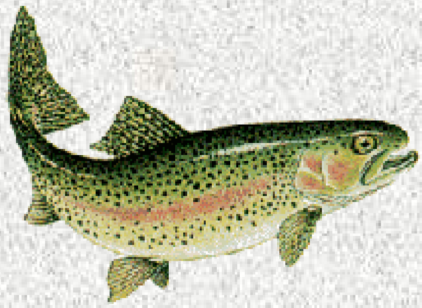
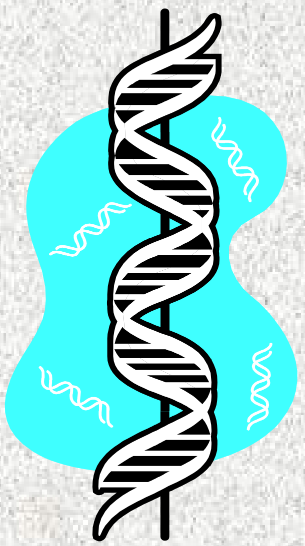
Trout Aquaculture Research at the National Center for Cool and Cold Water Aquaculture

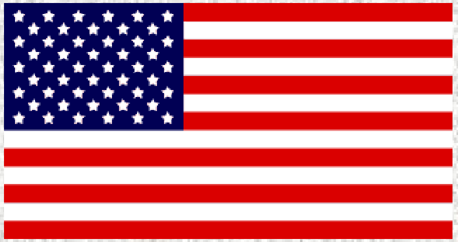


Caird Rexroad

USDA/ARS
National Center for Cool and Cold Water Aquaculture

Leetown, West Virginia





USDA
Secretary of Agriculture

Under Secretary for Research, Education, and Economics

Agricultural Research Service	Economics Research Service	National Agricultural Statistics Service	Cooperative State Research Education & Extension Service

National Center for Cool and Cold Water Aquaculture

To support and enhance the nation's cool and cold water aquaculture production through research and technology transfer.

US Trout Industry

- Rainbow trout are the most cultured coldwater fish in the US, 747 locations
- ~390 Commercial farms composed a ~$95 million industry in 2007
    - Foodfish ~$80 Million
        - Most sales to processors and restaurants
    - Stockers ~$5.84 Million
    - Fingerlings ~$1.7 Million
    - Eggs ~$7.5 Million
- ~433 State/Federal/Private locations with production aimed at conservation and restoration valued at an additional $102 million in 2007
- Majority of production in California, Wisconsin, Michigan, North Carolina, Pennsylvania, and Idaho (53% of sales of food size fish)
- Imported ~$83 million fresh and frozen trout products, ~$.5 million live in 2006
    - Compete also with Salmon, Shrimp, Catfish, and Tilapia

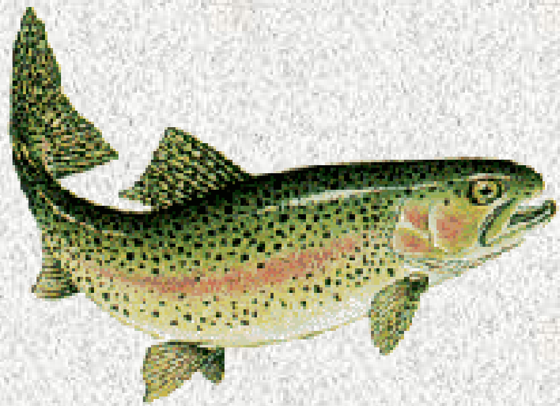
ERS Aquaculture Outlook, NASS Trout Production

US Rainbow Trout Industry

- **Large Operations**
  - **Troutlodge, Sumner, WA**
    - Egg producer
    - Large Breeding program – evaluating Hatch-out rates, Feed Conversion, Growth, Survivability, Uniformity, Flesh quality, Flesh yield, and Disease-resistance
  - **ClearSpring Foods, Buhl, Idaho**
    - Vertically Integrated – feeds, broodstock, production, processing, sales
    - Large Breeding program
- **Smaller Operations, USA**
  - USTFA
  - NAA
  - State Associations
- **Associated Industries**
  - Feed Production
  - Aquatic Health and Diagnostics
  - Genetic Services
  - Aquaculture Systems
  - Federal/State Hatchery Systems

Customer/Stakeholder Workshop: Issues faced by the US Rainbow Trout Industry

- Disease
  - Fp, IHNV, IPNV
  - Certifications
- Production Traits
  - Feed efficiency
  - Growth
  - Stress tolerance (handling, crowding, low O_2)
- Consumer Traits
  - Flesh color
  - Fillet quality
- Nutrition
  - Next generation of feeds
- Chromosome Set Manipulation
  - Tetraploid/triploid production



NCCCWA's role in supporting the US Rainbow Trout Industry

- **Two major companies from this industry have large breeding programs**

- **Several of the issues arising from the first NCCCWA Customer/Stakeholder meeting can be addressed through breeding**

- **NCCCWA Team Objectives:**
  - Develop and evaluating selective breeding strategies for trout, developing improved germplasm in the process, **complementing ongoing industry efforts**.

  - Conduct basic and applied research to understand environmental factors and biological mechanisms controlling traits of interest.

  Transfer technologies and germplasm to industry

Scientific Staffing

GENETICS and PHYSIOLOGY

- Research Physiologist – Dr. Greg Weber
- Research Geneticist – Dr. Timothy Leeds
- Growth Physiologist - Vacant

GENOMICS

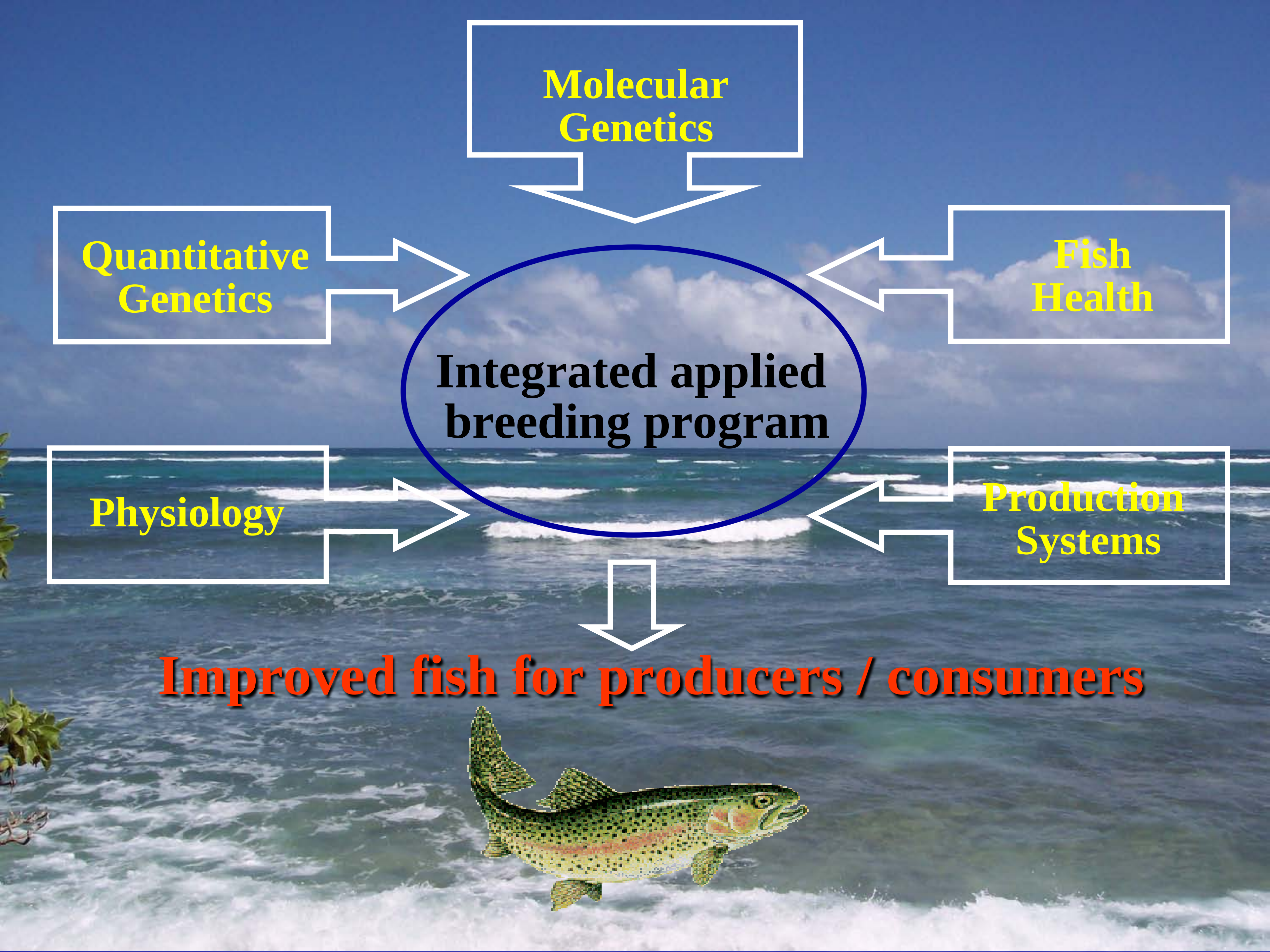
- Molecular Geneticist – Dr. Yniv Palti
- Molecular Biologist – Dr. Caird Rexroad III
- Molecular Biologist – Vacant
- Computational Biologist – Dr. Roger Vallejo

FISH HEALTH

- Molecular Immunologist – Dr. Greg Wiens
- Research Pathologist – Dr. Tim Welch
- Microbiologist – Dr. Jason Evenhuis

Scientific Support Staffing

- Research Technicians (13)
- Administrative Assistant (2)
- Information Technologist (1)
- Facilities Management (2)
- Water Systems Operator (1)
- Wet Lab Crew (5)


Molecular Genetics
Quantitative Genetics
Fish Health
Integrated applied breeding program
Physiology
Production Systems
Improved fish for producers / consumers

Selective Breeding

Hypothesis: Sufficient genetic variation exists in NCCCWA broodstock to realize genetic improvement through selection.

