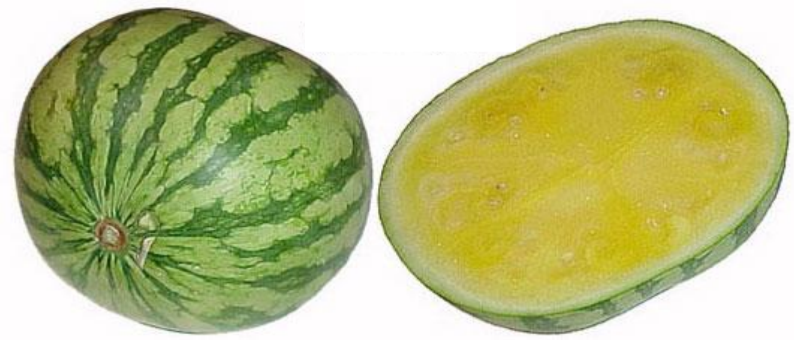
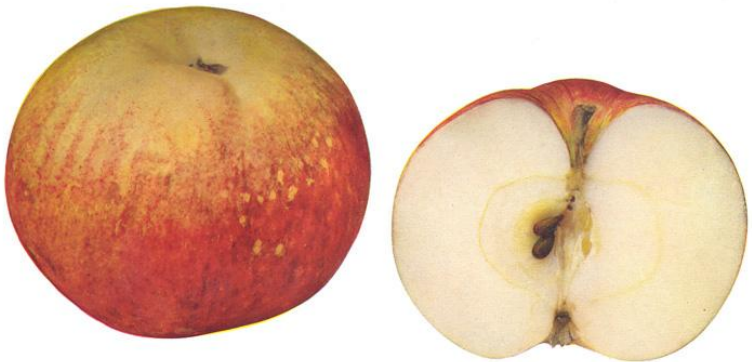
Triploid salmon:
Current knowledge, new concepts and further developments

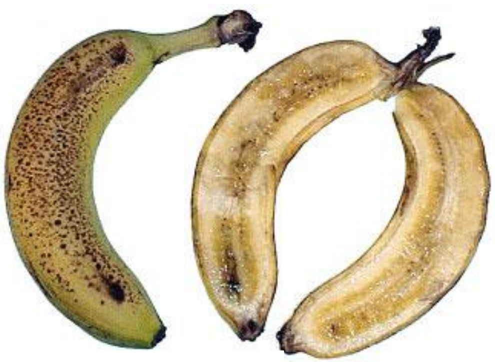
Dr. Herve Migaud

Institute of Aquaculture, University of Stirling





Gravenstein

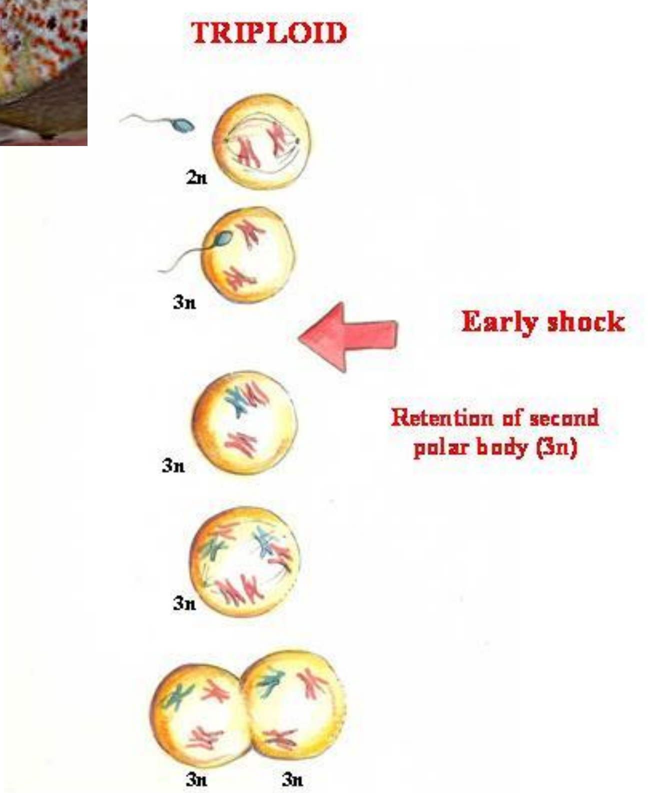


sugar beets, blueberries, bananas, watermelon, and even wheat.

UNIVERSITY OF STIRLING

Headlines

1. Sexual maturation
2. Why triploidy?
3. How is it done?
4. How triploid fish perform?
5. EC 7th Framework Program SALMOTRIP



UNIVERSITY OF STIRLING Why controlling maturation?

Production

- Arrest/Loss of growth
- Changes in flesh composition
- Changes in morphology
- Changes in colour/pigmentation
- Increased incidence of disease
- Death

Environment

- Escapees

Breeding companies

- Protection of IPR

How sexual maturation can be controlled?

- Photoperiod manipulations
- Monosex Production
- Selection
- Triploids = Sterile fish

These management techniques are species specific

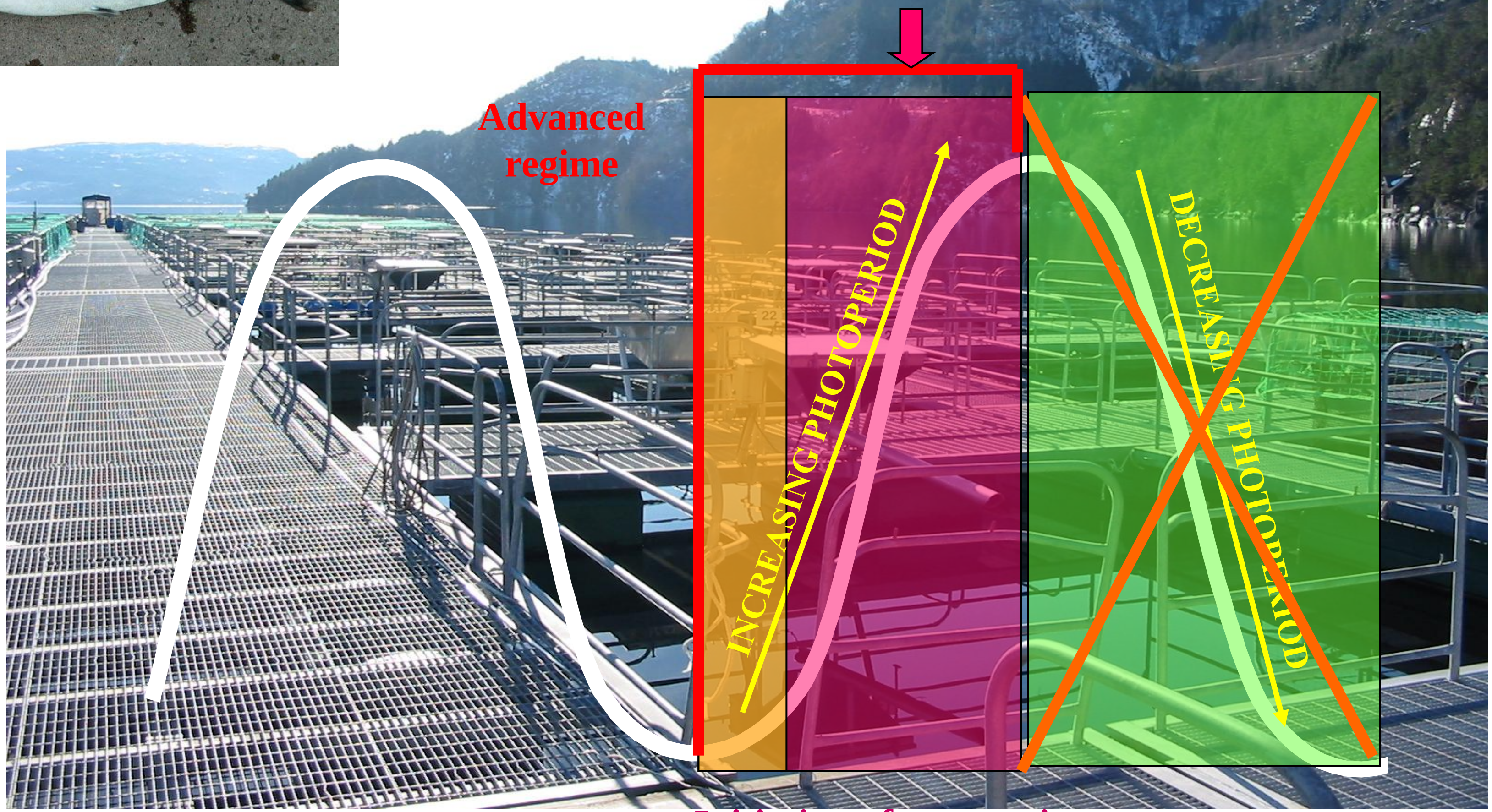
How sexual maturation can be controlled?

