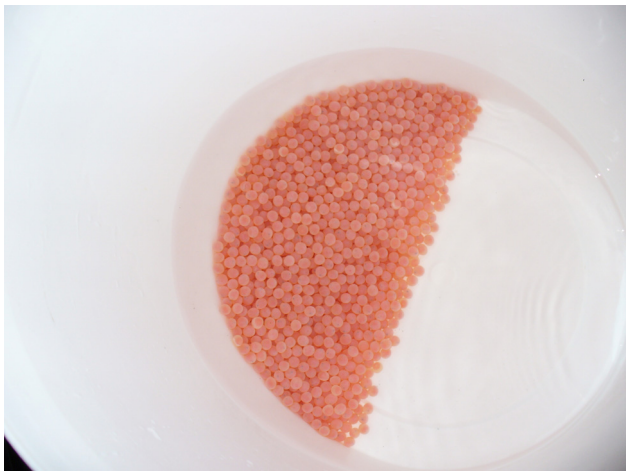
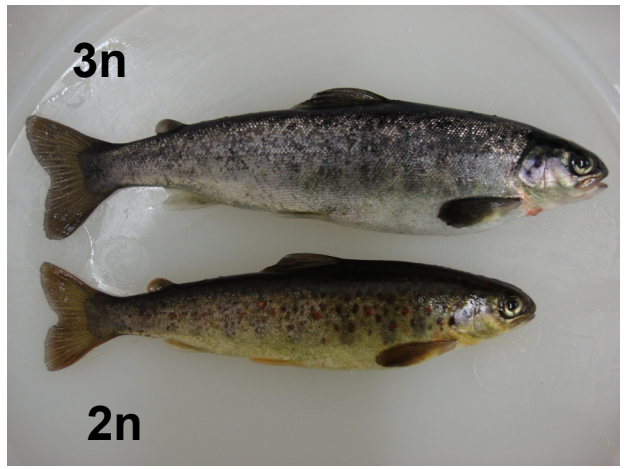
OUT OF SEASON PARR SMOLT TRANSFORMATION IN TRIPLOID ATLANTIC SALMON.





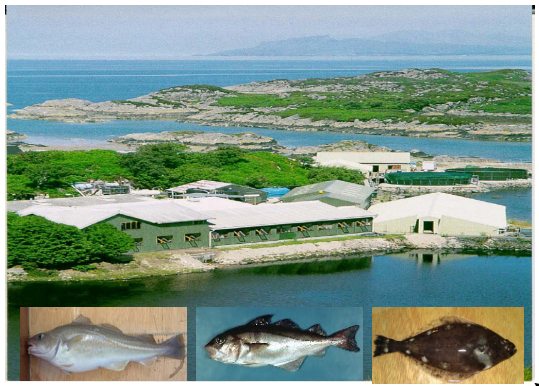
H. Migaud, J. Taylor, A.C. Preston, J. Bron, John Taggart

Institute of Aquaculture, University of Stirling, Stirling, Scotland, UK.

External Experimental facilities

http://www.fishresearch.co.uk/

SEAWATER



FRESHWATER

Current problems in salmon farming

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Genetic pollution by escapes

– Reduced fitness of wild population
– Reduced spawning (due to increased competition)
– Spread disease and parasite (ISA, BKD, IPN and Sea lice)

...ver the Orkney fish breakout has raised the profile of ecological concerns.

Stream of escaped farm fish raises fears for wild salmon

The escape of an estimated 100,000 farmed salmon in the Orkney Islands, off the north coast of Scotland, has highlighted mounting concerns about the ecological impact of such incidents on natural salmon stocks.

... currently investigating the impact of fish farming. But the Scottish Executive, which has been responsible for fishing and the environment since its establishment in 1999 ...

... the environment next month, will indicate where more research is needed to understand better the impact of escaped farm fish.

Although containment techniques for farm fish have improved, the number of ... expanding, says Black. According to environmental groups, about a million salmon have escaped from farms in Scotland since 1998.

"So far, the government has done noth-

Is Triploidy the solution?

- Tested in early 1990's to prevent pre-harvest maturation
- Abandoned in favour of photoperiod control

- Phenotypically similar, altered physiology?
  - Somatic growth- less/equal/high
  - Survival - lower
  - Deformity - greater
  - Flesh quality- similar to diploid
- Disease and stress resistance - comparable
- Environmental tolerance - triploid less tolerant to change

Industry is now keen to investigate this avenue again

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SALMOTRIP:

Feasibility study of triploid salmon production

FP6: EC Capacities Program (Jul 08 - Dec 10)

http://www.salmotrip.stir.ac.uk

5 Key areas of Research:

- Family-ploidy performance
- Culture sensitivity & deformity
- Out-of-season smolt production
- Commercial scale field trials
- Market Perception







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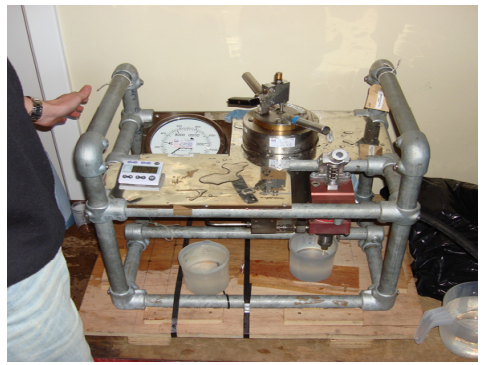
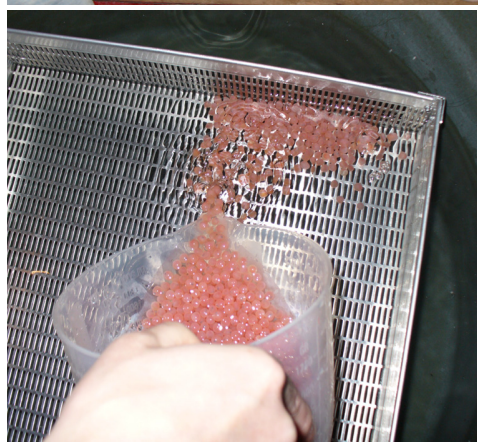
Materials & Methods

1) Experimental Trials

- 10 full-sib families (2 year Classes) 10♂:10♀
- 1st week December 2007, 2008
- Pressure induction (2500eggs/ploidy/family)
  - 9500PSI 5mins 30mins PF @ 10°C
- Individual family rearing up to tagging: Ploidy Discrete
- Random triplicated design
- Incubation: temp control 7.5 ± 0.8 °C
- Ongrowing: river water 12 ± 2.3 °C
- 1st feeding: Constant light & 24 hour feed





2) Commercial Trial (2008 only)

- 45 (full & half-sib) families 15♂:45♀
- 500 eggs / family / ploidy
- Communal family rearing: Ploidy discrete
- Incubation / ongrowing: river supply 10 ± 4 °C
- 1st feeding: Constant light & 24 hour feed