Goal: Develop and transfer technologies and germplasm to the aquaculture industry

Germplasm Research and Production

- Biology
  - High Fecundity
  - Cryopreserve Sperm
  - Response to Photoperiod Manipulation
- Crosses
  - Intraspecific crosses – within species
  - Interspecific crosses – crosses between two species
  - F1 Crosses – first generation, abundance of heterozygosity and uniformity
  - Backcrosses – cross of F1 and parent
  - Full Sib Crosses regular
  - Half Sib Crosses one common parent
- Genetic Manipulation
  - Transgenics insert DNA
  - Clonal lines – doubled haploids
  - Sex reversal
  - Chromosome Set Manipulation

Genetic Improvement of Aquaculture Species

1. Define trait
2. Identify variation in a trait directly due to variation in DNA sequence (Heritability)
   - Sequence variation can be in or around a gene and changes how the gene functions
3. Understanding of the basic genetics of the trait
   - (mode of inheritance)
4. Develop technologies designed to exploit positive genetic variation
5. Use those technologies to development of genetically improved strains

Quantitative Genetics Definitions

- ***Quantitative Genetics*** **- use of statistics to assign breeding values to broodstock used in selective breeding programs for the development of superior strains for aquaculture**

- ***Phenotype*** **– category or classification of a trait**

- ***Heritability*** **– the extent to which an animals breeding value can be predicted from its phenotype, = V_G/V_P**

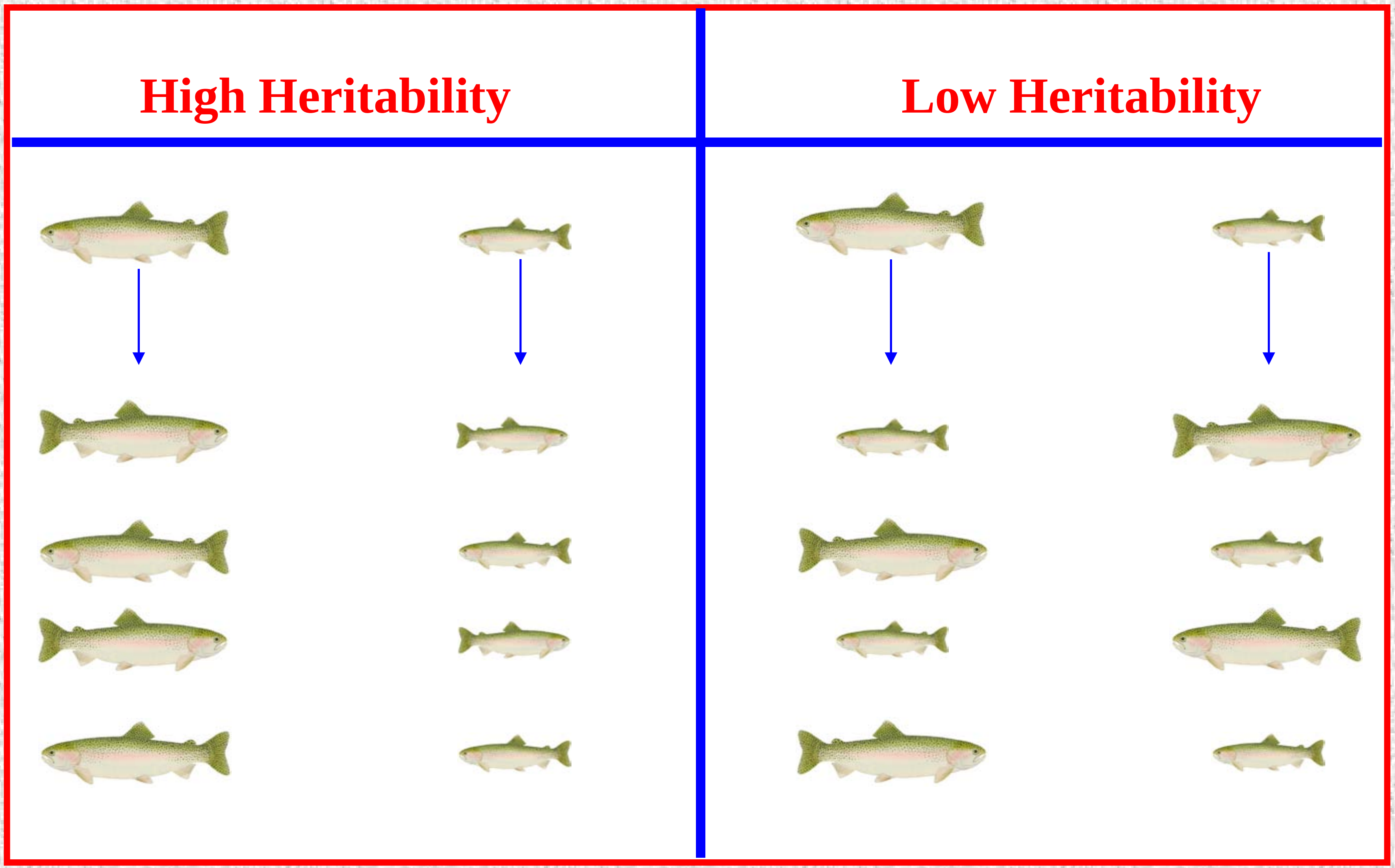
- ***Germplasm*** **– biological resource material**

Quantitative Genetics

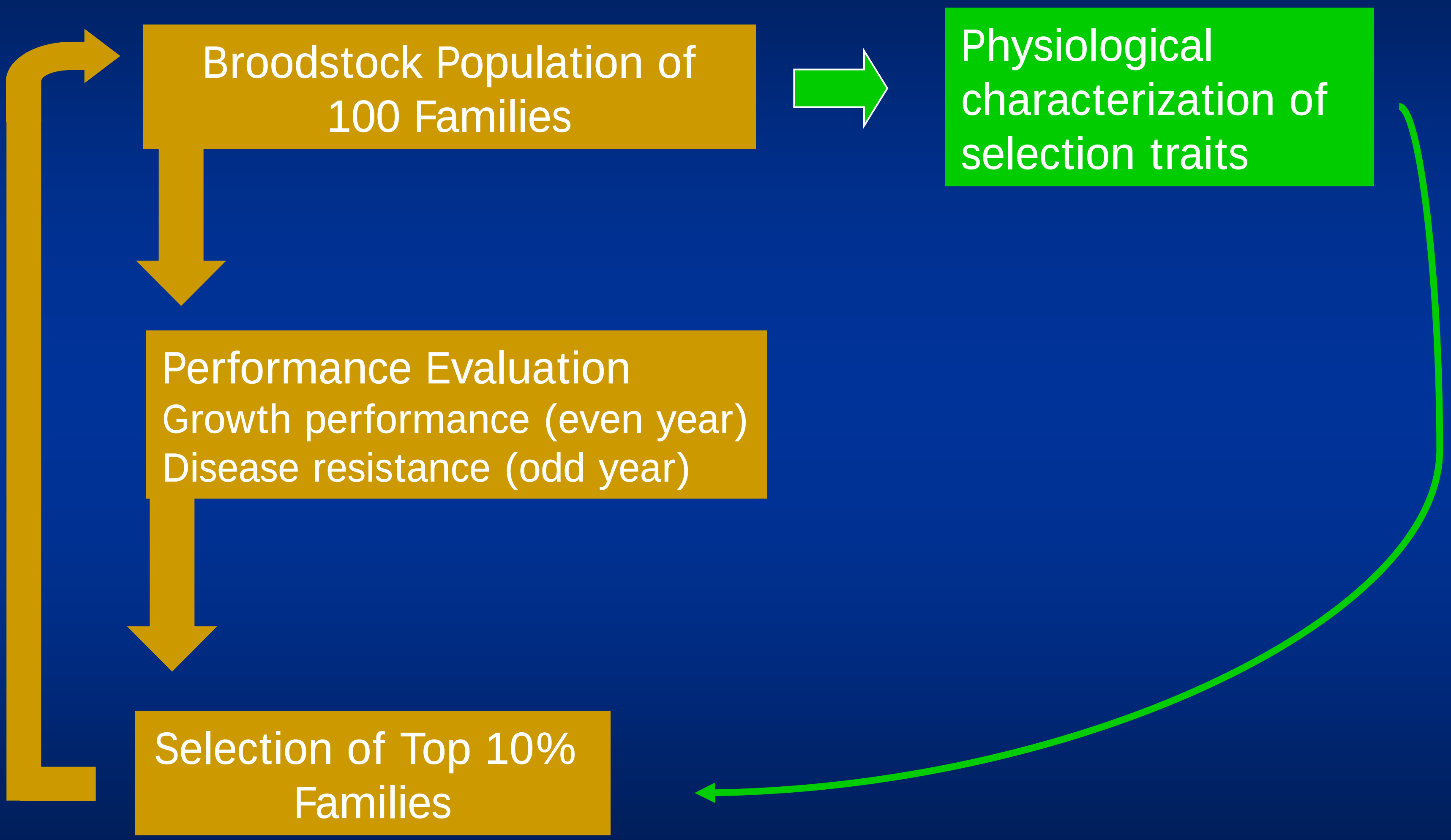
$$V_P = V_G + V_E + V_{GxE} \cdots$$

- V_P= variation in phenotype
- V_G=variation due to genetics (V_A+V_D +V_{AxD}…)
- V_E=variation due to environment (V_N+V_T+V_{PP}…)
- V_{GxE}=variation due to gene x environment interactions
- Continuous variation
- Discontinuous variation
- Use of specific crosses to determine heritability of a trait and to determine breeding values