Photoperiod manipulations

- Non invasive
- One/both sexe(s) mature prior to harvest
- Seasonal temperate species
- Used commercially routinely in salmon
- Not 100% and expensive

UNIVERSITY OF STIRLING

Photoperiodic inhibition of early maturation in salmon



Window shortened and thus reduced number of fish initiating maturation

Initiation of maturation

(decision)

Gonadogenesis

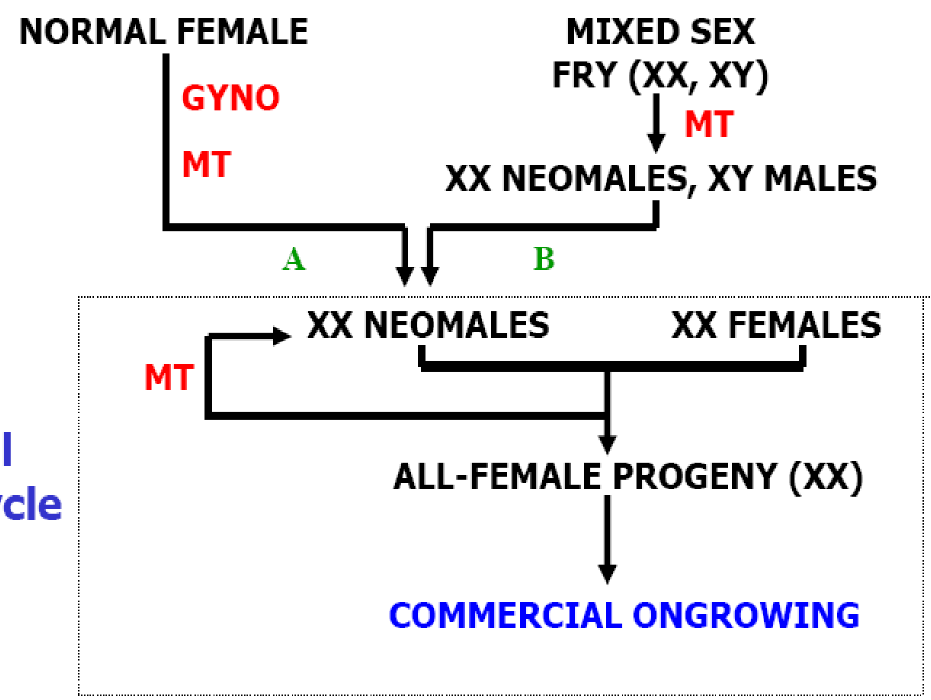
Migaud et al., 2005

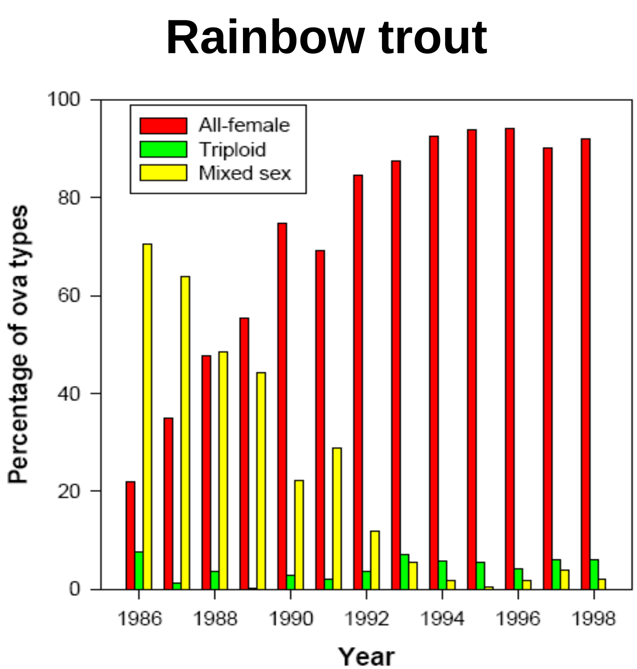
How sexual maturation can be controlled?

Monosex Production

- One sex reaches harvest size before maturation
- Involves hormonal treatment (indirect, consumer/environmental concerns)
- Used commercially in trout for portion size production

Commercial production cycle



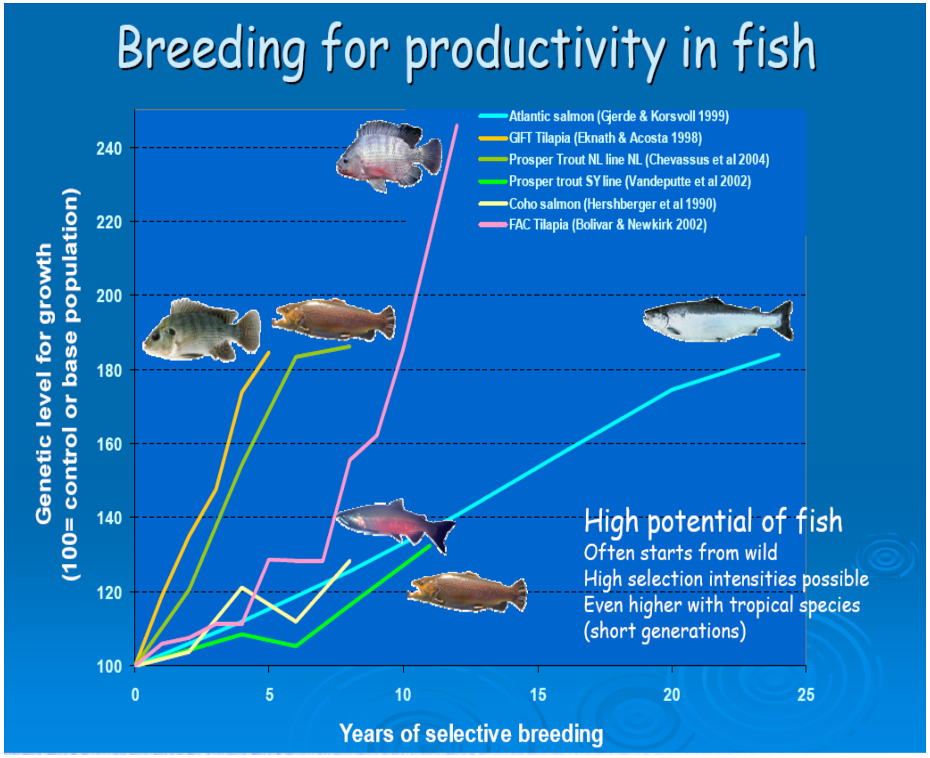


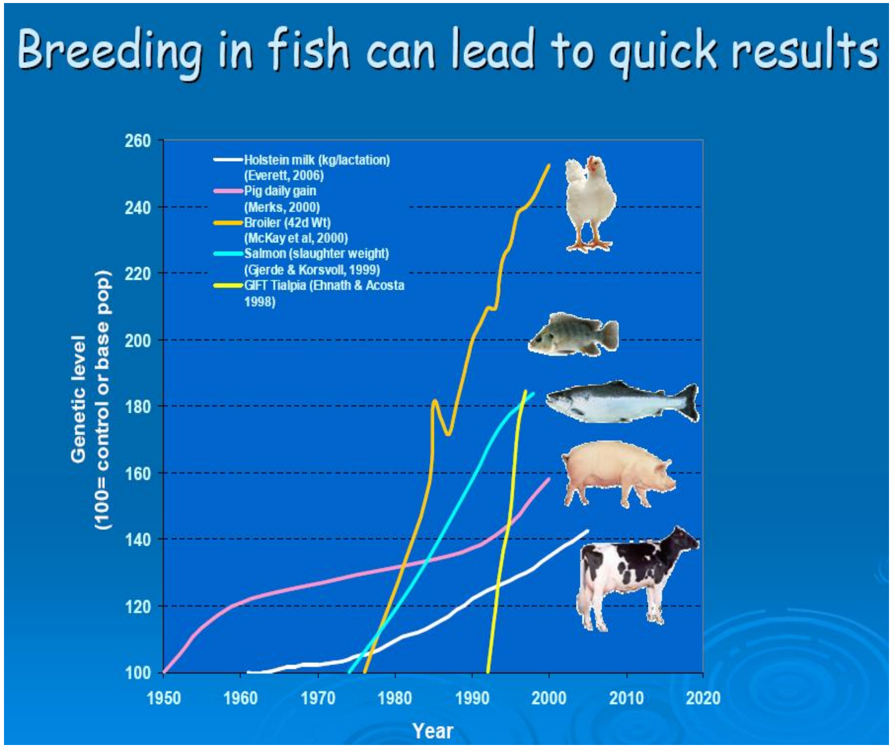
GYNO = meiotic gynogenesis; MT = 17a-methyltestosterone

How sexual maturation can be controlled?