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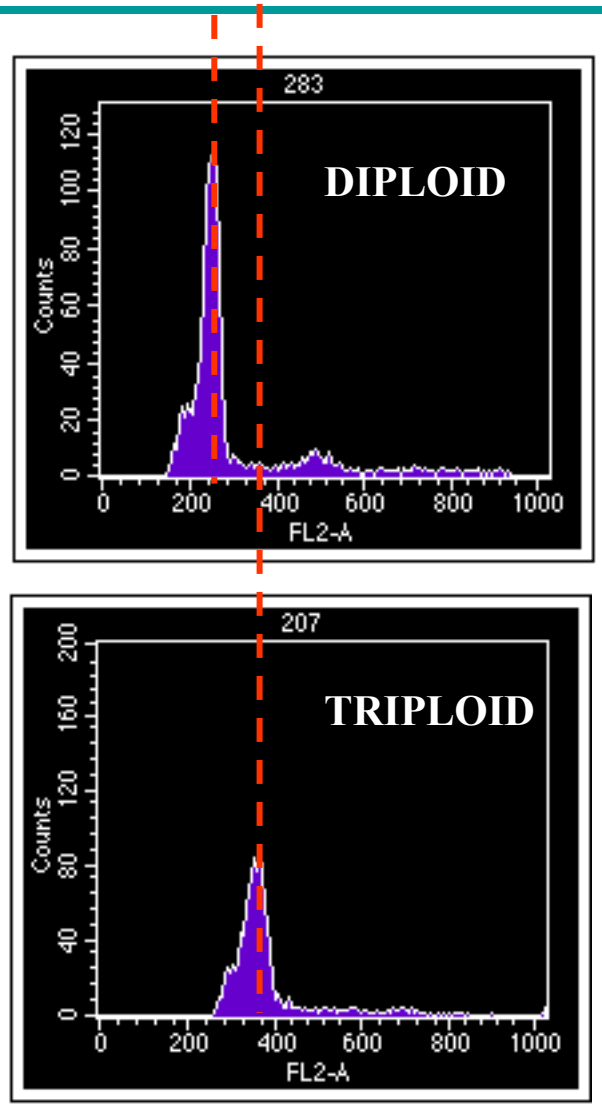
Materials & Methods

Sampling Procedure

- **Oocyte diameter** (no correlation to fertilisation)
- **Daily egg picking / mortalities ~ survival**
- **Daily counting / classification deformity from hatch**
- **1-2 weeks Wt-L measurement (n=20-50)**

Ploidy Verification

- **Flow cytometry & blood smears (n=100-200)**
- **100% triploid rate**



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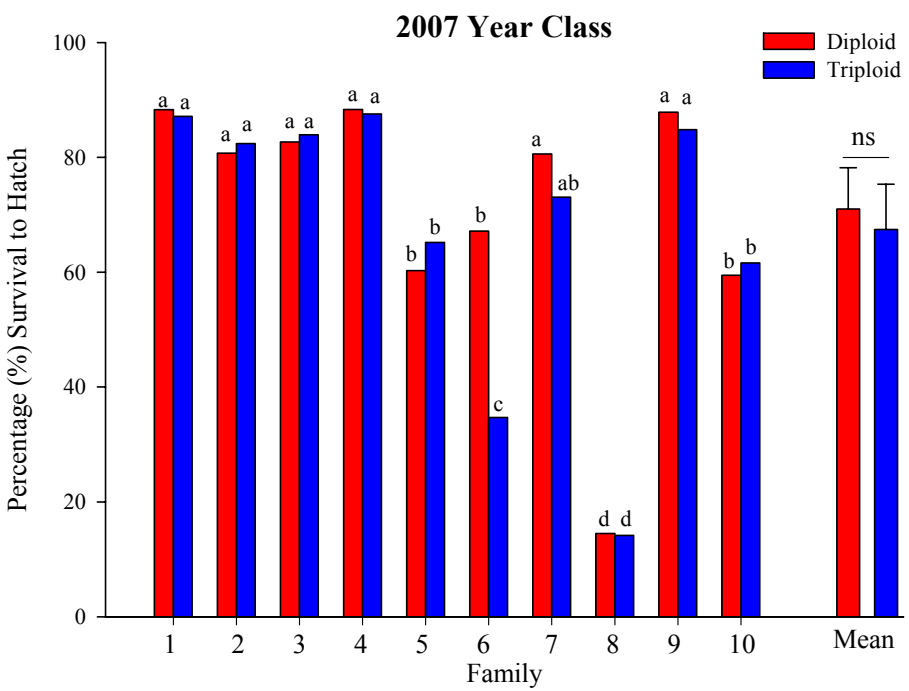
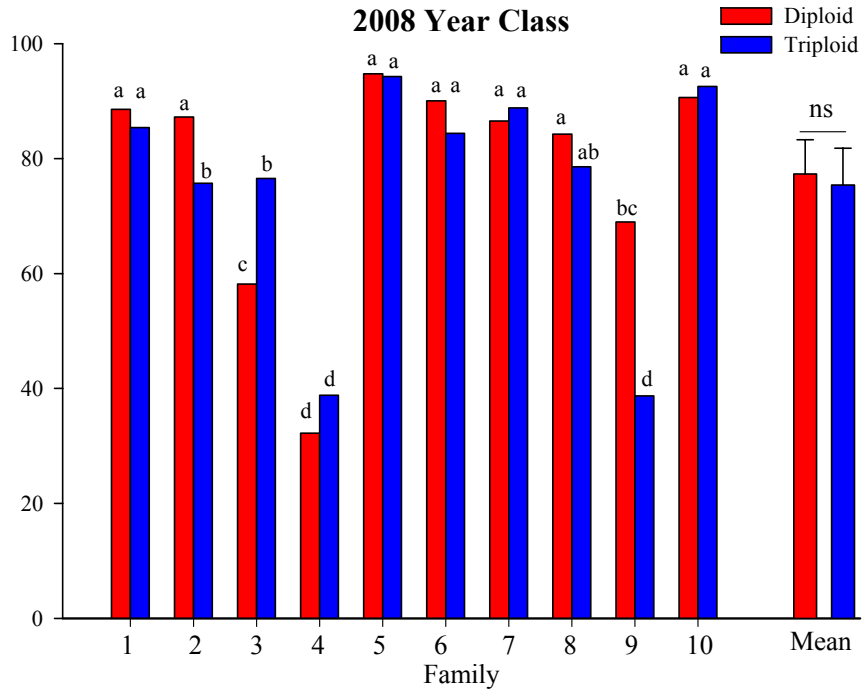
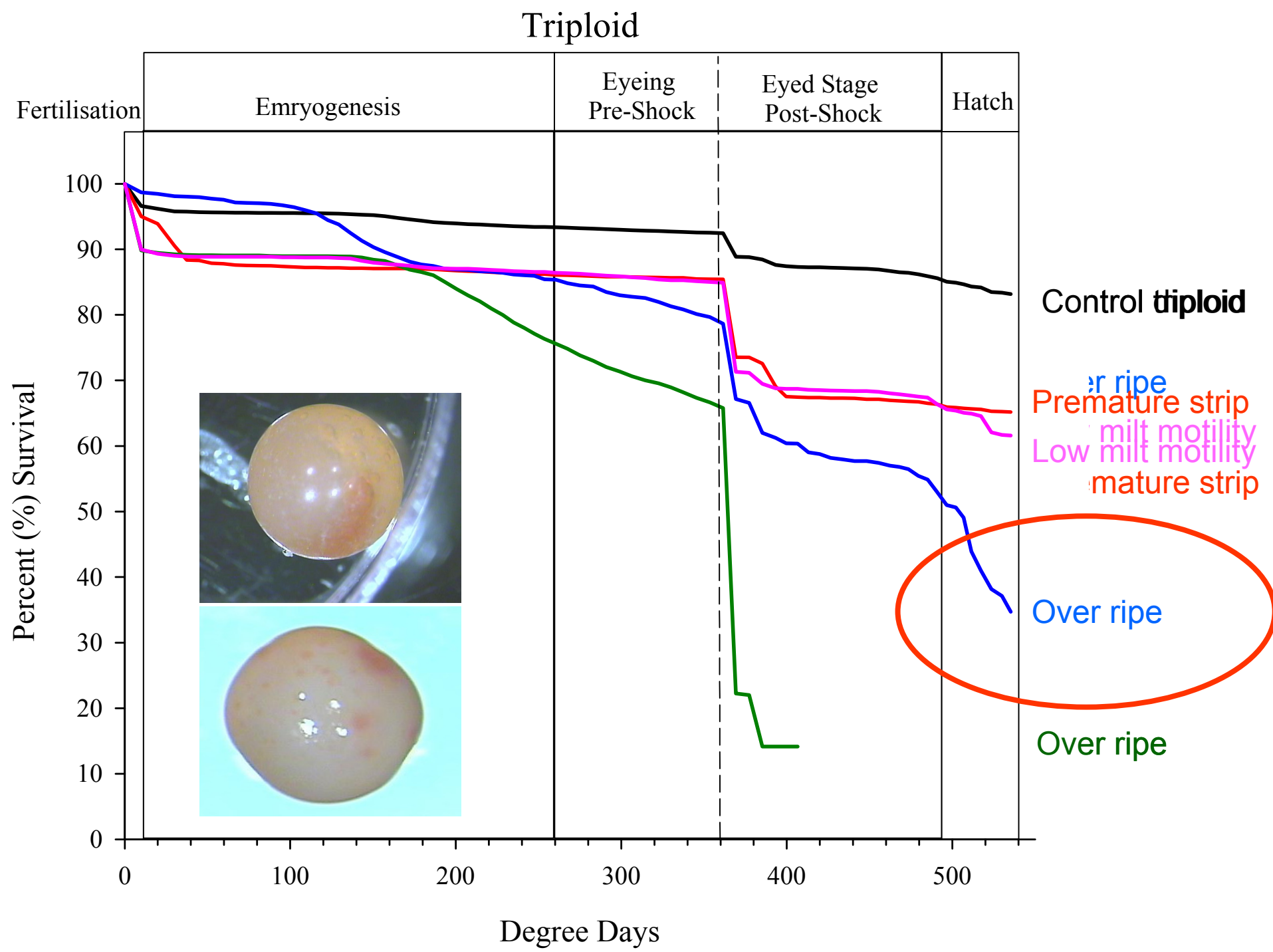
Survival to Hatch