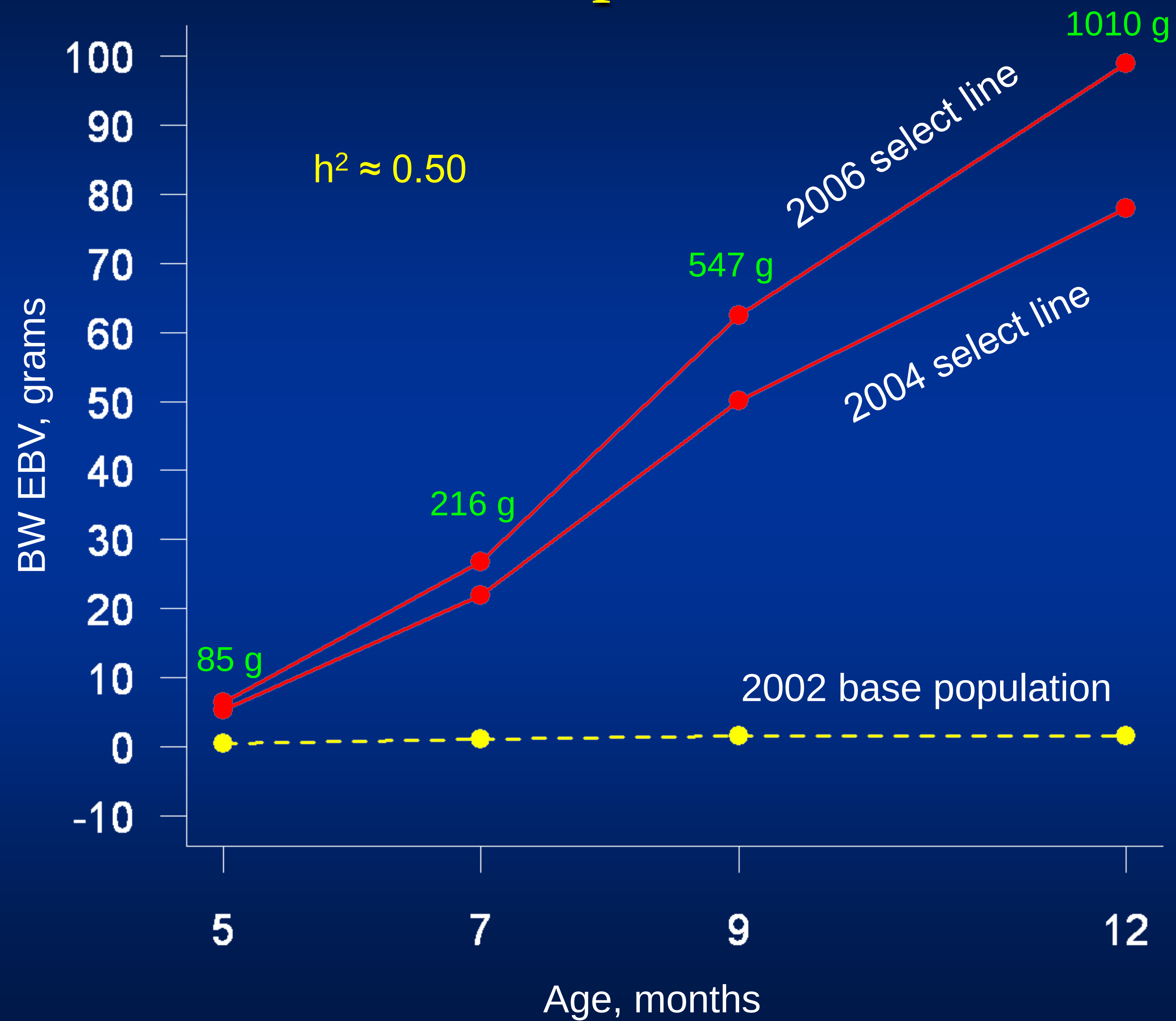
Mapping Traits - Heritability



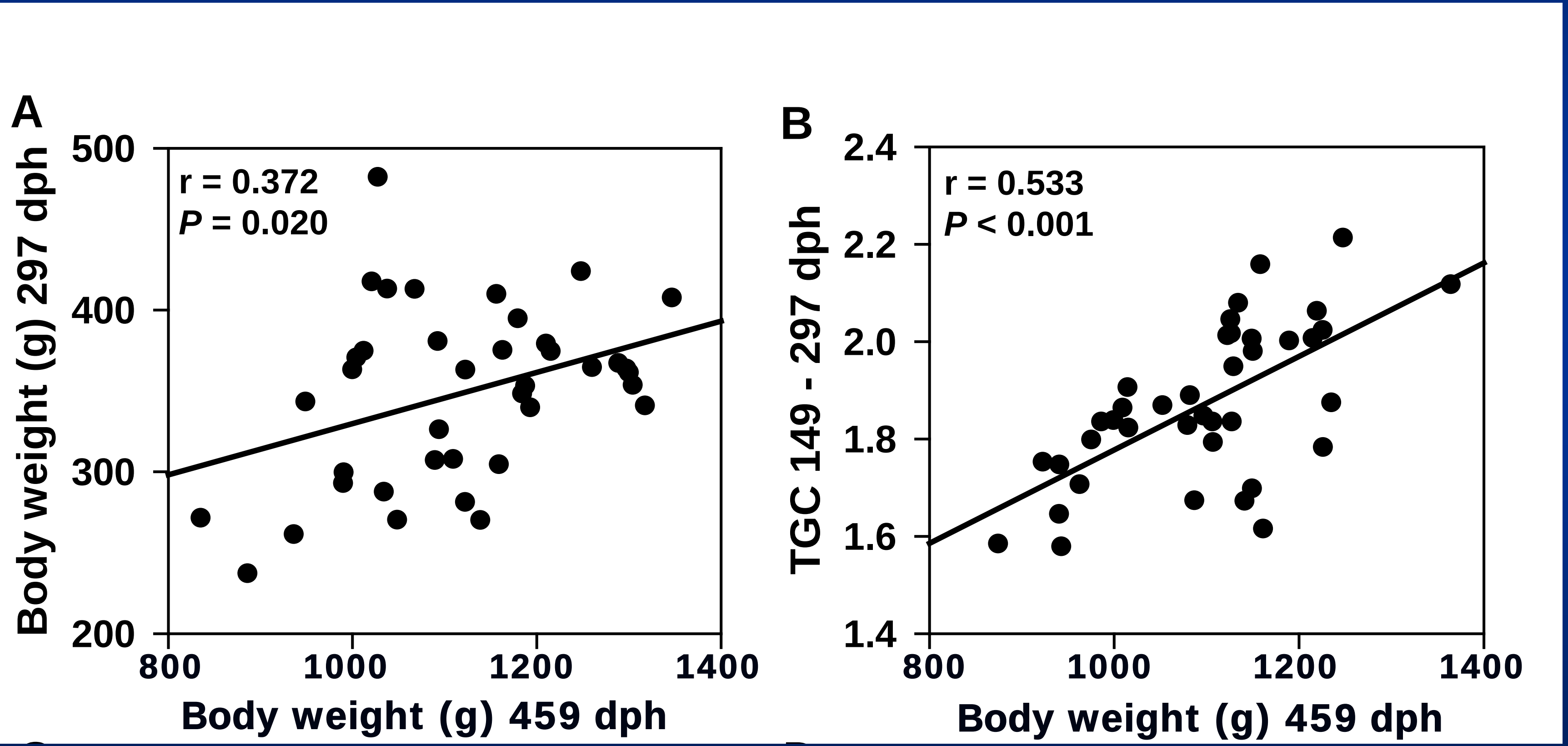
Selection Approach



Growth Improvement Line



Trait Characterization: Thermal Growth Coefficient and Future Growth Performance

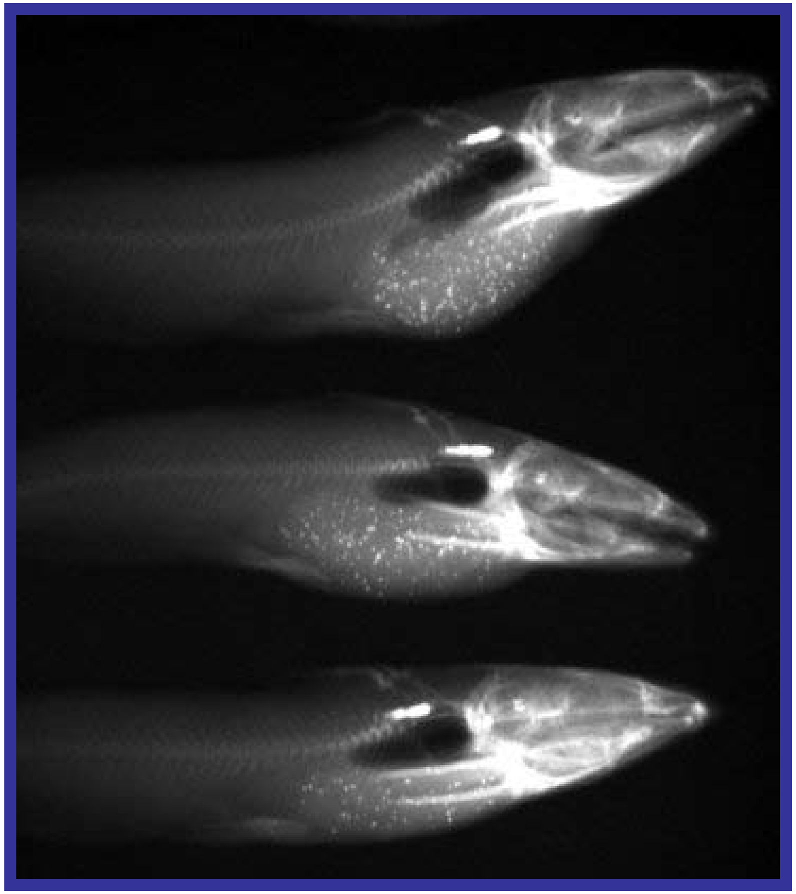


(Lankford and Weber 2006)

Preliminary heritability estimate
TGC 9-12 months: $h^2 = 0.32$

Feed Intake

- Fed labeled diet
  - 73 full-sib families
  - 30 fish per family
  - 3 occasions
- Intake of each fish quantified
  - Beads counted