Selection

- **Long expensive process (yearly progress)**
- **Selection for late maturation possible but not for sterility**





How sexual maturation can be controlled?

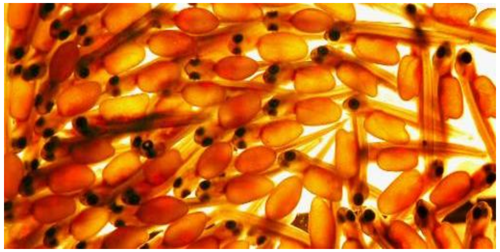
Triploid induction

- Not a new concept (tested in the 80-90s)
- Involves stripping and egg shocking (temperature or pressure)
- Both sexes mature prior to harvest
- Only female fully sterile (males can develop gonads)
- Used in the trout industry (90% of rainbow trout in France is triploid)

Polyploidy in aquaculture

Species	Country	Application
Atlantic salmon	Canada	B, P
Rainbow trout	Canada, France, Japan	B, P
Sea bass	(France)	I
Sea bream	-	-
Atlantic cod	-	-
Meagre	-	-
Turbot	(Spain)	I
Halibut	(France)	I
Sole	-	-
Grass carp	USA	B, P*
Nile tilapia	-	-

Polyploidy applied in breeding programs (B), practical culture (P) or initial work (I).
*In the USA. In China, still experimental



UNIVERSITY OF STIRLING

Why triploidy in salmon farming?

At present triploidy offers the only “commercially acceptable“ means of sterility

Sterility would:

- Alleviate the negative impact that escapee fish present to the environment,
- Improve fish welfare and performances during on growing,
- Give a mean to protect domesticated stocks for salmon breeding companies
- Overall make the salmon industry more sustainable.

Data generated from this project will also aid legislative decision making regarding future aquaculture policies and the use of triploidy within the salmon industry.

Directive 90/220/CEE, April 1990

Increased public awareness of escapee potential impacts

LIVE LI BBC NEWS CHANN

Last Updated: Monday, 20 October,

E-mail this to a friend

Farm threat to wild s

By Helen Briggs
BBC News Online science reporter

Repeated escapes of farmed endangered populations of w extinction, say scientists in t

There has been concern over tl domesticated salmon are breedi changing the genetic make-up ability to survive in the natural

Until now, there has been little direct scientific evidence but, according to a report published in the journal Royal Society Proceedings B, the fears of environmentalists may be justified.

In a 10-year study, researchers from Ireland, Northern Island and Scotland, found that wild salmon were vulnerable to extinction becaus pressures from farmed fish.

Experiments with wild and farme marine water showed that the interbred had a much lower sur fish died in the first few weeks

Overall, farmed salmon were mu in the wild compared with nativ return to rivers to spawn.

Fisheries and Oceans Canada | Pêches et Océans Canada | Canada

Français | Contact Us | Help | Search | Canada site
DFO Home | What's New | About Us | Media Room | Info for:
FAQs | Fast Facts | Site Map | Regions
Science Home | Publications | Facilities | Career | Links

FISHERIES AND OCEANS CANADA
Biotechnology

Science Information By:
- A-Z Index
- Activity
- Audience
- Organization

Science Stories
- Feature Articles
- Subscribe to our Feature Article mailing list

Find Info on:
- Centres of Expertise
- Science Awards

Biotechnology to Help Protect Wild Salmon Stocks –

The Triploid Approach

Salmon farming is the largest sector in Canada's growing aquaculture industry. One of the challenges facing Atlantic salmon aquaculture development, however, has been preventing farmed fish from escaping into the wild. The worry on both the east and west coasts, is that the escaped salmon will interbreed with wild salmon, which could alter the genetic makeup of these stocks as well as their ecological interactions.

One technique being looked at to address these concerns is the use of triploid salmon (see also 'Biotechnology to help Protect British Columbia's Wild Salmon Stocks – the All-Female Approach'), i.e., salmon with three sets of chromosomes (the threads of DNA that carry genetic information) instead of the normal set of two. The extra set of chromosomes prevents development of viable eggs or sperm so, if the triploid fish escape, they can't reproduce. Triploid salmon are occasionally found in wild and cultured populations, and are relatively easy to mass produce.

What is the issue?

One problem with triploid salmon is that some show deformities, such as lower jaw defects, slower growth and higher mortality than their normal (diploid) relatives. This has tended to discourage the aquaculture industry from using triploid salmon.

DFO researchers and their partners are working to discover the cause(s) of these undesirable traits, in order to find ways to fix or minimize them. Because triploid salmon may escape, scientists will also assess how triploid salmon perform under wild environmental conditions, e.g., do they show mating behaviour or competition for other resources.

The research plan

Researchers think that triploids have more defects because their nutritional requirements may be different than those of diploid salmon. They are, therefore, studying the total energy, protein, fat, mineral and vitamin profiles

---

Health

IOLOGY | SCIENCE | HEALTH | SPORTS | OPINION

ARCH | FITNESS & NUTRITION | MONEY & POLICY | VIEWS

ıeering Comes to the Rescue

E-MAIL
PRINT
REPRINTS
SAVE
SHARE

d scientists dedicated to ıgh predictions of juicy s consisting entirely of ıce that genetic disguised and cheerfully as we know them.

ent appearance on their ılmon that can be rendered sterile, cheaply embryonic, egg stage of their development. If cial aquaculture industry can be persuaded to ficant threats to the dwindling, stressed stocks the genes of domestic and wild fish -- can be

l Whoriskey, vice president of research and tion, "in that taking the genetic, reproductive a cost-effective way would really protect wild he salmon farming industry."

agic bullet sought by a conservation n once by false hopes.

accelerating the viability and growth of fish- eil of extinction slowly closing around wild

Farmed/wild salmon interactions

UNIVERSITY OF STIRLING

Only few studies done but strong evidence that farmed salmon can interact with wild stocks

- **Migration behaviour of 2n/3n salmon using microtagged fish released (Cotter et al., 2000, Ireland)**

  - 2.25% of the mixed sex diploid (0.6 for triploid) returned to the river system where released as smolt and 7% to the coast (1.6 for triploids)

  - None released at cage site returned to the river system but 5 to 9% returned to the coast

- **Return of farmed salmon escaped as juveniles in freshwater (Lacroix and Stokesbury, 2004, Canada)**

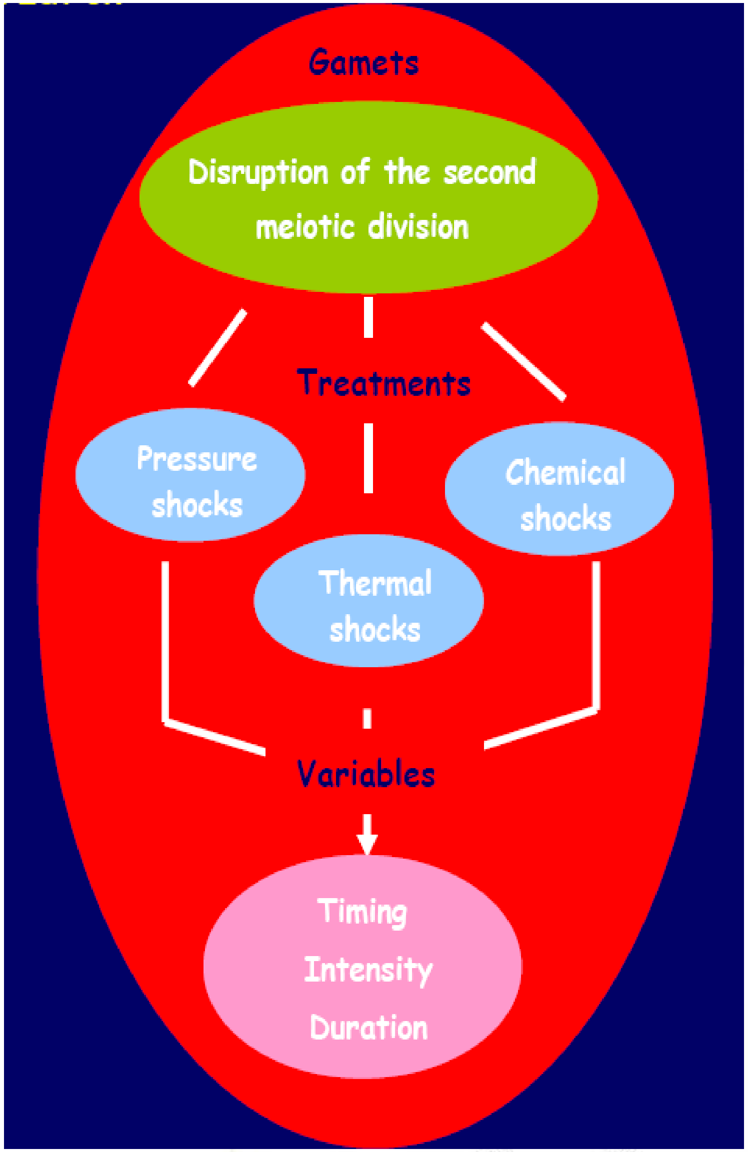
  - Adult returns to the river system was 57% farmed fish escaped from sea cages, 34% wild fish and 9% farmed fish escaped as juveniles from the hatchery