- No overall ploidy effect on survival
- Significant family effect on survival
- Correlation between gamete quality and survival


Triploid
Fertilisation
Emryogenesis
Eyeing
Pre-Shock
Eyed Stage
Post-Shock
Hatch
Percent (%) Survival
100
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
500
Degree Days
Control triploid
er ripe
Premature strip
milt motility
Low milt motility
mature strip
Over ripe
Over ripe

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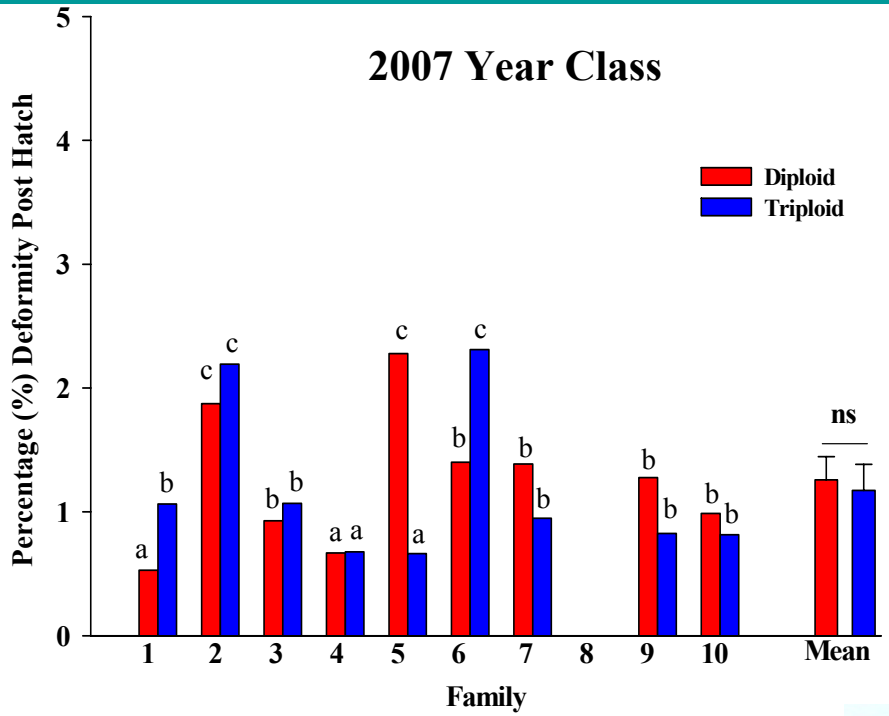
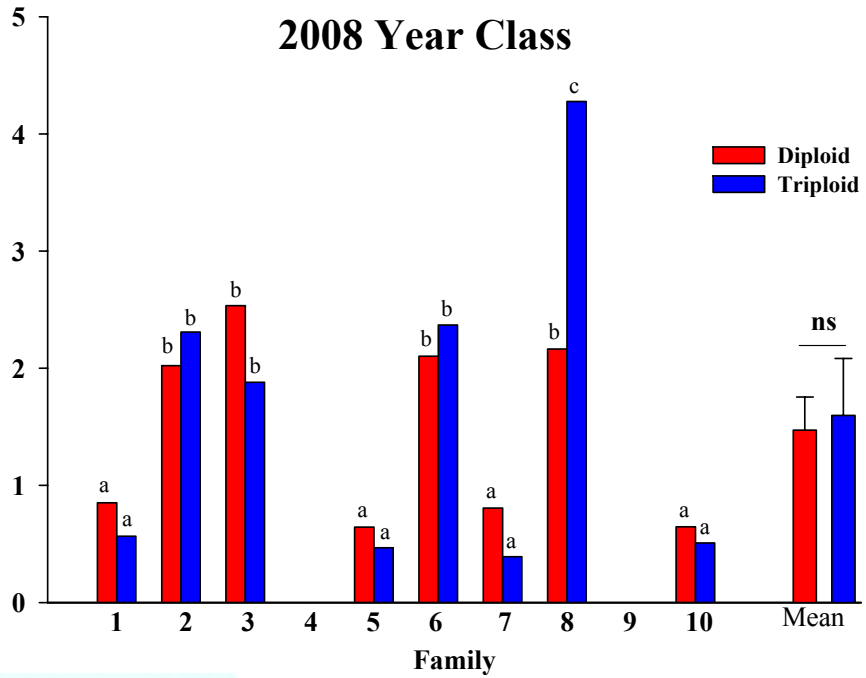
Post-Hatch Deformity