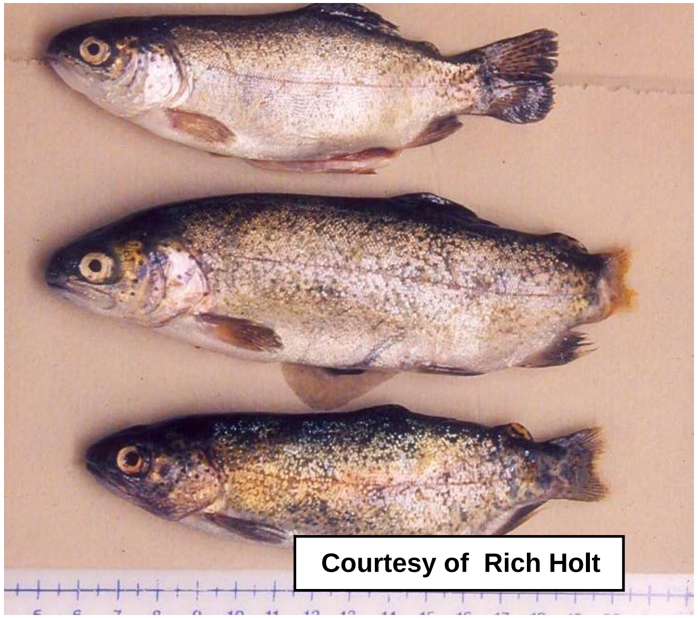
Diseases

Bacterial Coldwater Disease

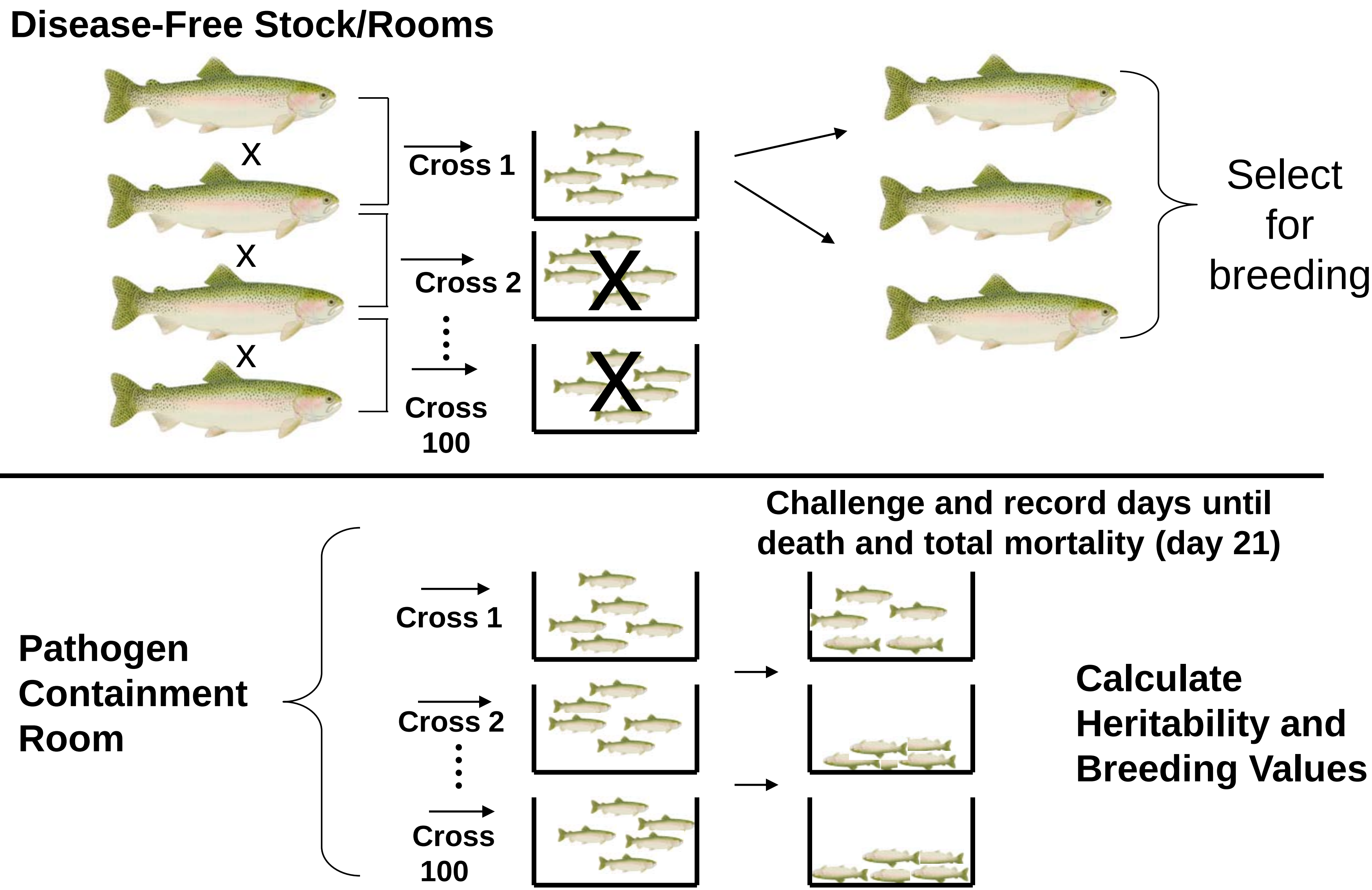
- ***F. psychrophilum***
- **Economic impact**

Enteric Red-Mouth Disease

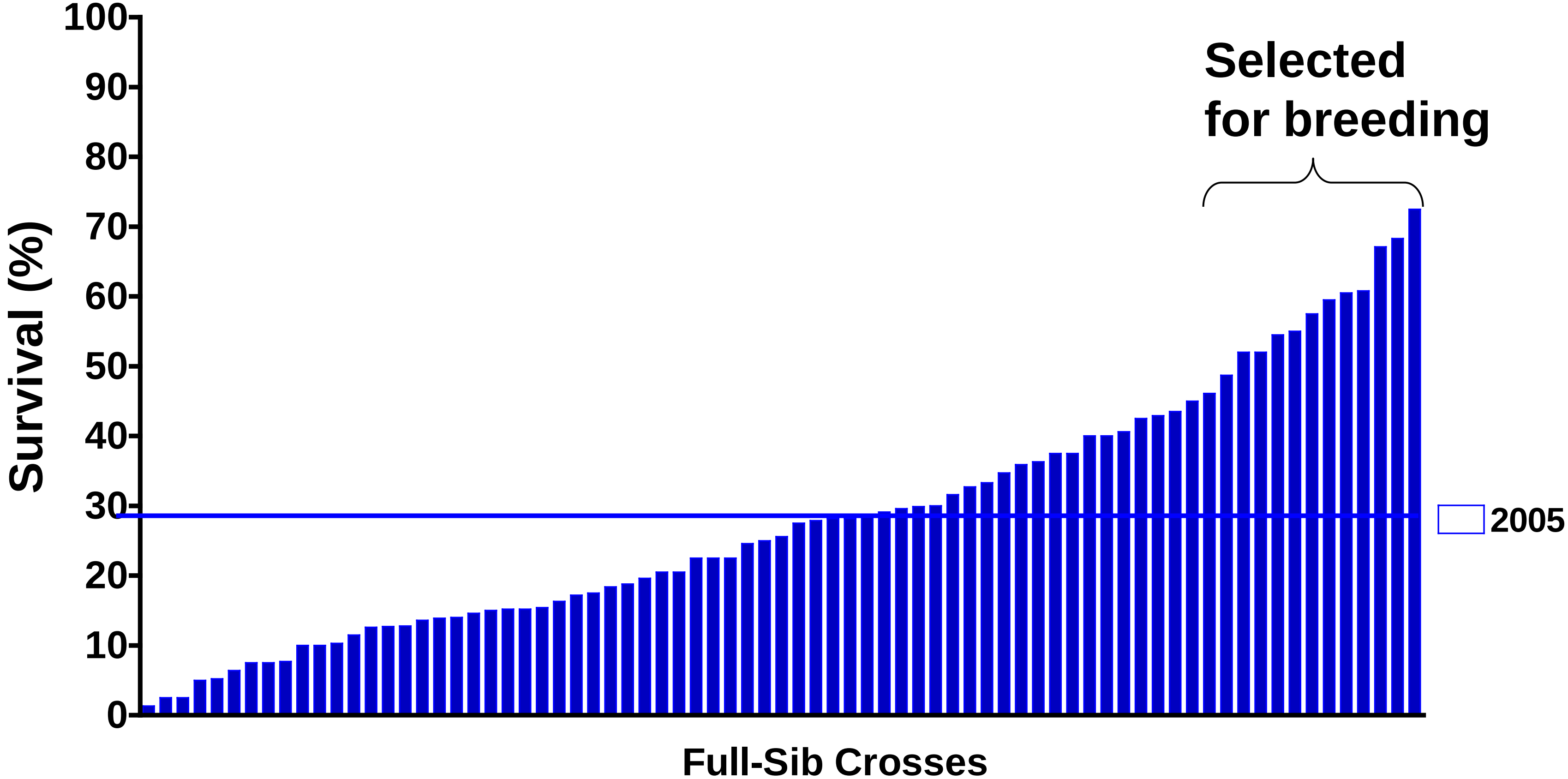
- ***Y. ruckeri***
- **Vaccination model**
- **Recently emerging biotype II**