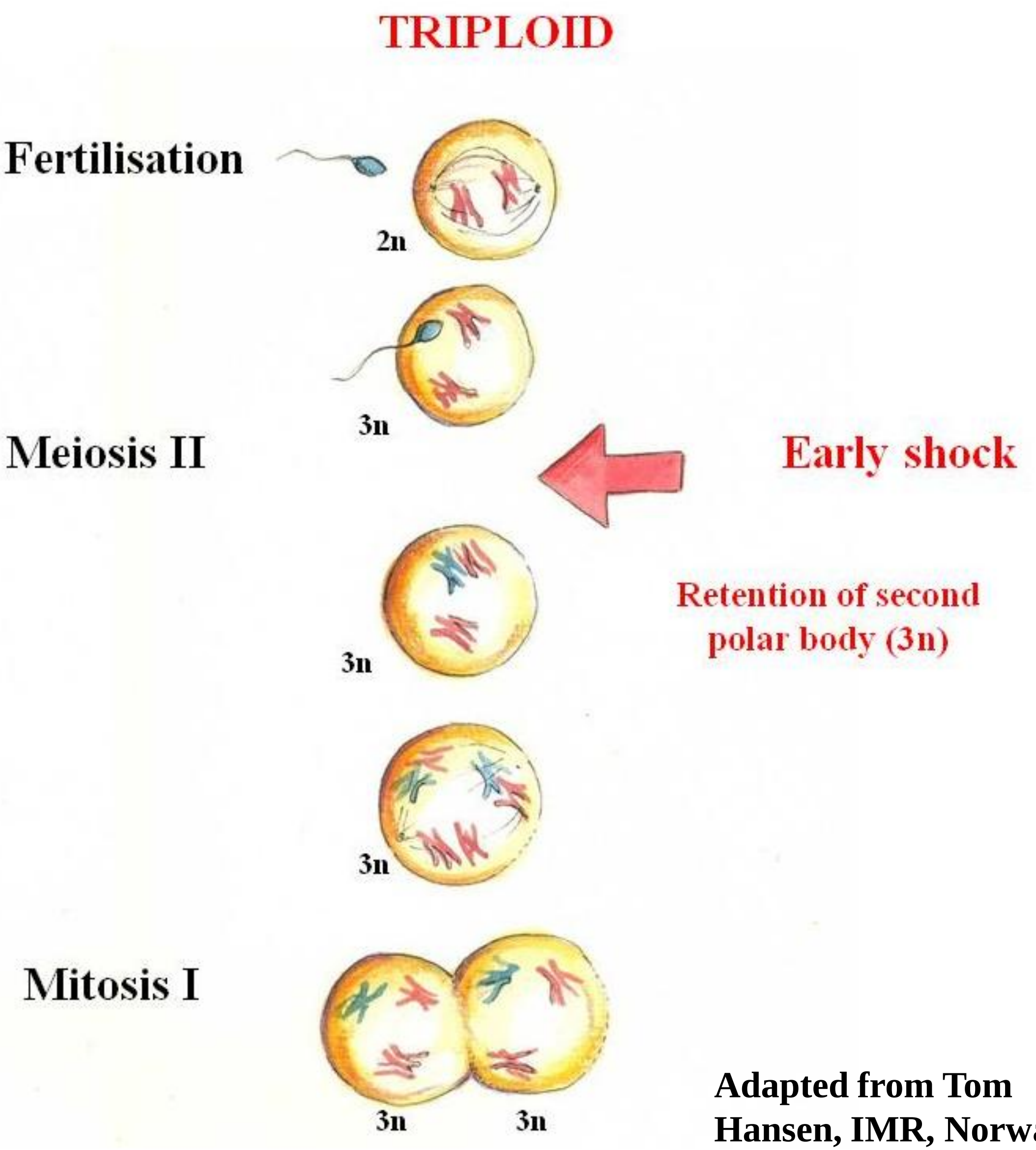
Genetic assignment can identify farm of origin for salmon escapees (Glover et al, 2008, Norway)

- Genotyping (15 microsatellites loci) from 7 cage sites and 29 escapees

- 21 escapees from one site

How to produce triploid?



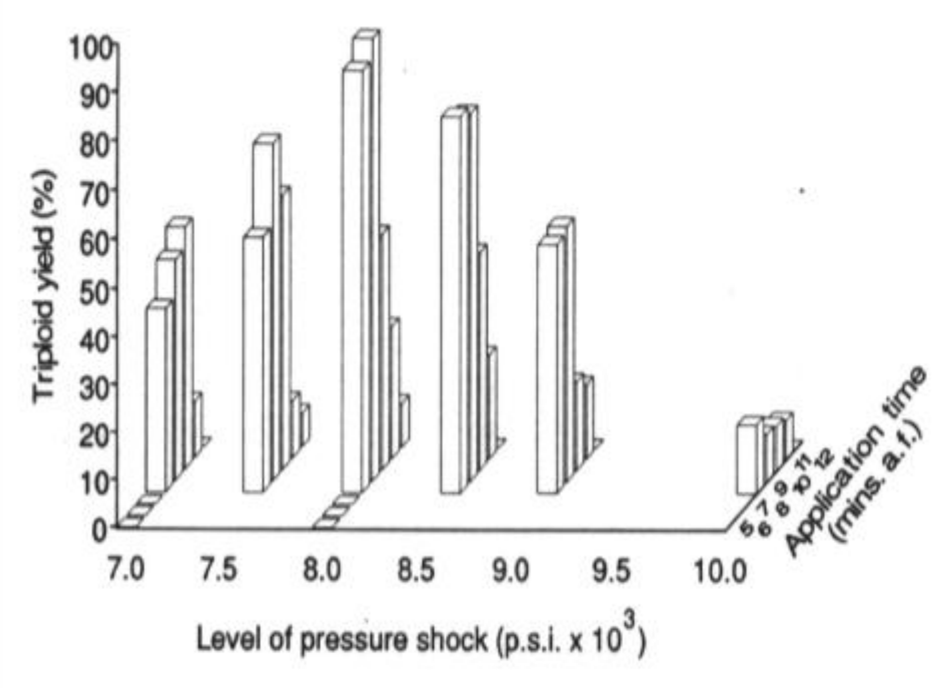


Adapted from Tom Hansen, IMR, Norway

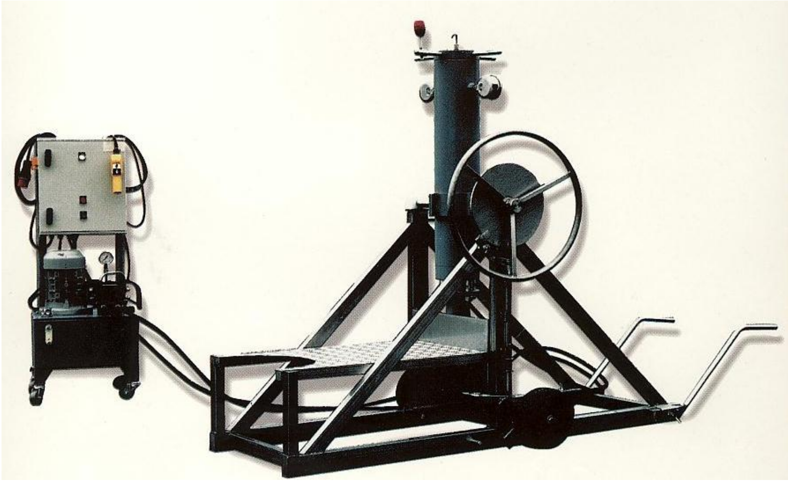
Pressure shock

Three main variables (temperature dependent):

- **Time of application after fertilisation (WHEN?)** **at 10º C 30 MPF (300ºmins)**
- **Level of pressure (HOW MUCH?)** **9500 psi**
- **Duration of the shock (FOR HOW LONG?)** **5 mins**