Kyphosis | Spiral Tail | Siamese | Lordosis | Scoliosis | Twin-Head

- No ploidy effect
- Significant family effect

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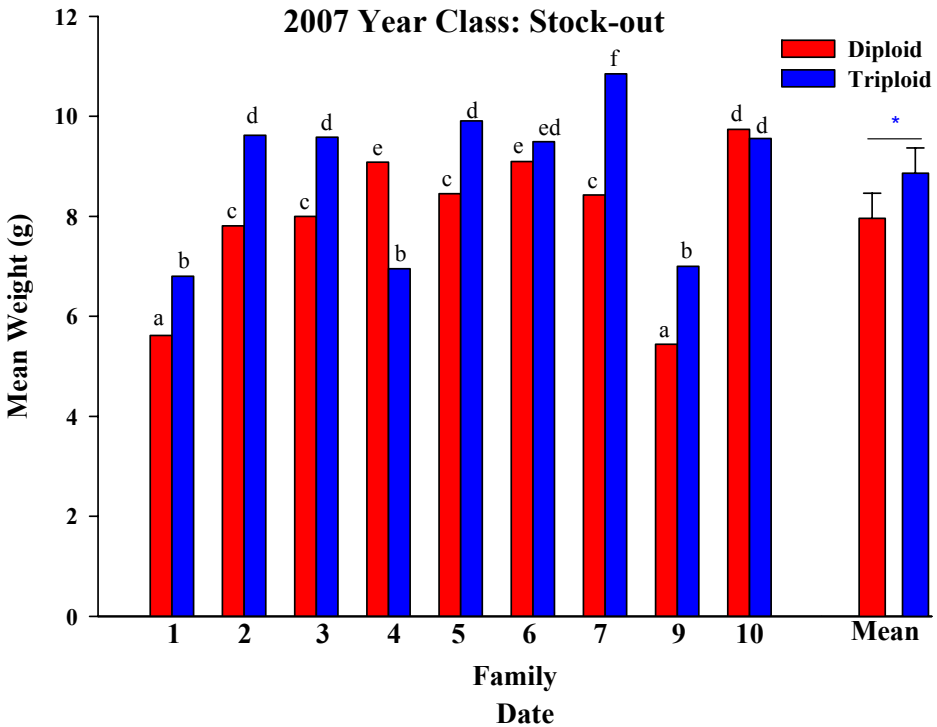
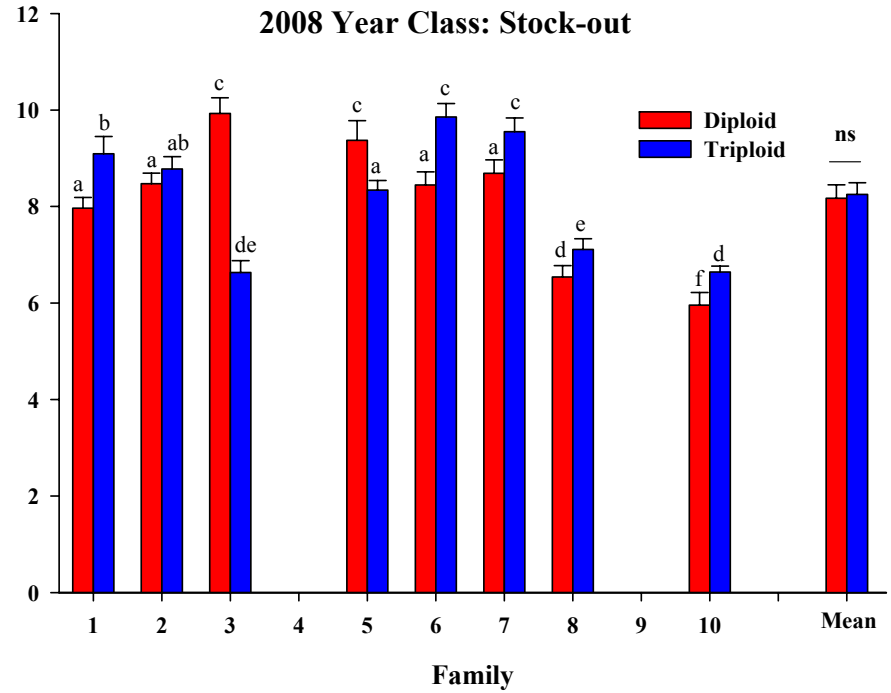
Post-Hatch Growth





- Triploids significant smaller at hatch/first feeding
- Triploids higher SGR: comparable or higher weight at stocking
- Comparable mortality 2%
- Comparable deformity 4%
- Significant family effect on size at hatch and growth

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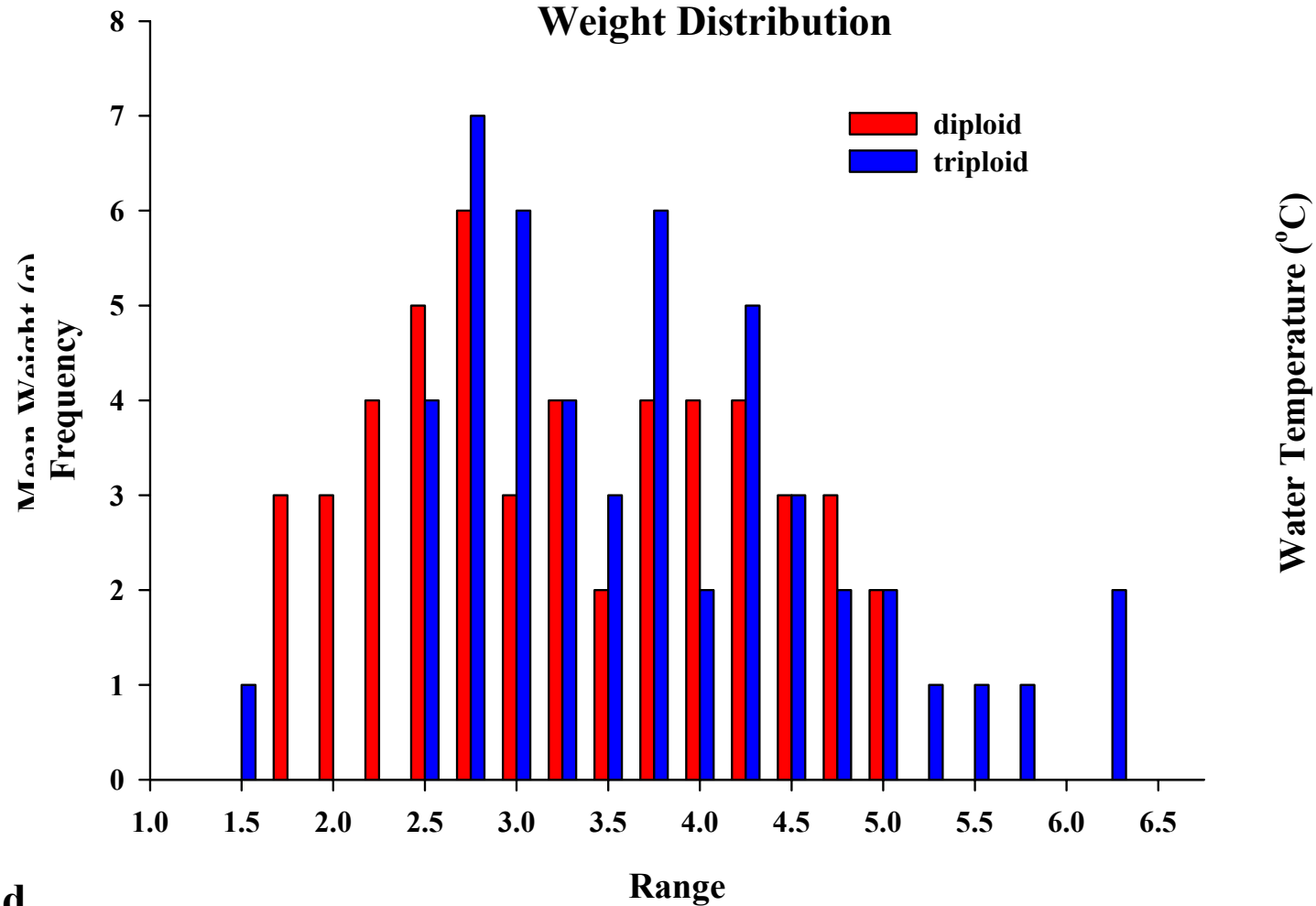
Commercial Trial



- Triploid
- Higher SGR
- Lower deformity 50 vs 75%: Opercular shortening: site specific?
  - Greater variability in triploids
  - But family effect unknown (genotyping)