Family-Based Selective Breeding for Disease Resistance

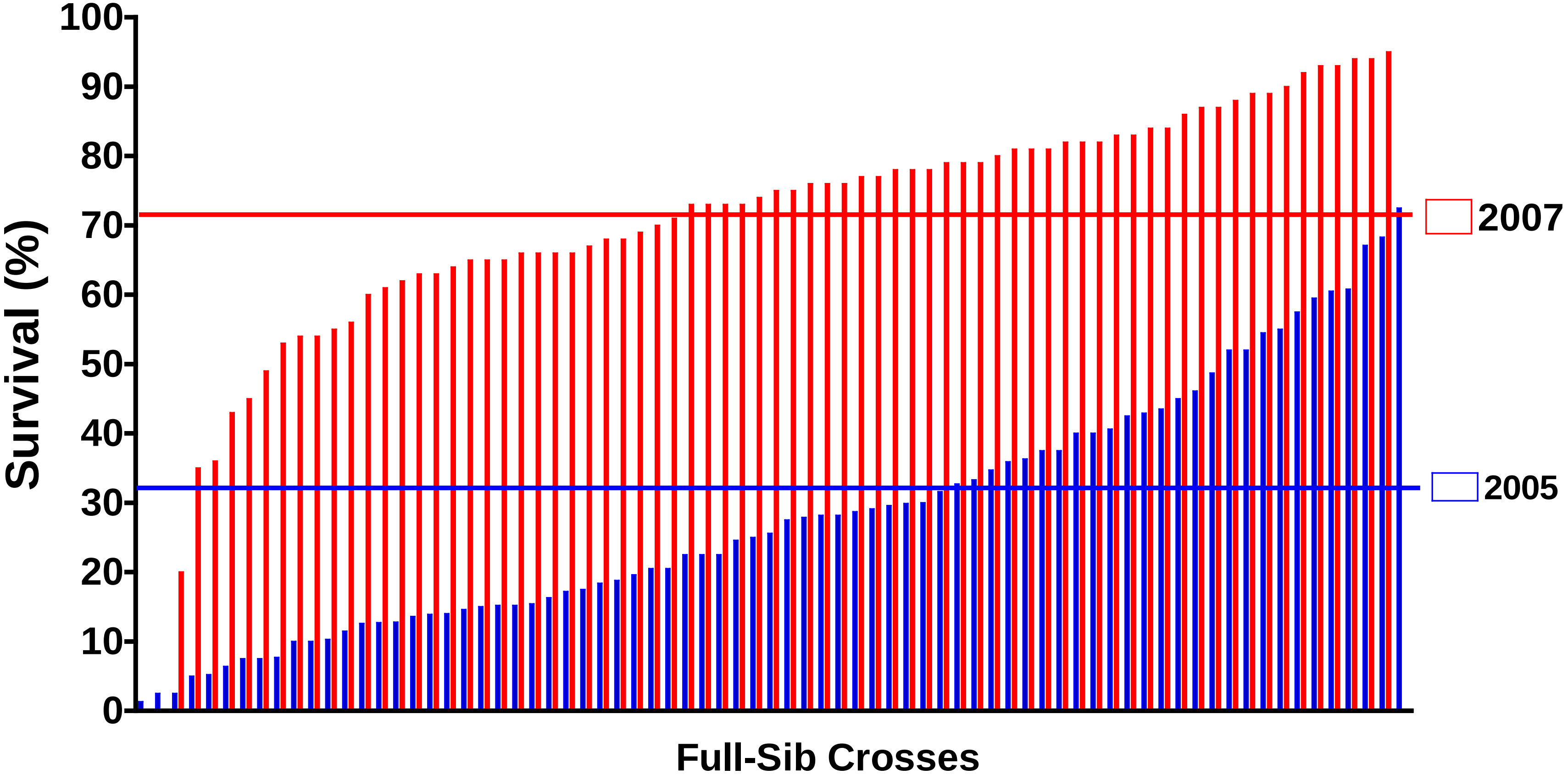


Large Variation in Resistance to *F. psychrophilum* challenge (2005 year-class).



- Silverstein, J.T., Vallejo, R., Palti, Y., Leeds, T.D., Rexroad, C.E. III, Welch, T.J., Wiens, G.D. and Ducrocq, V. 2008. Journal of Animal Science. Submitted
- Leeds, T.D., Silverstein, J.T., Vallejo, R.L., Palti, Y., Rexroad, C. E. III, Welch, T.J. and Wiens G.D. Journal of Animal Science. In Preparation

Selective breeding increased average survival (2007 year-class).



- Silverstein, J.T., Vallejo, R., Palti, Y., Leeds, T.D., Rexroad, C.E. III, Welch, T.J., Wiens, G.D. and Ducrocq, V. 2008. Journal of Animal Science. Submitted
- Leeds, T.D., Silverstein, J.T., Vallejo, R.L., Palti, Y., Rexroad, C. E. III, Welch, T.J. and Wiens G.D. Journal of Animal Science. In Preparation

Characterization of Resistant Fish

- **Are fish resistant throughout life cycle?**
  - **Yes – 2 g, 10g and 800g evaluation**
- **Are fish resistant to other *Fp* strains?**
  - **Yes –2 other strains tested**
- **What is the mechanism of resistance?**
  - **More resistant to BCWD= bigger spleen**
  - **Differences in immune gene expression**

Hadidi, S., Glenney, G., Welch, T.J., Silverstein, J., Wiens, G.D. 2008. The Journal of Immunology. 180:4156-65.

Genotype by Environment

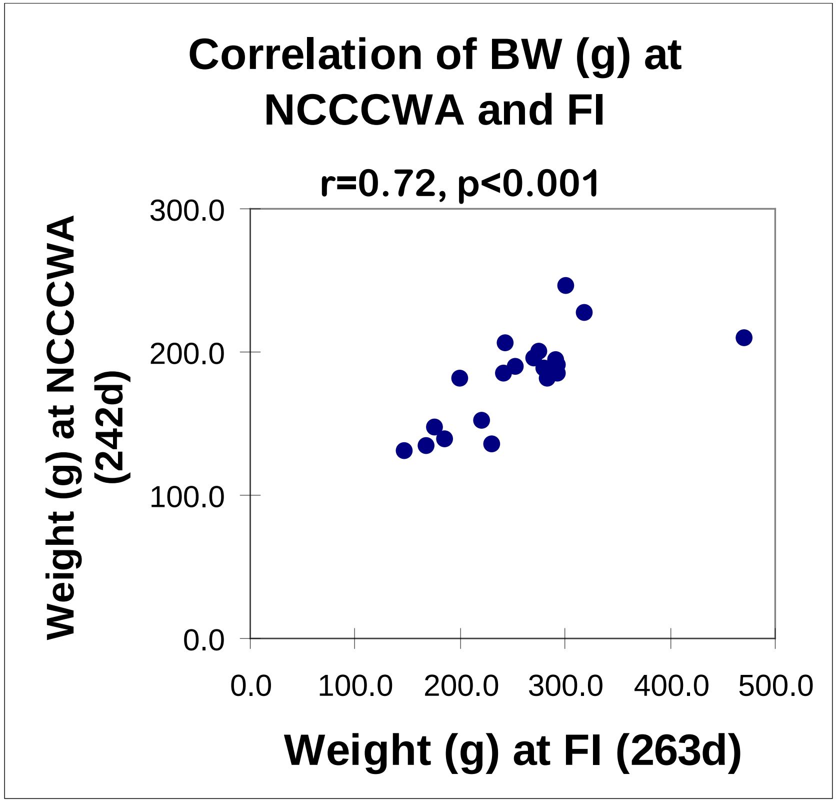
- High intensity recirc environment vs. traditional raceway
  - Will fish grow as well as , or better than in “traditional” system?
    - Environmental effect
  - Will the fish that grow best in traditional system be the same fish that grow best in high intensity system?
    - Genotype x environment effect

G x E : a real concern?

- Tolerance:
  - Water hardness
  - Temperature
  - Waste product concentration
  - Fish density
- Examine performance of families across different environments
  - NCCCWA, CFFI, NCSU
  - Flow through vs. Partial Re-use

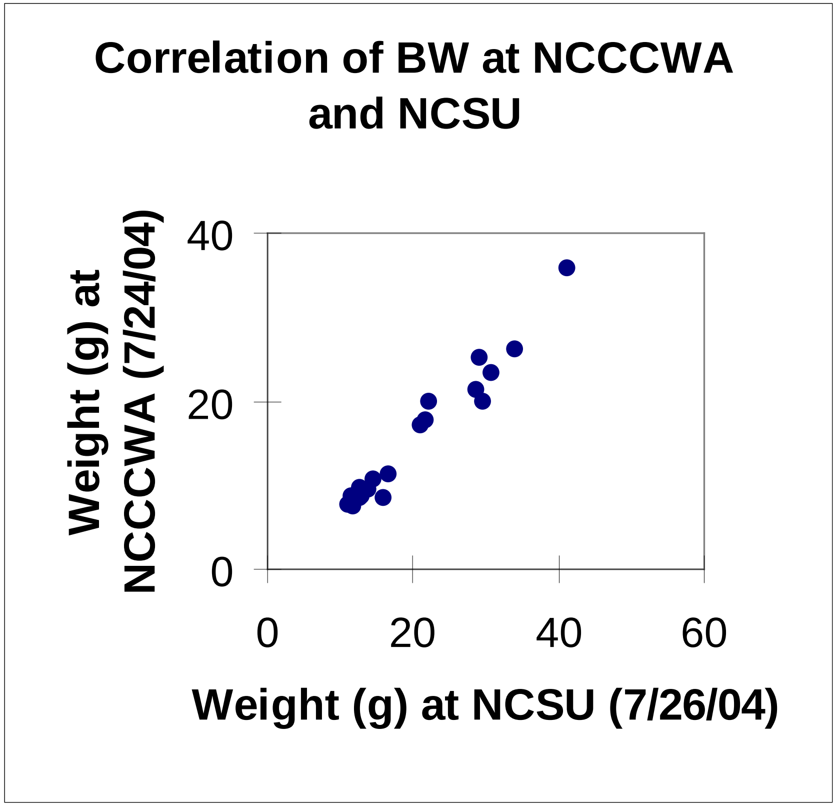
Comparison of families in different environments

Partial Re-use systems



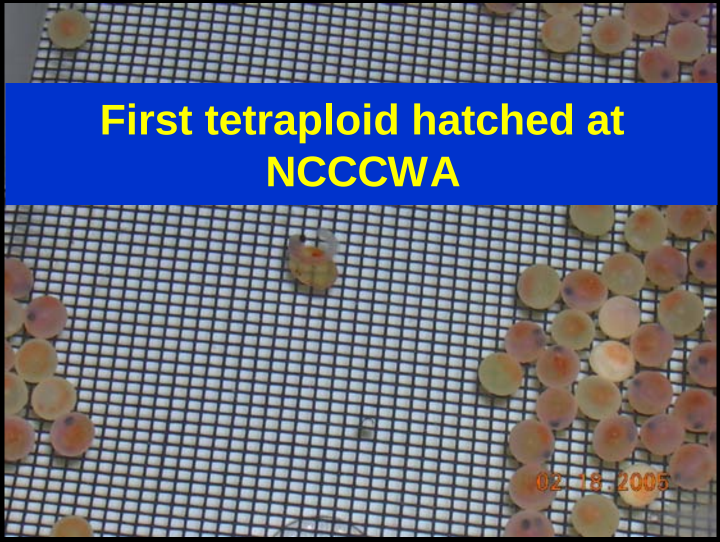
Silverstein and Summerfelt (unpublished)

Flow Through systems



Silverstein and Hinshaw (unpublished)