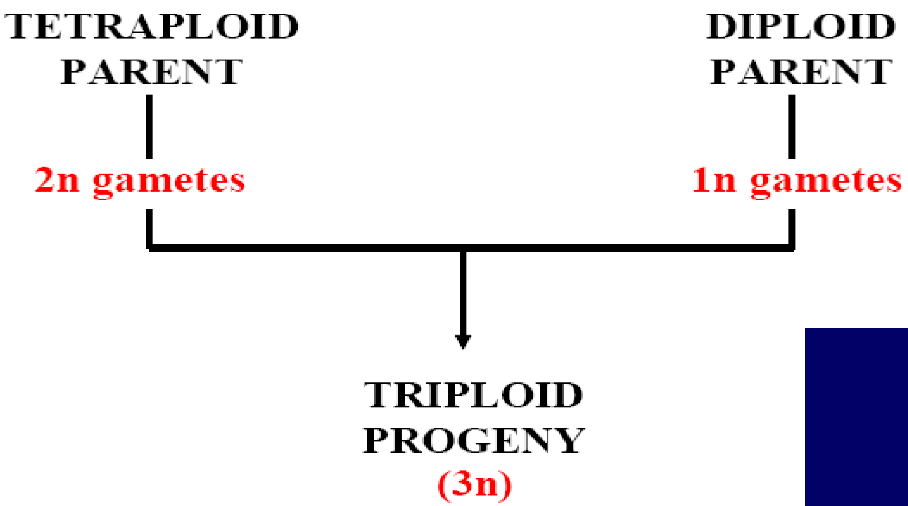
Benfey *et al*., 1988



UNIVERSITY OF STIRLING

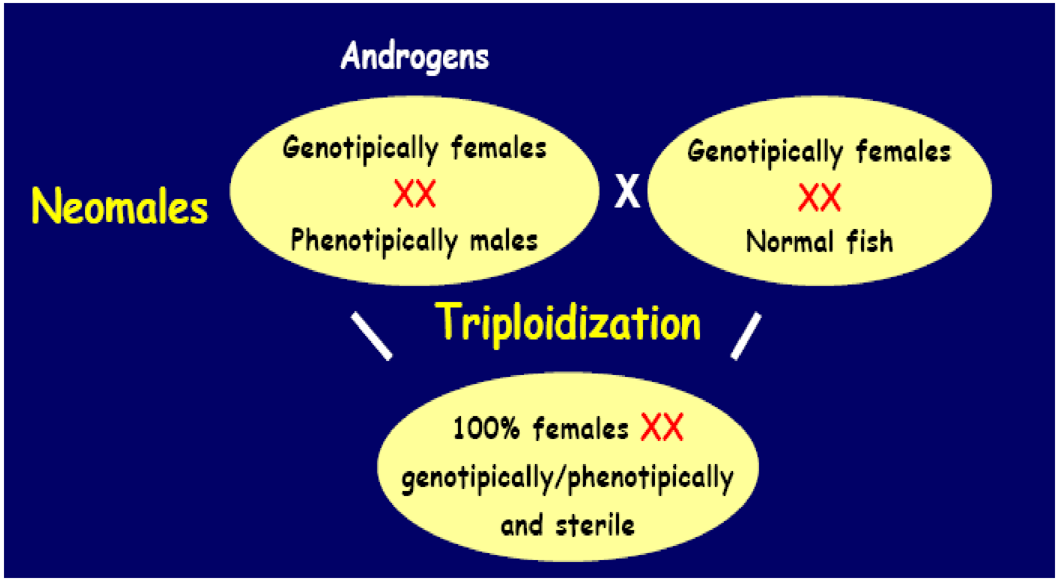
How to produce triploid?