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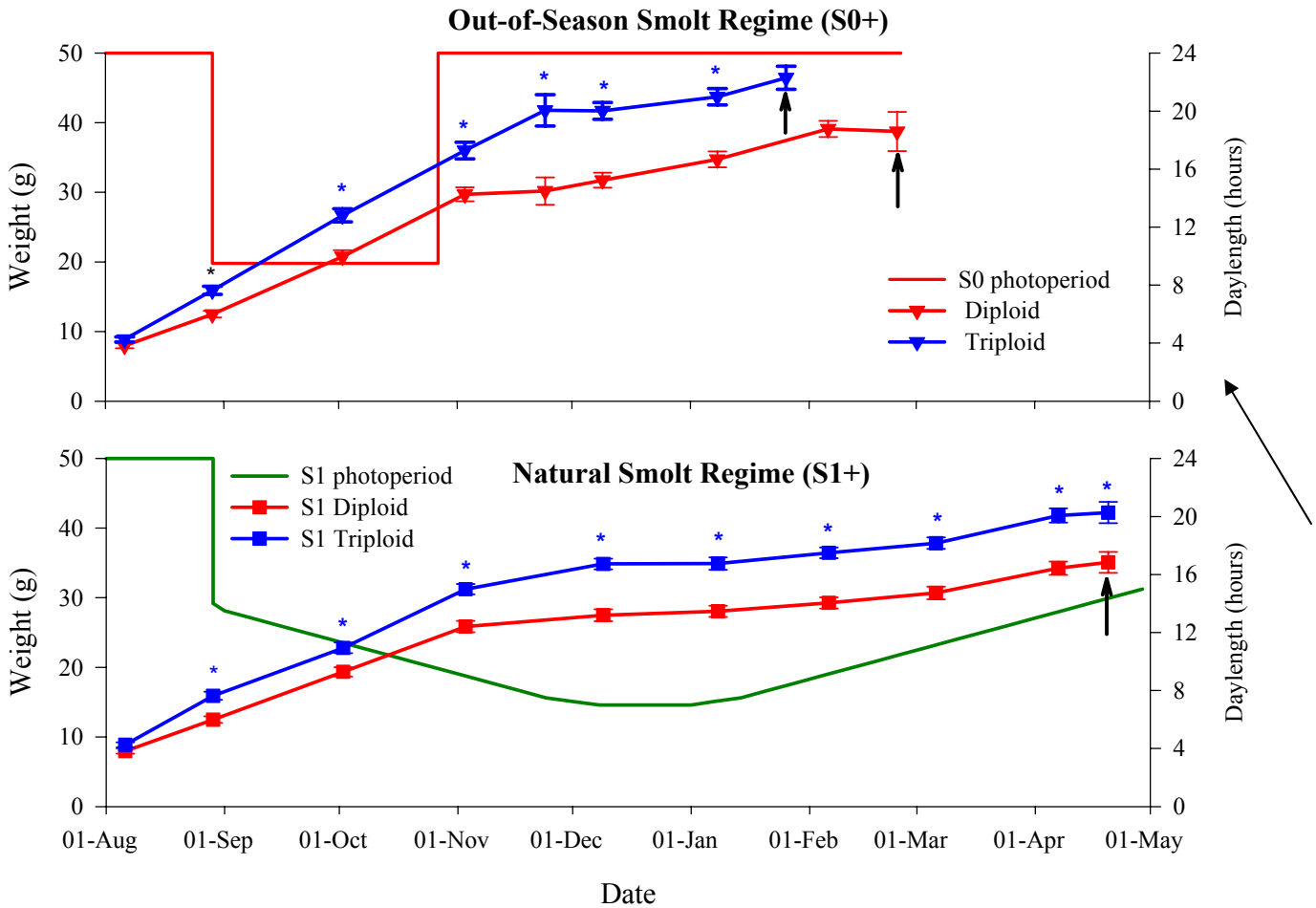
Freshwater Growth



DIPLOID S0+

TRIPLOID S0+

Sea transfer

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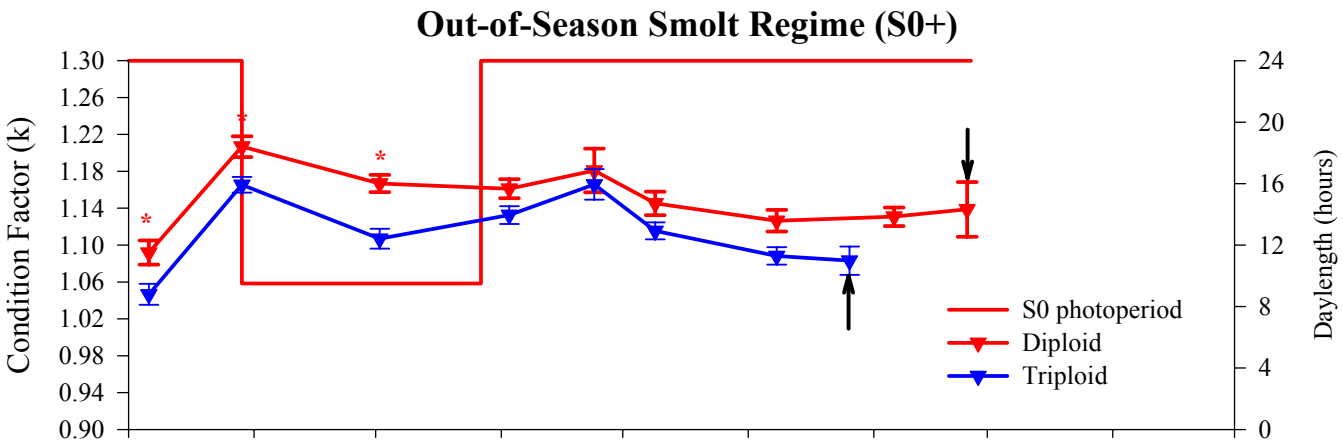
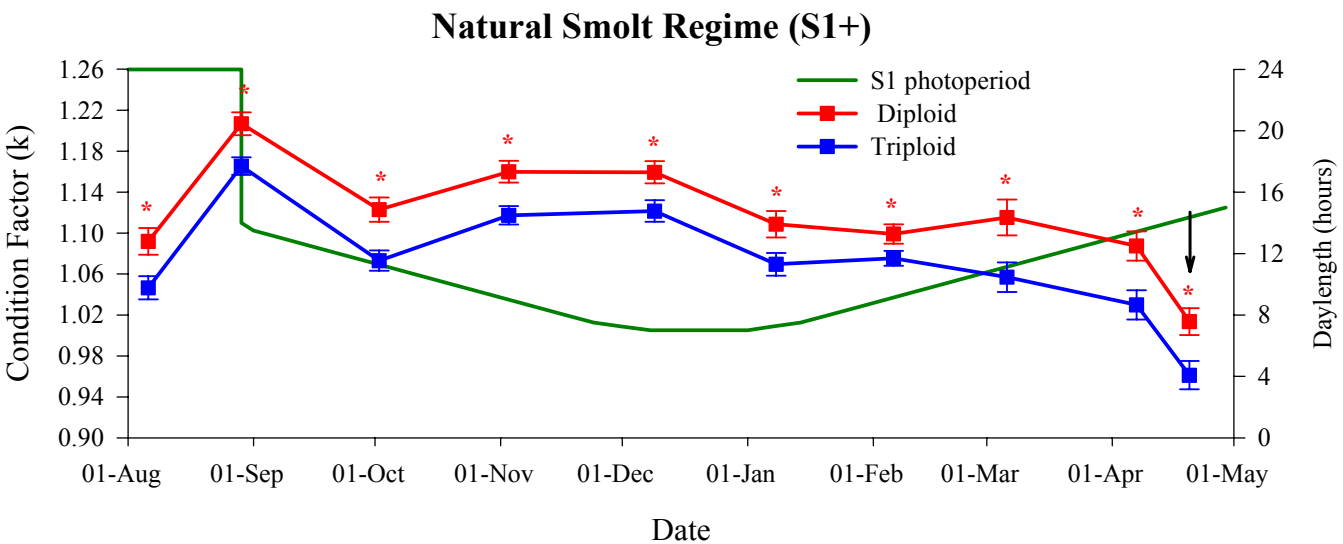
Freshwater Condition





