require the development of methods for biological containment.
- The development of containment techniques will help to ameliorate some of the gene flow concerns about the interbreeding of genetically engineered fish with native populations.

Conclusions



- No genetically engineered animal species have yet been commercialized
- Even if approved at the Federal level, there are additional State-specific regulations related to the rearing and possession of genetically engineered fish in California
- Unlikely there will be any GE aquaculture or animal agriculture in CA in foreseeable future