Triploids from tetraploid x diploid crosses



But not successful in most species due to very low viability of tetraploids

Production of all female triploids

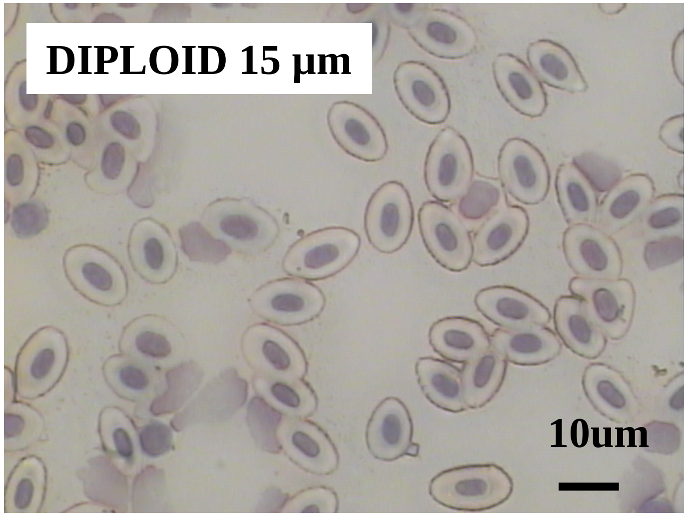


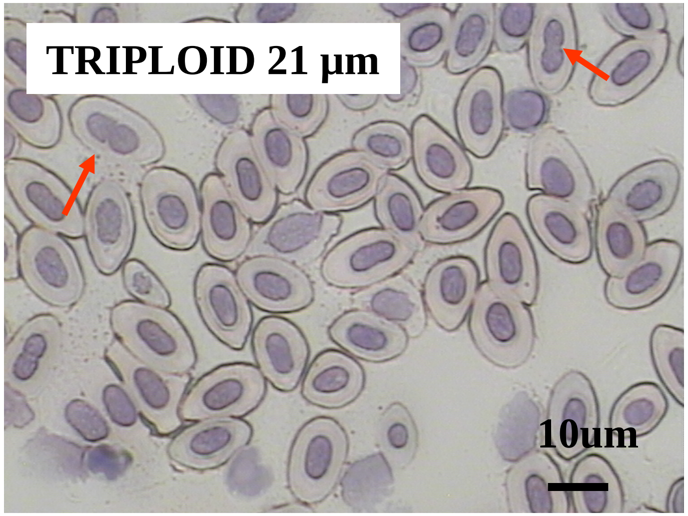
Triploid confirmation: Flow cytometry

UNIVERSITY OF STIRLING

Measures the fluorescence of stained DNA

RBC SMEARS

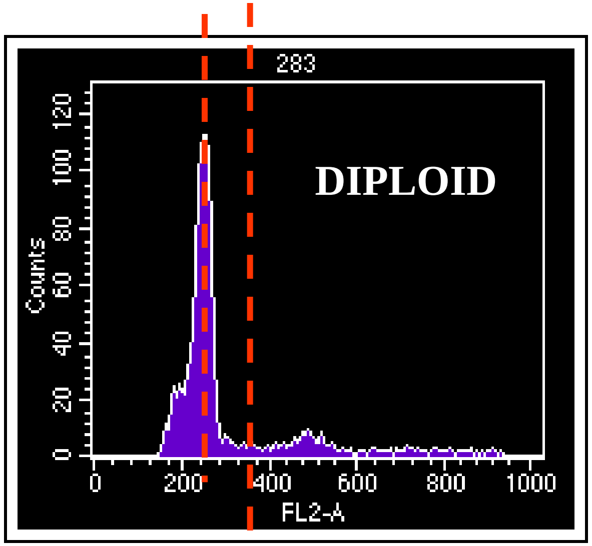


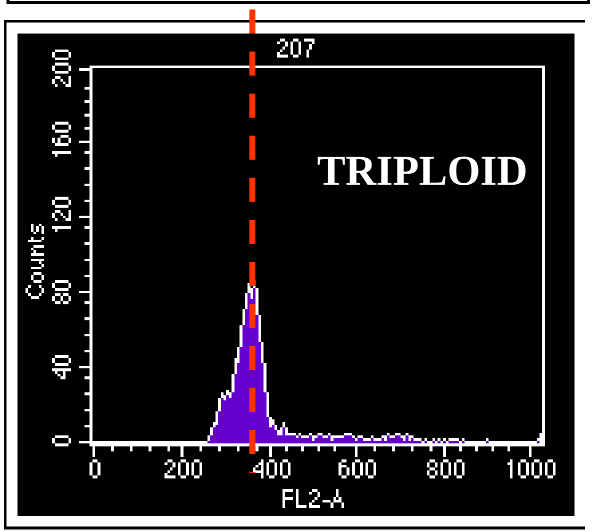


30 individuals per ploidy per family assessed

100% triploid rate in fish processed so far

FLOW CYTOMETRY



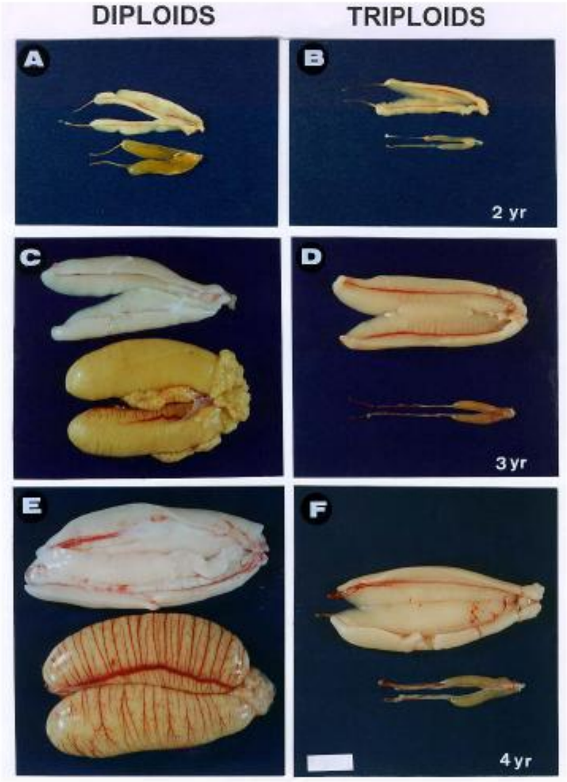


Gonadal development

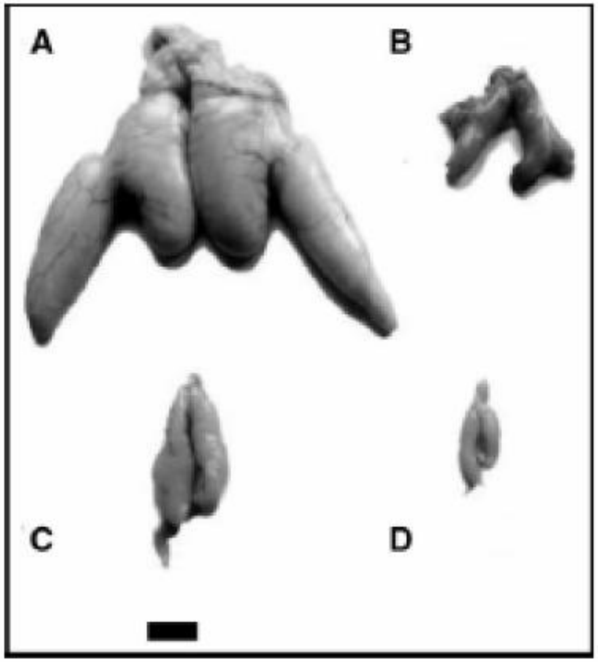
TRIPLOIDY EFFECTS

✓ Evaluate triploid performance in terms of reproduction

Felip *et al.* (2001)



Cal *et al.* (2006)



Degree and permanence of gonadal sterility in triploids

UNIVERSITY OF STIRLING

Triploid performances

Five key areas of concern have been identified within the literature and industry reports:

1) **Higher mortalities,**
2) **Tolerance of sub-optimal environments,**
3) **Morphological deformities,**
4) **Poorer growth performances and**
5) **Consumer and business-to-business market issues with respect to the communication and acceptance of new technologies.**

Mortalities

- **Triploidy previously associated with higher mortality**
  - **First-feeding**
  - **Seawater entry**

- **More recent studies (<1.5% Oppedal *et al*., 2003)**

- **Possible reasons:**
  - **Cellular physiology**
  - **Extreme environments**
  - **Incomplete smoltification**
  - **Inappropriate diets**
  - **Stock specific deformities**
  - **Communal rearing**

Tolerance of sub-optimal environments

- **Cells and tissues have lower surface area: volume ratio**
  - Active transport of ions?
  - Blood oxygen capacity?
  - Hormonal signalling?
  - Immune function?

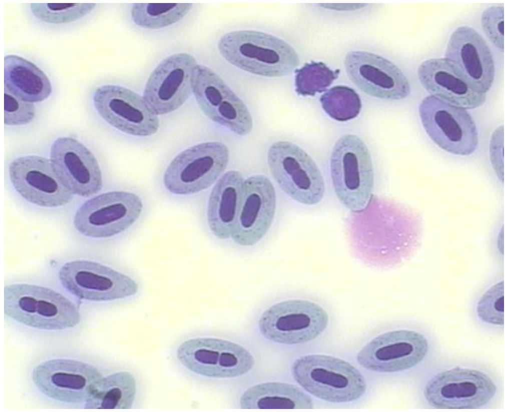
- **Potential Gill Deformity**
  - Osmoregulation?
  - Gas exchange?

- **Minimum size for successful smoltification?**
  - Correlation Size vs. SW survival

- **Metabolic Physiology 2n = 3n**

  - problems at extreme environmental range

RBC



Shortened gill filaments

UNIVERSITY OF STIRLING

Slower Exercise Recovery

Hyndman *et al*., 2003

Triploid Mortality 90%

No reduction muscle Phosphocreatine (PCr) in 3n

= anaerobic pathway problems

= temperature limiting biosynthesis

Slower muscle ATP recovery in 3n

High temp = rate limiting

Lower O2 consumption = rate limiting

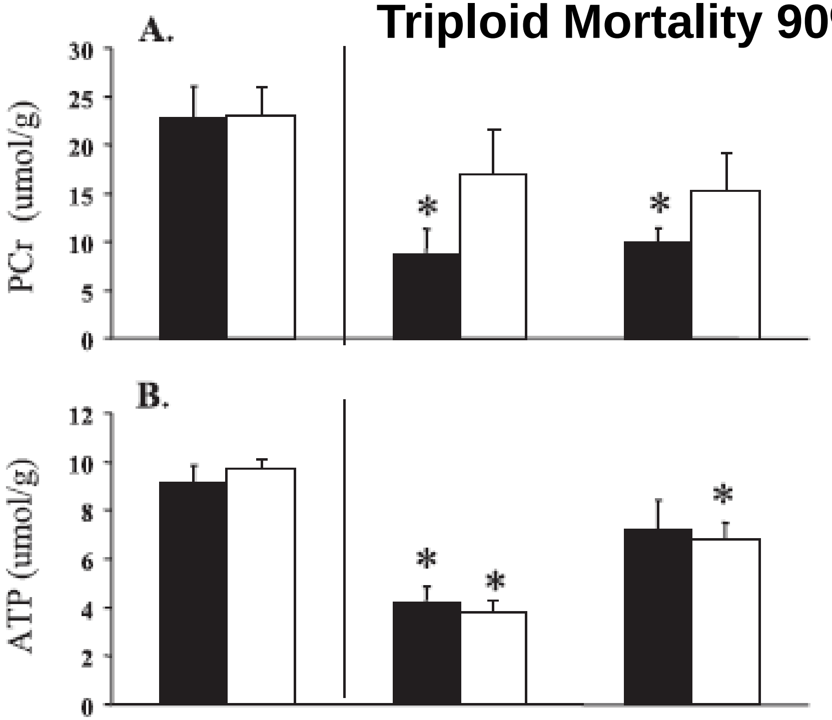


Fig. 3. White muscle PCr (A), ATP (B) and glycogen (C) of diploid (black bars) and triploid (open bars) brook trout before and after 5 min of exhaustive exercise (dashed line). (N=8 for triploids at all times, whereas N=9 for diploid controls, N=7 for diploid 0 h and N=5 for diploid 2 h.) An asterisk (*) denotes a significant difference from the control and the plus sign (+) denotes a significant difference between ploidies within the time interval; $p<0.05$.

Anaerobic capacity of triploids maybe lower but results not conclusive

Effect not apparent with "normal" environmental range (9°C = no mortality)

Lower metabolic rate at higher temp (Atkins & Benfey 2008)

Osmotic stress

Freshwater (<5 mOsm kg^{-1})

- ***Osmotic influx***
- ***Ionic efflux***
- ***No drinking***
- ***Dilute urine***
- ***Active uptake of ions***

Seawater (~1000 mOsm kg^{-1})

- ***Osmotic efflux***
- ***Ionic influx***
- ***Copious Drinking***
- ***Concentrated urine***
- ***Active extrusion of ions***

Freshwater Parr

Saltwater Smolt

Internal Fluids (~300-400 mOsm kg^{-1})

- ***Must be maintained***
- ***Ionic/osmotic balance***
- ***Acid/base balance***
- ***Normal cellular function***

UNIVERSITY OF STIRLING

Seawater Adaptation – *Acute Responses*

Seawater Transfer

Osmoregulation?

- *Cortisol & IGF-I*
- *Increased chloride cell size and number*
- *Increased Na^+, K^+, ATPase activity*

Immune?