Sea transfer

DIPLOID S0+

TRIPLOID S0+

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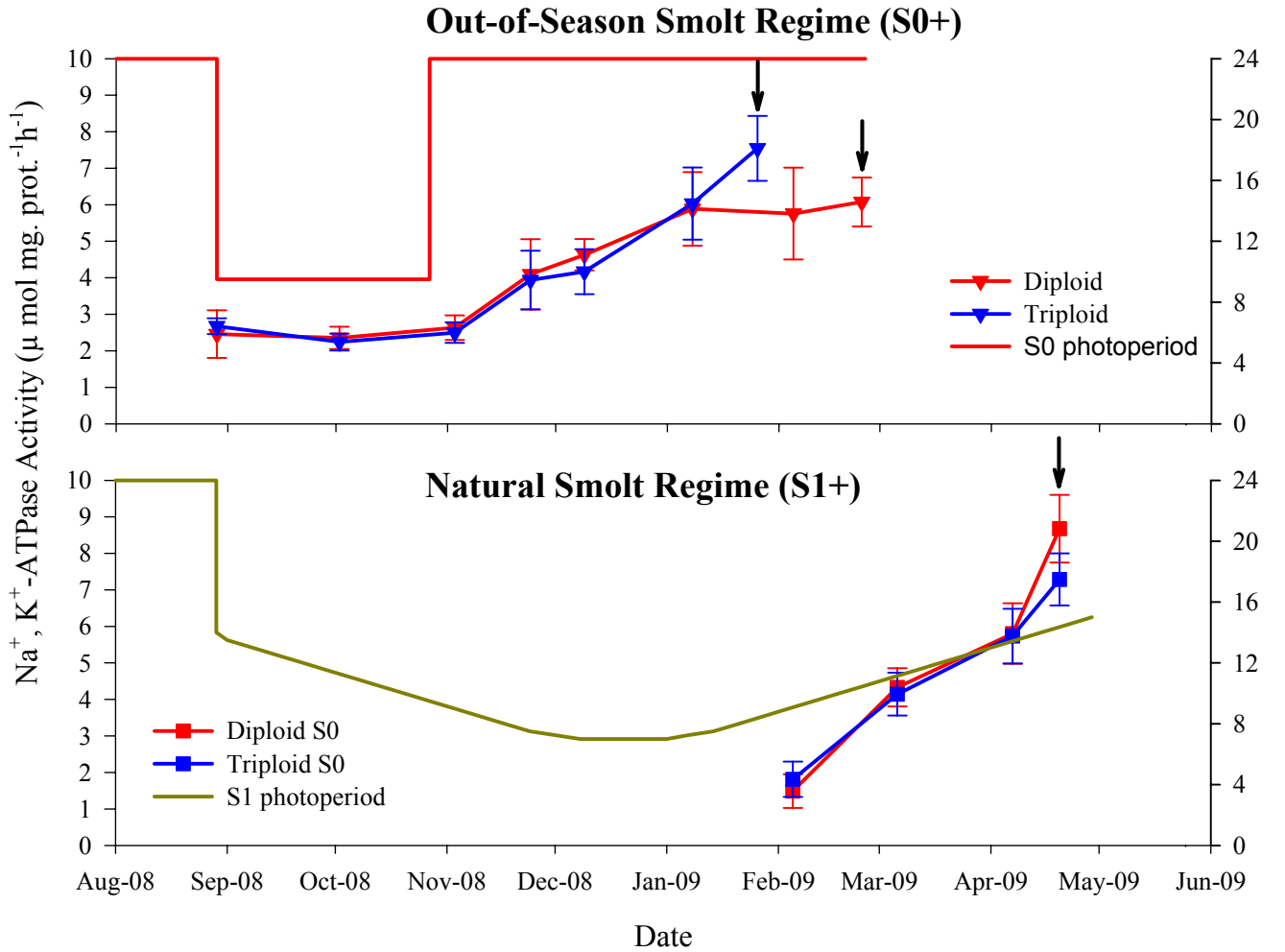
ATPase Activity



↕ **Sea transfer**

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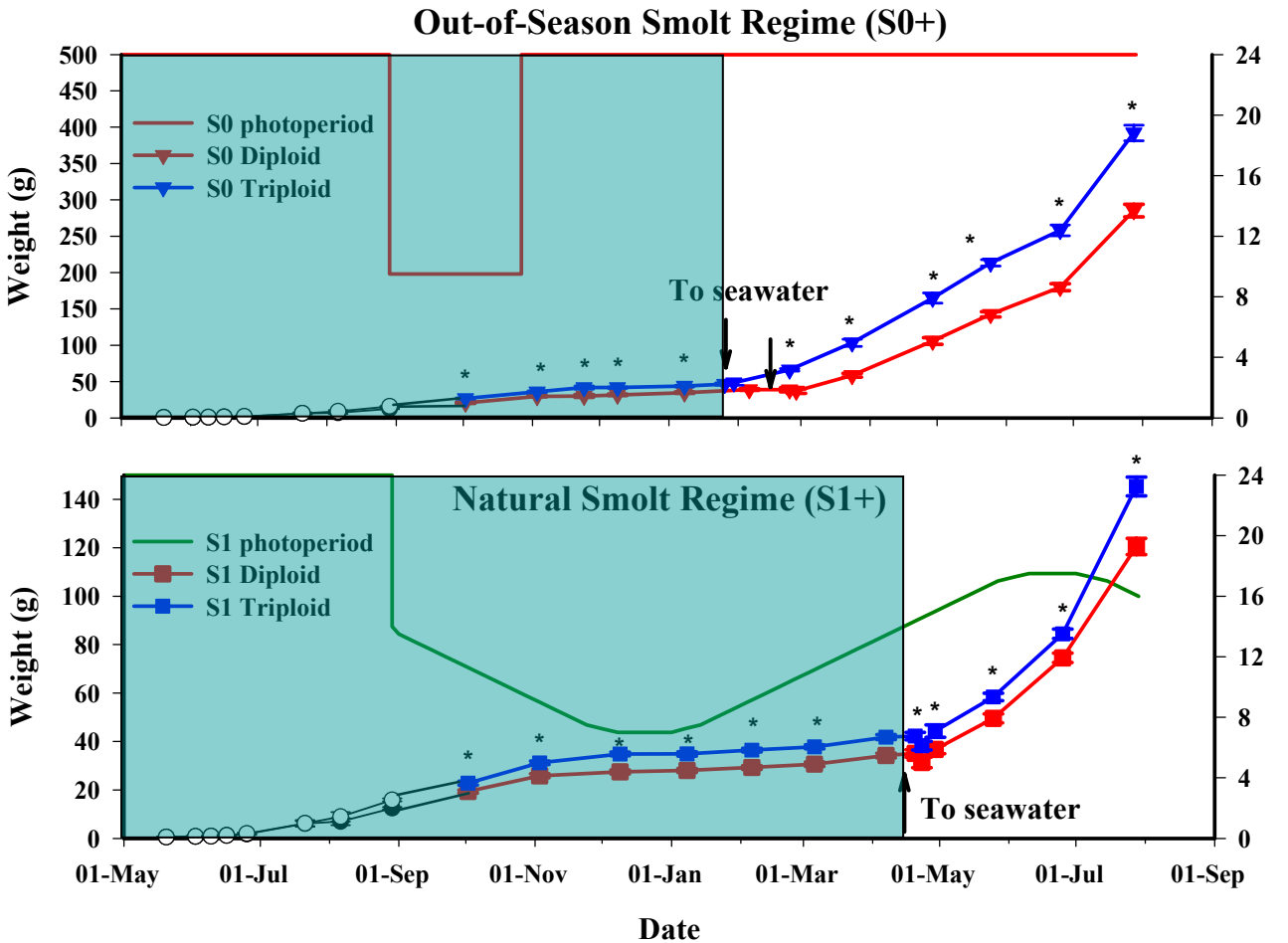
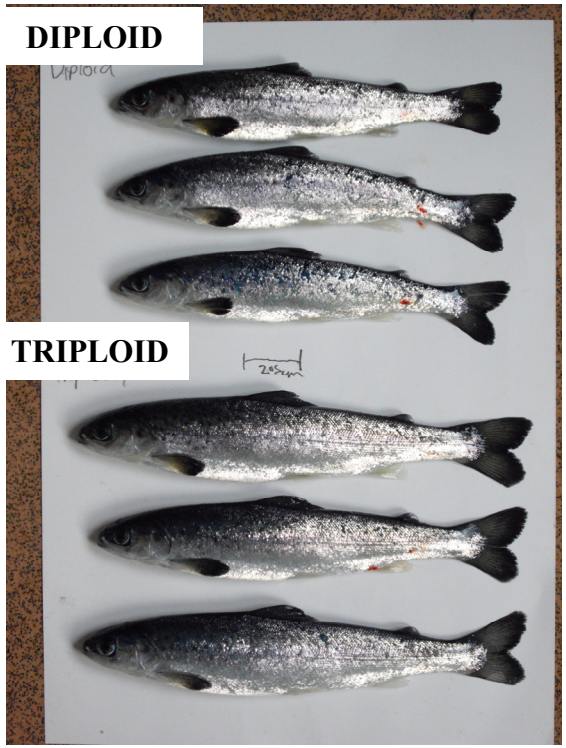
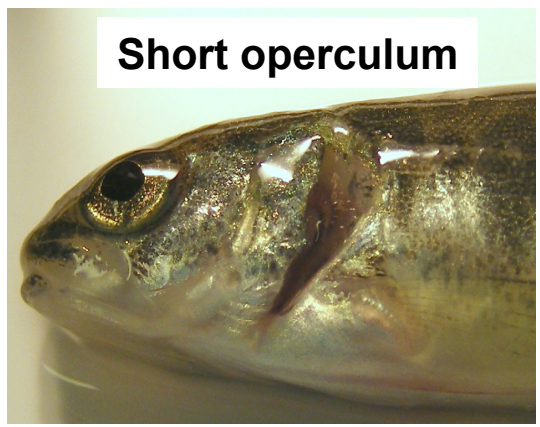
Post-Hatchery Growth 2007 year class