Creation of Tetraploids



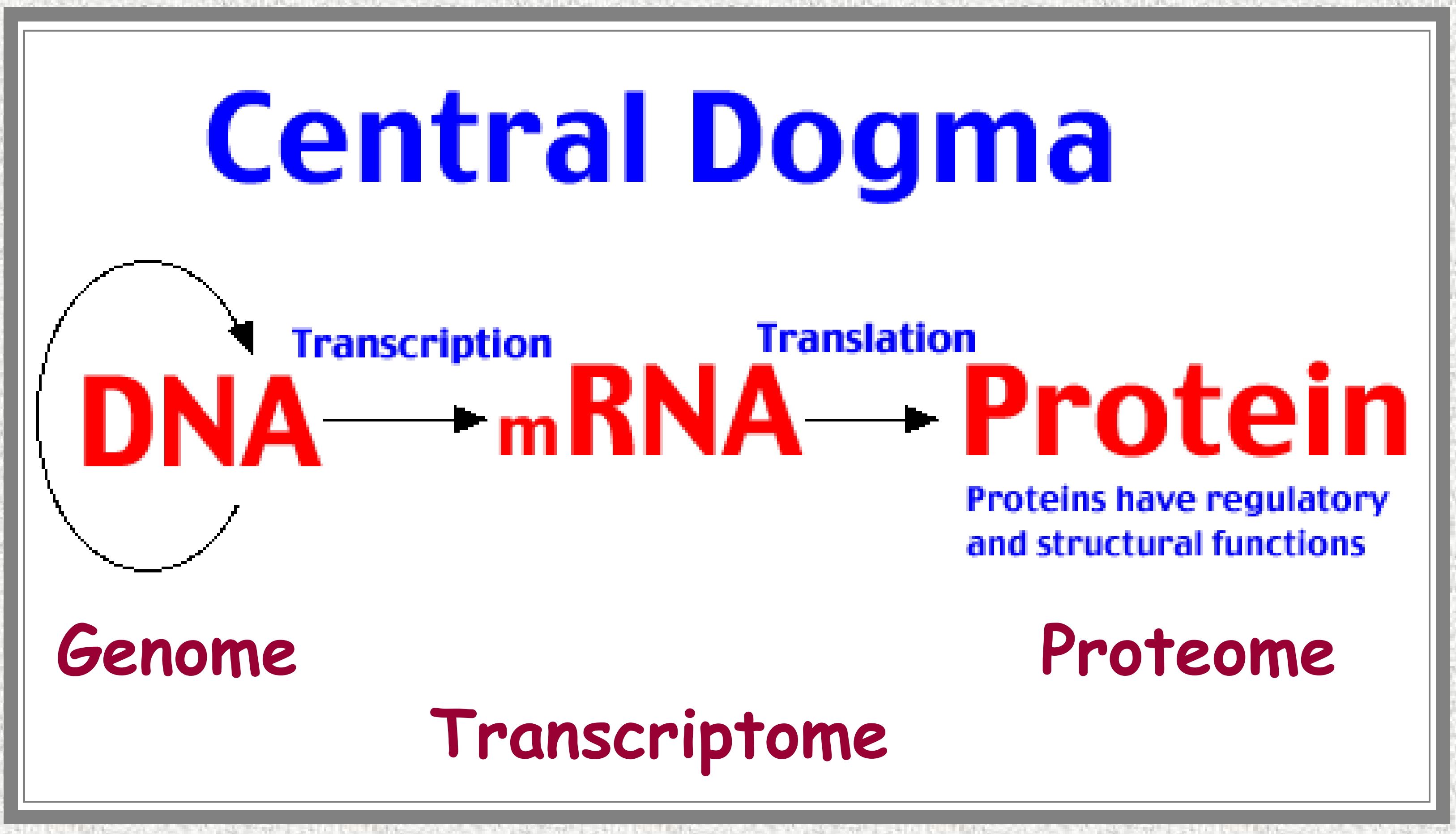
First tetraploid hatched at NCCCWA

Chromosome Set Manipulation can produce rainbow trout which are sterile

Triploid Production

- Tripliod Benefits
  - More energy to growth and not reproduction
  - Protect germplasm/breeding strategies
- Similar protocol to developing tetraploids
  - Low efficiency, not 100% (diploid contamination)
- Cross Tetraploids with Diploids
  - High efficiency
  - Less defects

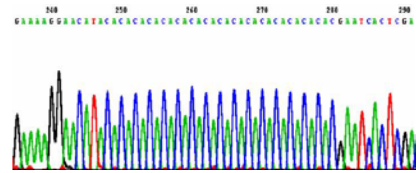
What are the genes?

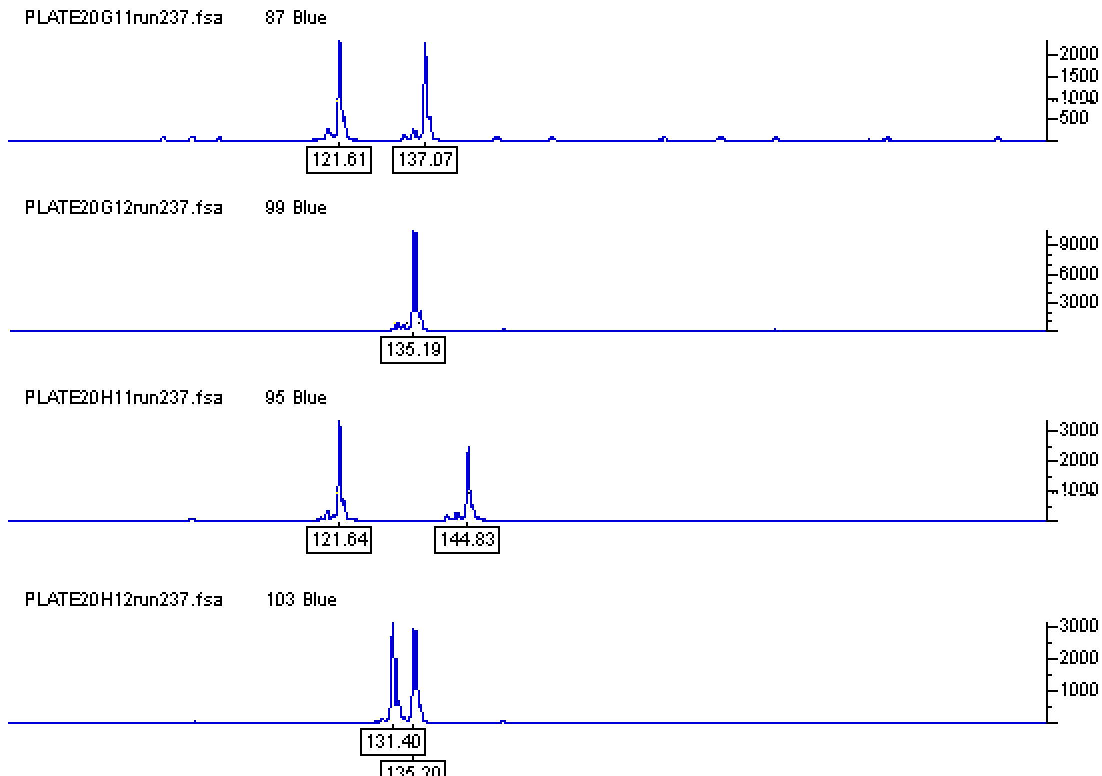


Use of Molecular Genetics for the Improvement of Rainbow Trout for Aquaculture Production

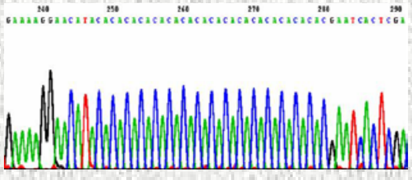
- Maintain genetic diversity thru selection
- Evaluation of families in common garden, ability to identify parentage
- Identify genes for selection which affect traits which are expensive or difficult to measure or require sacrificing fish
- Introgression – introgression of haplotypes associated with a phenotype into a population
- Multi-trait selection, especially where multiple traits can not be evaluated on individuals
- Association with a breeding program including "commercially relevant" germplasm to facilitate use of that information - NCCCWA broodstock

Genotyping Microsatellites





Genetic Markers

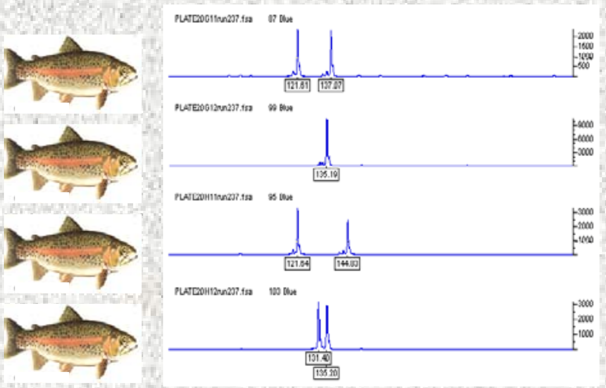


Useful for determining identifying individuals, parentage, characterizing population structures (migration, inbreeding, strain identification), estimation of genetic variation, conservation, evolutionary studies) and genetic maps having the goal of identifying genes affecting traits

Rainbow trout

- ~835 anonymous
- 181 from BAC clones
  - Physical map integration
- 334 from genes
- Therion DNA, Int., Saratoga Springs, New York

- **Striped bass**
  - n=498
  - Kent Sea Tech Corporation
- **Collaborations**
  - Spanish mackerel
  - Greater amberjack
  - Red drum
  - Pacific sardine
  - Cobia