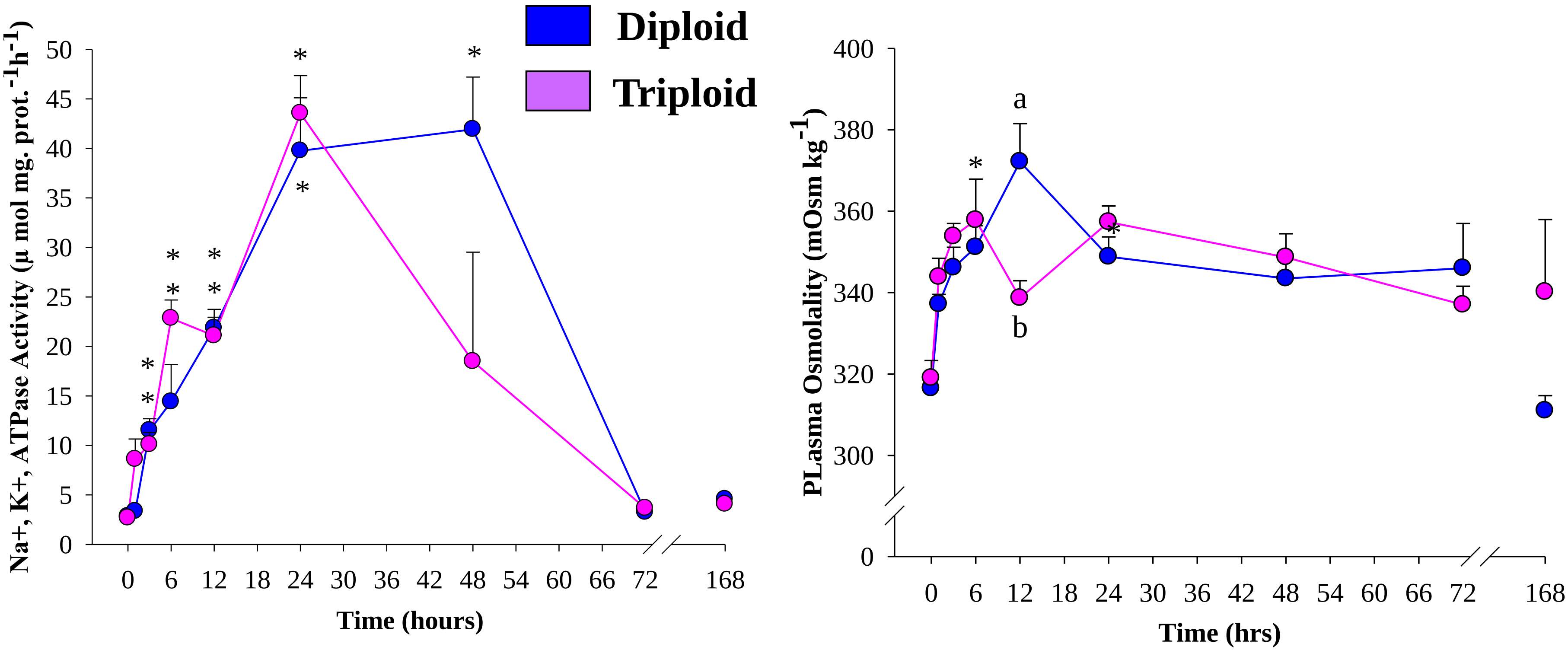
- *Cortisol*
- *Non-specific immune system?*

Stress Response?

- *1º Cortisol*
- *2º Glucose*
- *2º Haematology*

UNIVERSITY OF STIRLING

Smoltification: ATPase Function



190g rainbow trout 3n / 2n = 95-98 % SW survival

100g rainbow trout 3n = 0% 2n = 90 % SW survival

Clear importance of size for SW Tolerance ~ cellular function & ion transport

Taylor et al., 2007

UNIVERSITY OF STIRLING

Triploid concerns

Five key areas of concern have been identified within the literature and industry reports:

1) **Higher mortalities,**
2) **Tolerance of sub-optimal environments,**
3) **Morphological deformities,**
4) **Poorer growth performances and**
5) **Consumer and business-to-business market issues with respect to the communication and acceptance of new technologies.**

Morphological deformities

- **Numerous deformities reported**

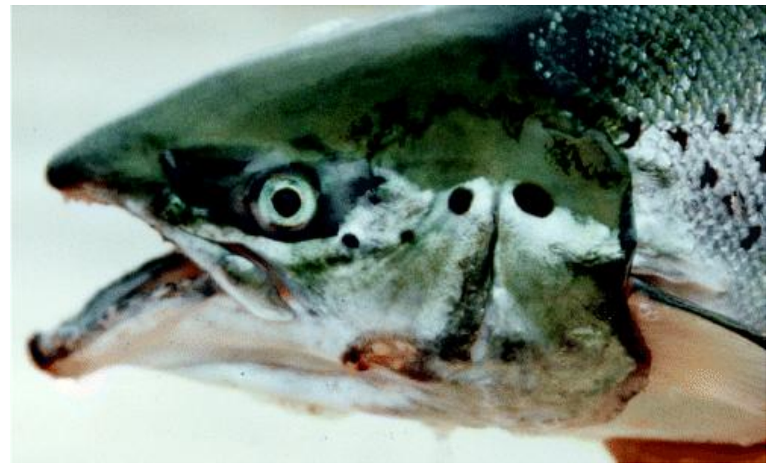
Lower Jaw, Cataract, Short Operculum, Compressed Spine, Reduced no. Gill Filaments

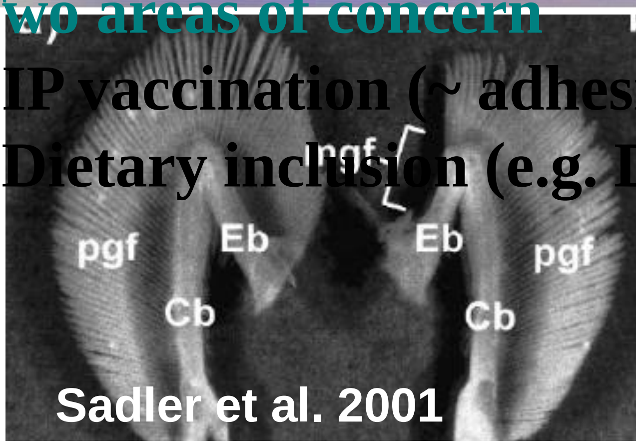
Stock or strain specific?
Genetic component?
More prevalent in saltwater?

Two areas of concern

- IP vaccination (~ adhesions & d[illegible]rmity)

- Dietary inclusion (e.g. Dietary P[illegible] skeletal deformity)