- Triploids higher SGR
- Significant family effect
- Survival 98-99%
- Deformity <4%

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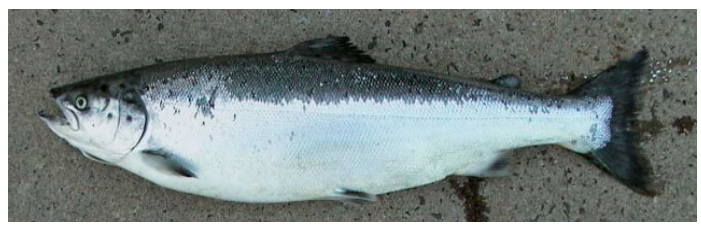
Conclusions

- **Triploids:**
- **Comparable survival**
- **Lower hatch weight**
- **Higher SGR during hatchery rearing**
- **Comparable deformity prevalence**
- **Strong family component**
- **Out of season**
- **Optimal gamete quality essential**
- **Differential gene expression**
  - **Life stage specific**



- **Future of triploid salmon looks promising**
- **Will be essential to monitor long-term performance**

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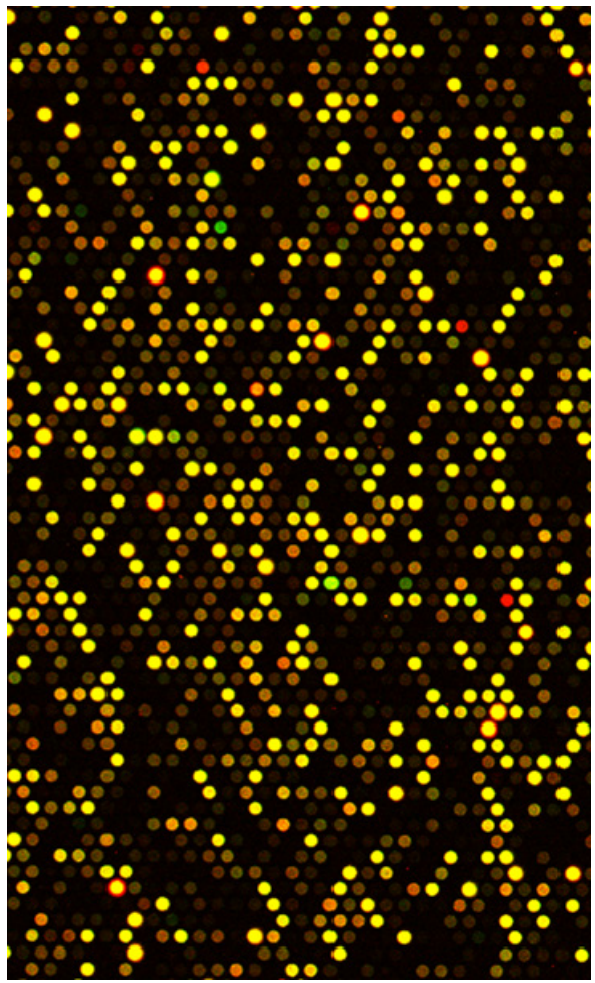
Microarray Analysis

- For microarray, a preliminary study examined two experimental conditions:

1. Yolk sac fry stage - triploids vs diploids for pooled families. 4 replicate pools per ploidy

2. Yolk sac fry and 68 day old fed fry –diploids vs triploids for single family. 3 replicate pools x 2 timepoints (fry / fed) x 2 states (dip/trip)

- Analysis platform was the new 44K probe Salar_1 Agilent oligo microarray designed with colleagues from U.K., Norway and France. This array is considered to be immune probe enriched.



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Microarray Results

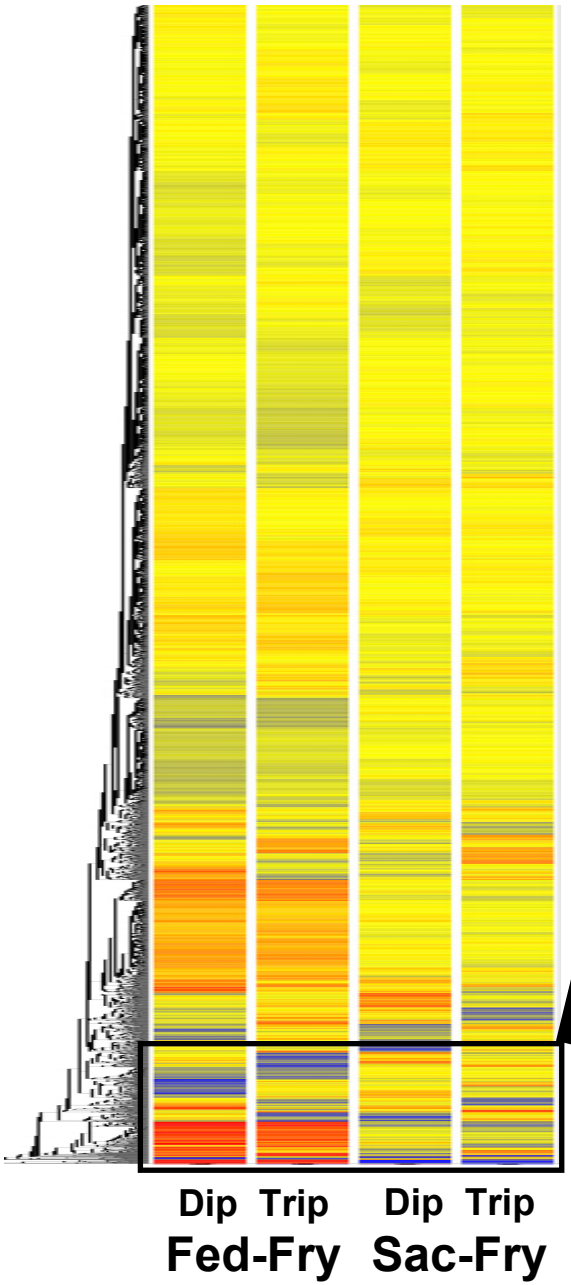
Differentially expressed genes in two preliminary microarray experiments (out of 38,379 features passing quality filters).