Relationship Matrix Between Individuals

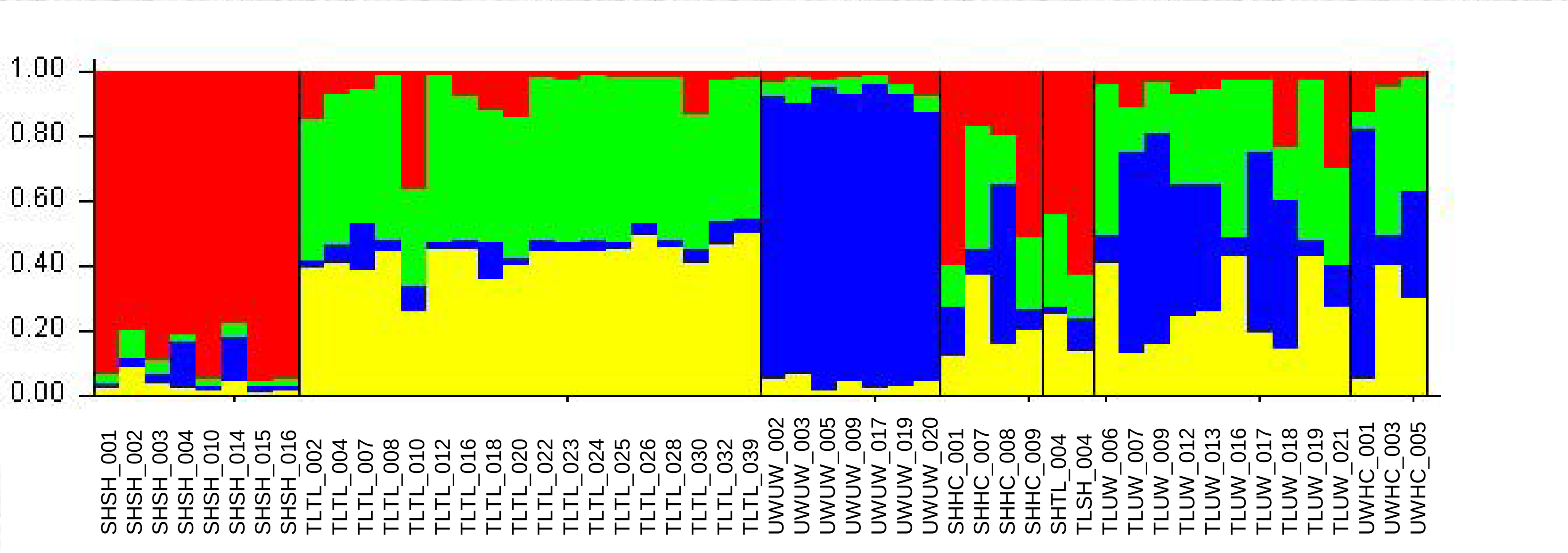
	YRO1041	YRO1131	YRO1561	YRO2235	YRO2308	YRO3596	YRO3752	YRO3764	YRO3766	YRO3775	YRO3861	YRO3865	YRO3891	YRO3894	YRO3906	YRO3920	YRO3948	YRO3973	YRO3980
YRO1131	0.4																		
YRO1561	0.5	0.3																	
YRO2235	0.2	0.3	0.2																
YRO2308	0.5	0.3	0.4	0.2															
YRO3596	0.3	0.3	0.3	0.2	0.2														
YRO3752	0.2	0.3	0.2	0.3	0.3	0.2													
YRO3764	0.2	0.3	0.2	0.3	0.3	0.3	0.4												
YRO3766	0.3	0.3	0.2	0.3	0.2	0.3	0.3	0.7											
YRO3775	0.2	0.3	0.2	0.4	0.2	0.3	0.3	0.4	0.4										
YRO3861	0.3	0.3	0.2	0.4	0.3	0.3	0.3	0.4	0.4	0.4									
YRO3865	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3								
YRO3891	0.3	0.4	0.2	0.3	0.3	0.2	0.4	0.3	0.2	0.3	0.3	0.4							
YRO3894	0.3	0.3	0.3	0.2	0.3	0.2	0.3	0.3	0.2	0.3	0.3	0.3	0.3						
YRO3906	0.3	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.4	0.2	0.4	0.4	0.3					
YRO3920	0.2	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.4				
YRO3948	0.2	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.5	0.5	0.5	0.3	0.2	0.3	0.3	0.3			
YRO3973	0.2	0.2	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3		
YRO3980	0.2	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.3	
YRO4054	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.2	0.3	0.2	0.2	0.3	0.2	0.3	0.3

Population Substructure

•Using LD data, we were able to determine that we have an effective breeding size (Ne) of ~150 in 2005 and 2006

•Actually used ~320 fish

- ratio of .48 actually contribute unique genetic variation



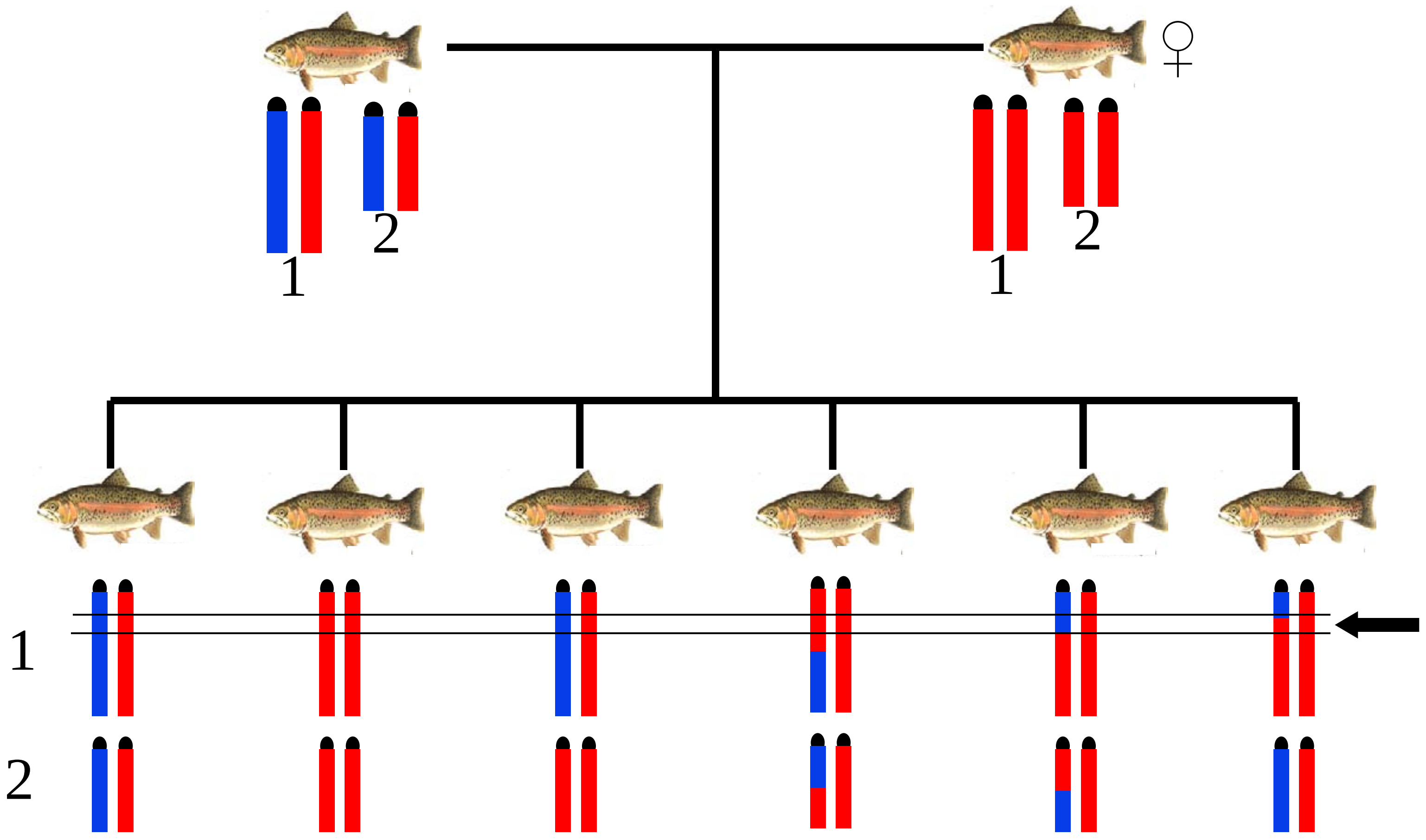
Genetic and Diet Effects on Growth Rate and Reproduction in the Rainbow Trout Strains of Troutlodge

- Palti et al., 2006. Evaluation of family growth response to fish meal and gluten-based diets in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 255(1-4):548-556.

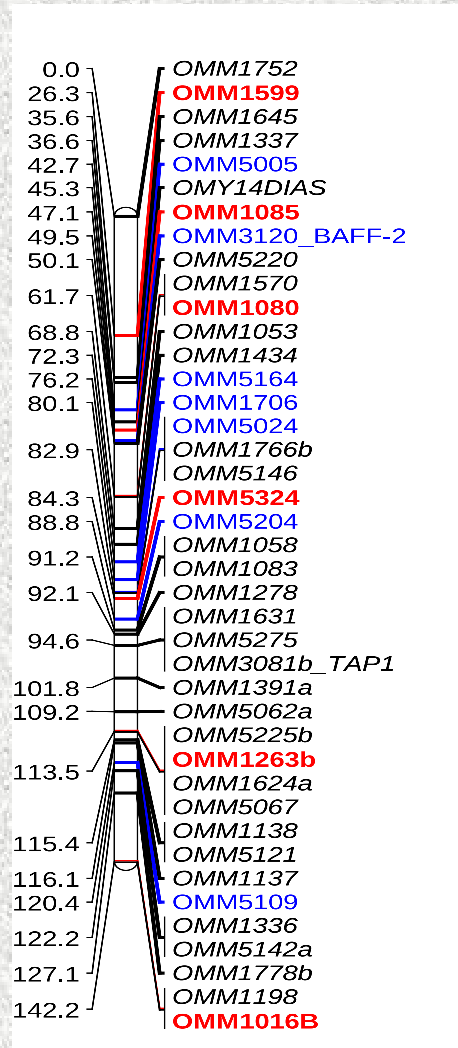
- Johnson et al., 2007. Development and evaluation of a new microsatellite multiplex system for parental allocation and management of rainbow trout broodstocks. Aquaculture 266:53-62.

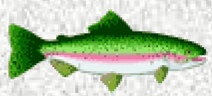
- Pierce et al., 2008. Family growth response to fishmeal and plant-based diets shows genotype x diet interaction in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 278:37-42.