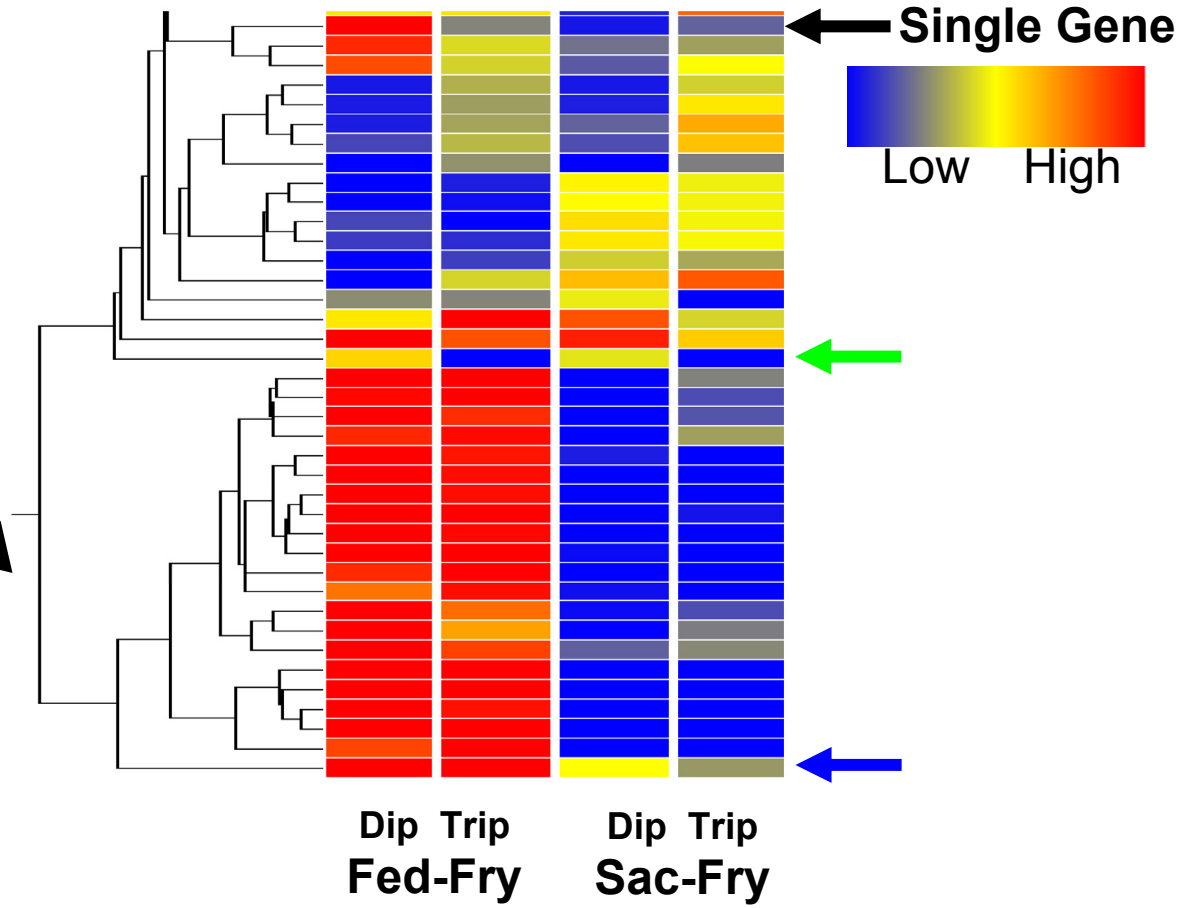
	Dip vs Trip Pooled Families	Fry vs Fed Dip vs Trip
Ploidy effect	2174**	4868*
Ploidy x Fry/Fed		3186*

* 2 way ANOVA p=< 0.05, no False Discovery Rate Correction

** T- test unpaired unequal variance (Welch) p=< 0.05 Fold-Change > 1.2

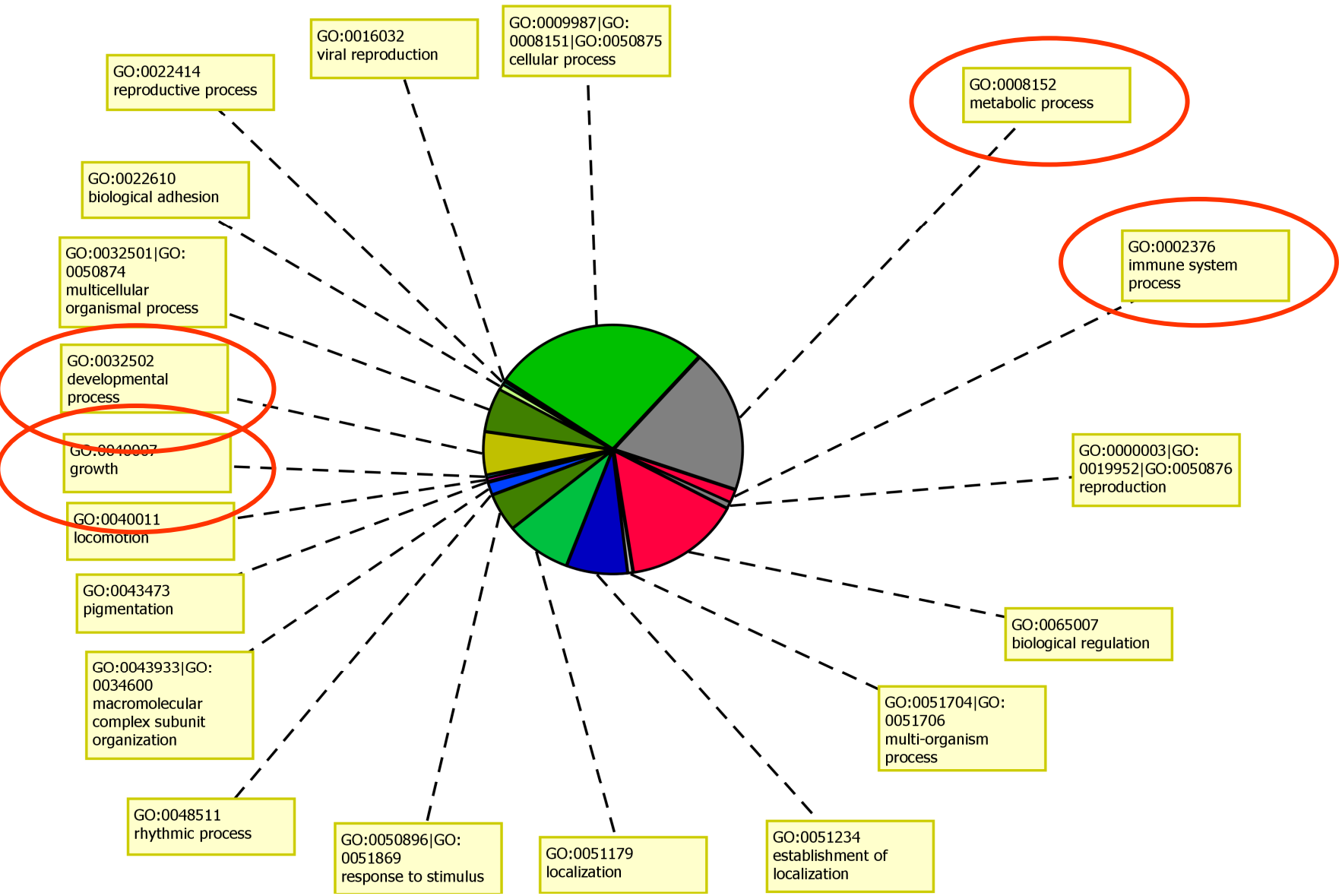
Significantly differentially expressed genes clustered by similar behaviours (High / Low Expression) with respect to Fry Stage and Ploidy





- Fed and Sac Fry express different genes (surprise!!)
- Diploids and Triploids show different expression **within** given states and **according to** state

Biological GO terms for genes found to be differentially expressed between diploid and triploid fed and sac fry.



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Future Directions

- **Future work to focus on:**
  - Family selection programs
  - Nutritional aspects
  - Immune function
  - Physiology & Endocrine function
  - Molecular mechanisms
- **SALMOTRIP**
  - Many components examined
  - Knowledge transfer to industry
  - Protect wild fisheries

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Acknowledgements

- Salmotrip project:
  - Dr. John Taylor
  - Andrew Preston
  - Eric Leclercq
  - Dr. Iain Berrill
- Genomic study
  - Dr. John Taggart
  - Dr. James Bron
  - Dr. Andrew Davie
  - Elsbeth McStay

Salmotrip network

- Tom Hansen, IMR, Norway
- Adriaan Kole, WUR, Netherlands
- Derrick Guy, LNS, Scotland
- Arne Storset, AG, Norway
- Olav Brek, CAC, Norway
- Dougie Hunter, MH Scotland
- Gavin Moss, Salmo, France