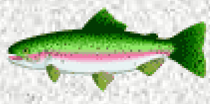
Mapping Traits

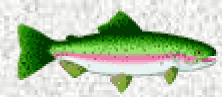


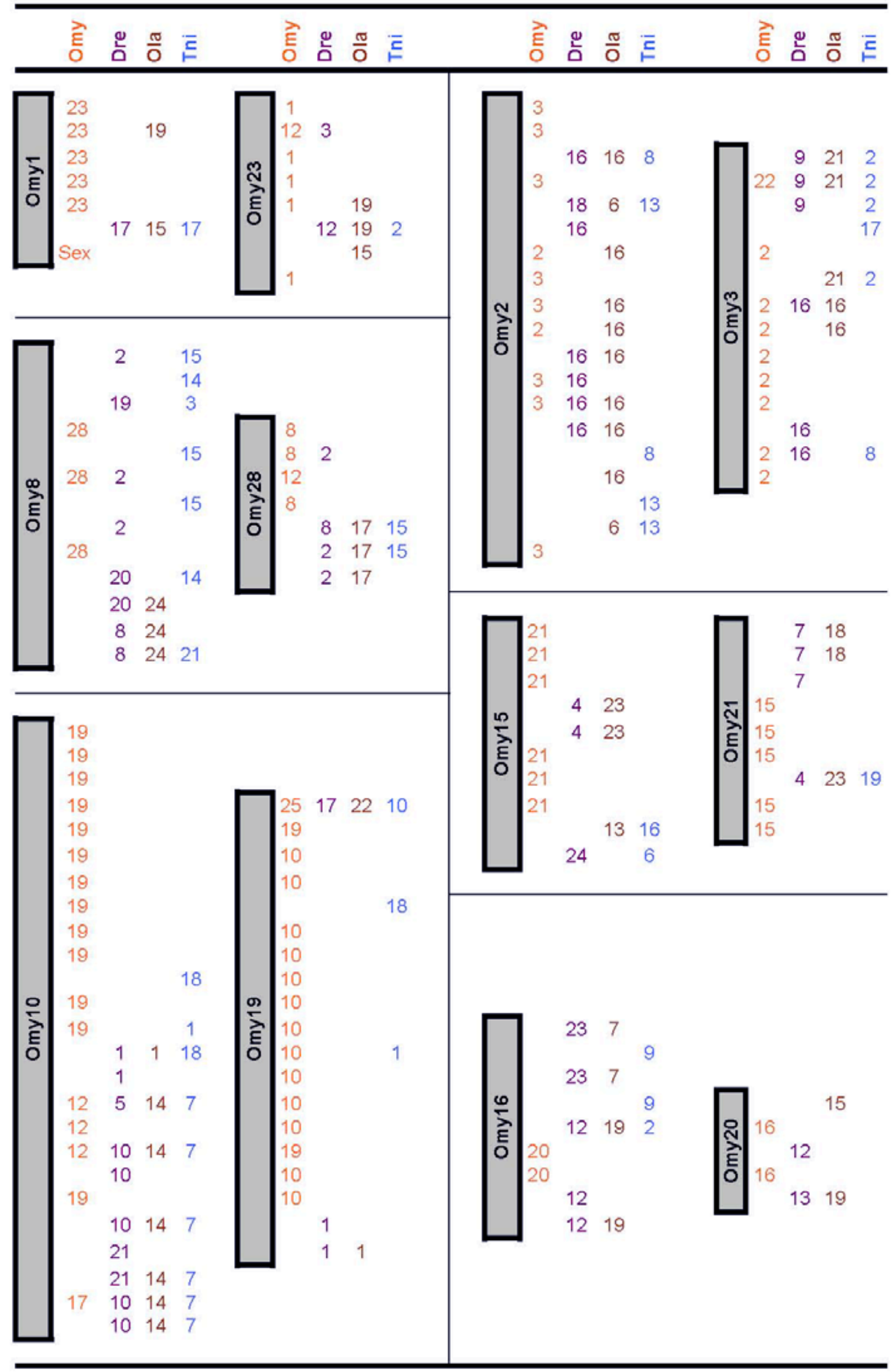
Rainbow Trout Comparative Map



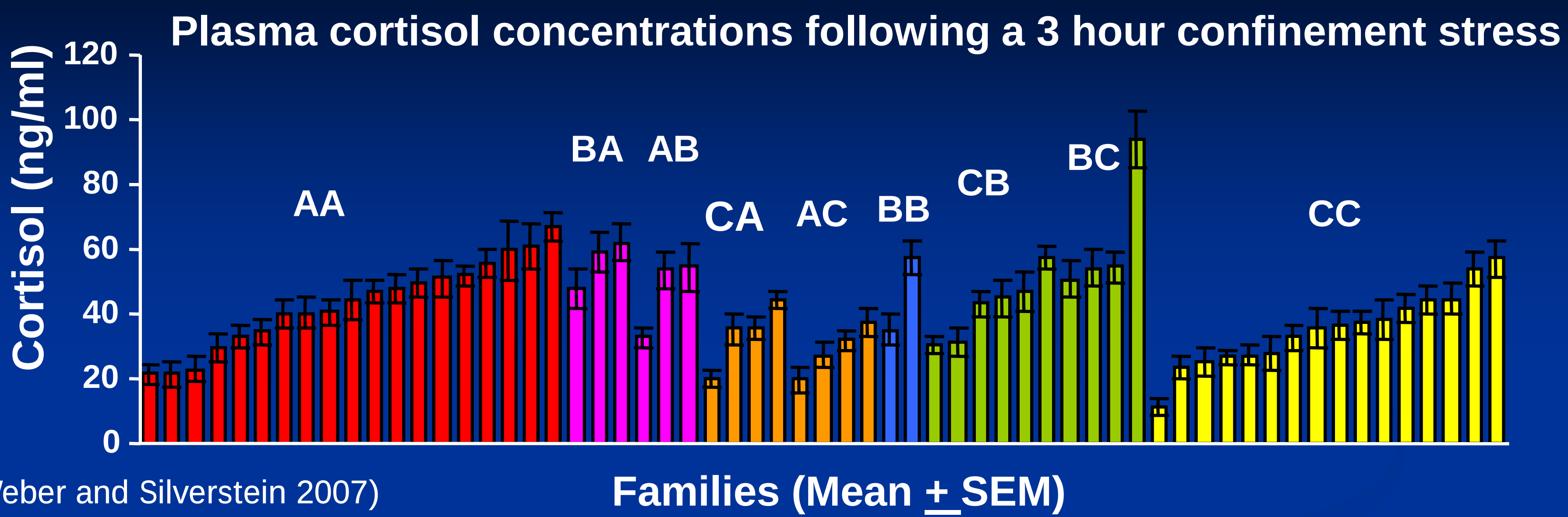






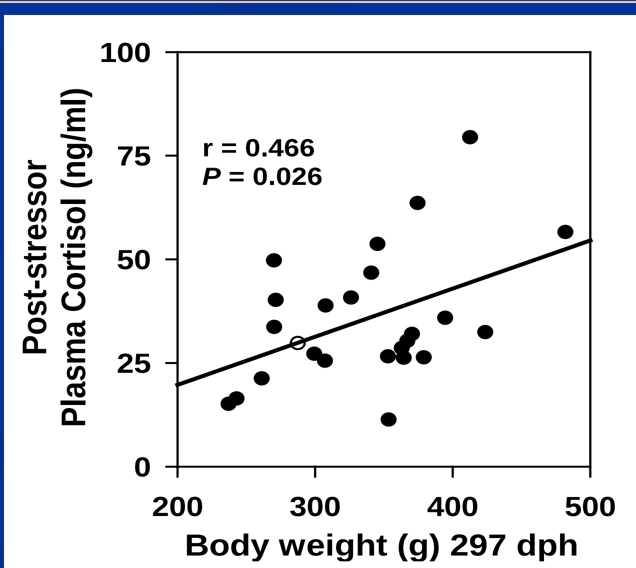


Trait Evaluation: Stress Response



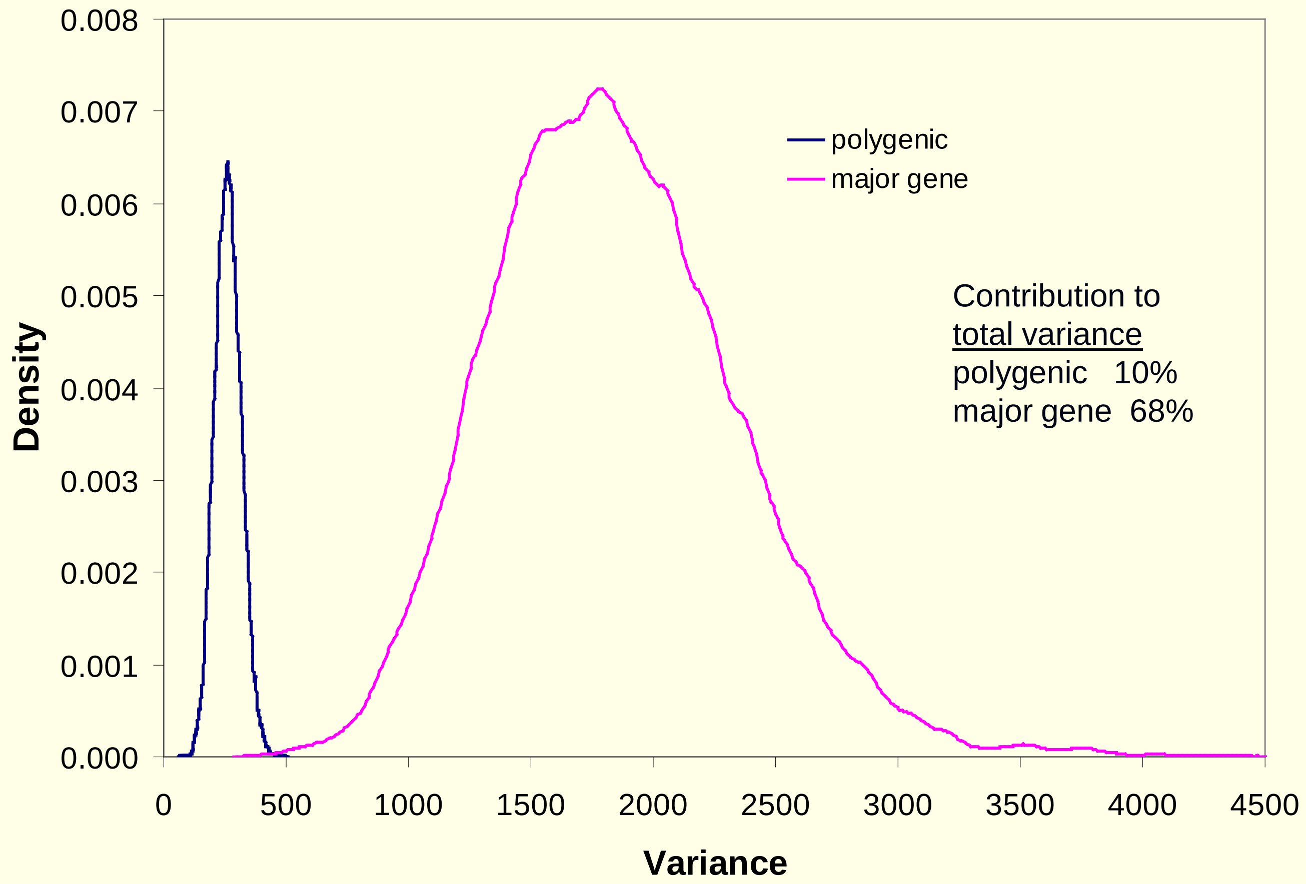
(Weber and Silverstein 2007)

Heritability: $h^2 \geq 0.40$



(Lankford and Weber 2006)

Contribution of Major Genes to Post-stressor Plasma Cortisol Levels



Marginal posterior densities* for polygenic and major gene variance from the mixed inheritance model for plasma cortisol (Gibbs sampling: samples per chain=500,000; burn-in=250,000; thinning=5,000; chains= 7)

USDA ARS
National Center for
Cool and Cold Water Aquaculture
Leetown, West Virginia