Sadler et al. 2001

Typical Deformities Observed pre-first feeding

Kyphosis

Lordosis

Scoliosis

Spiral Tail

Siamese

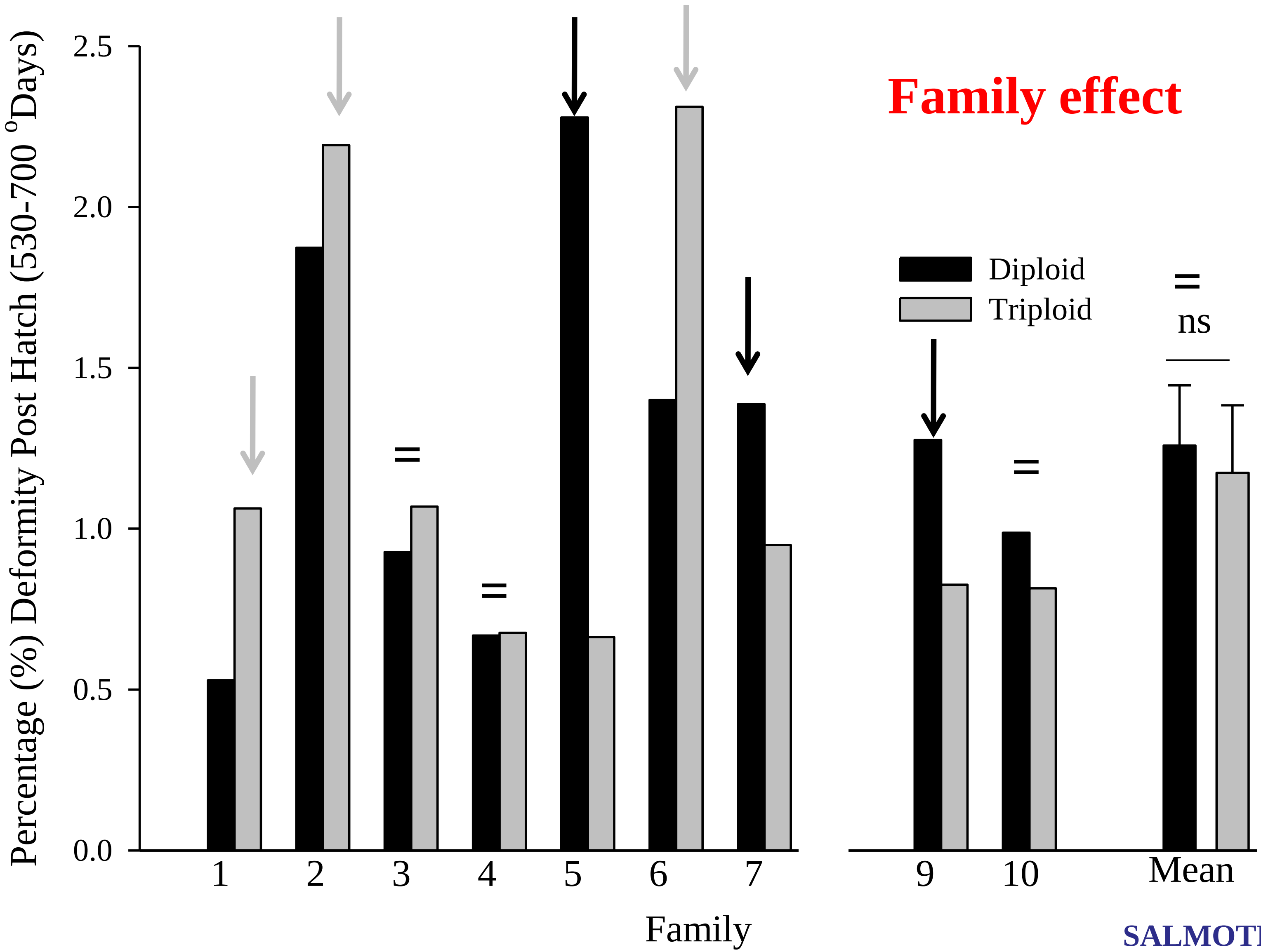
Twin-Head

NB: All deformities non-cranial pre-first feeding

All deformities are lethal

UNIVERSITY OF STIRLING

Percentage Deformity Post-Hatch within morts (Pre-1st Feeding)



SALMOTRIP project

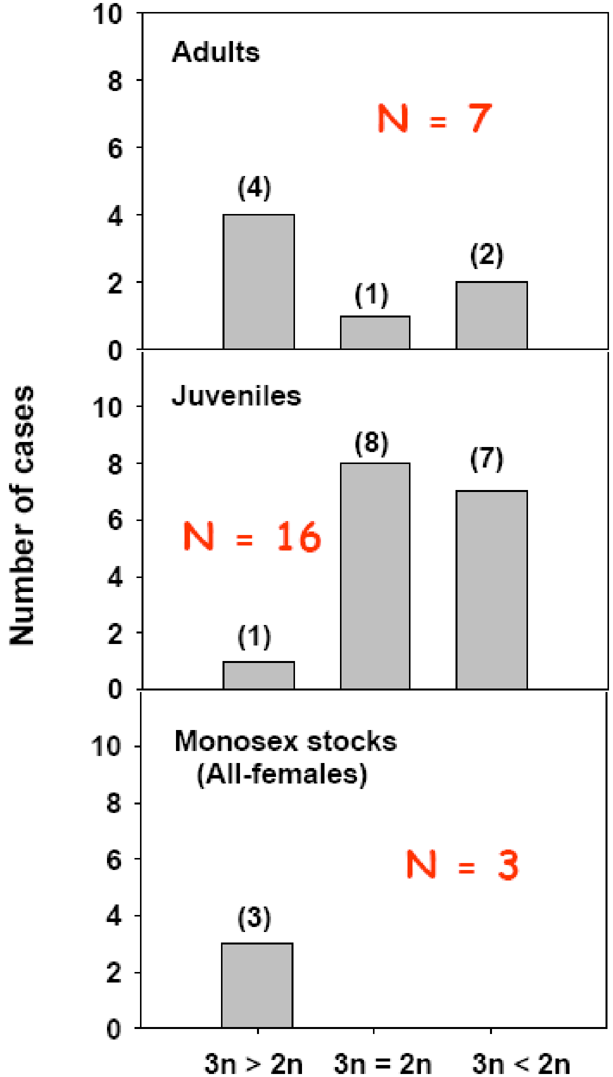
UNIVERSITY OF STIRLING

Triploid concerns

Five key areas of concern have been identified within the literature and industry reports:

1) **Higher mortalities,**
2) **Tolerance of sub-optimal environments,**
3) **Morphological deformities,**
4) **Poorer growth performances and**
5) **Consumer and business-to-business market issues with respect to the communication and acceptance of new technologies.**

Growth performances



Felip (2000)

Performance of triploids is species specific

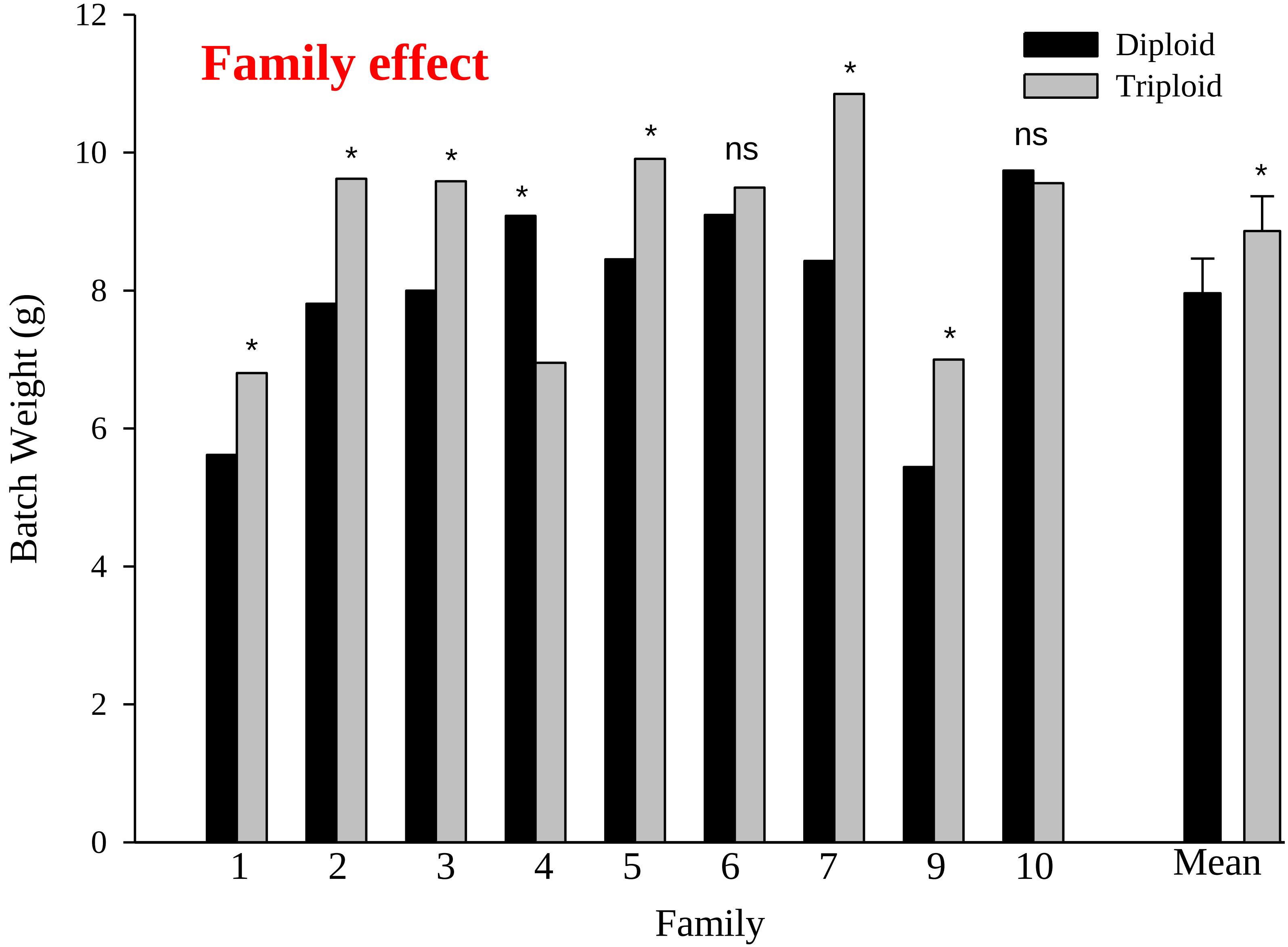
Species	Growth	Reference
Ictalurus punctatus	+	Wolters *et al.* (1982)
Oreochromis niloticus	+	Bramick *et al.* (1995)
Heteropneustes fossilis	+	Tiwary *et al.* (1997)
Oreochromis aureus	+	Valenti (1976)
Silurus glanis	+	Krasznai & Marian (1986)
Oncorhynchus mykiss	-	Solar *et al.* (1984)
Lepomis gibbosus	-	Kerby *et al.* (1995)
Oncorhynchus kisutch	-	Withler *et al.* (1995)
Salmo salar	-	Galbreath *et al.* (1995)
Misgurnus mizolepis	NC	Kim *et al.* (1994)
Salmo salar	NC	McGeachy *et al.* (1995)
Oreochromis niloticus	NC	Hussain *et al.* (1995)
Oreochromis aureus	NC	Chang *et al.* (1993)

(+) positive, (-) negative, (NC) no change

Basant *et al.* (2004)

UNIVERSITY OF STIRLING

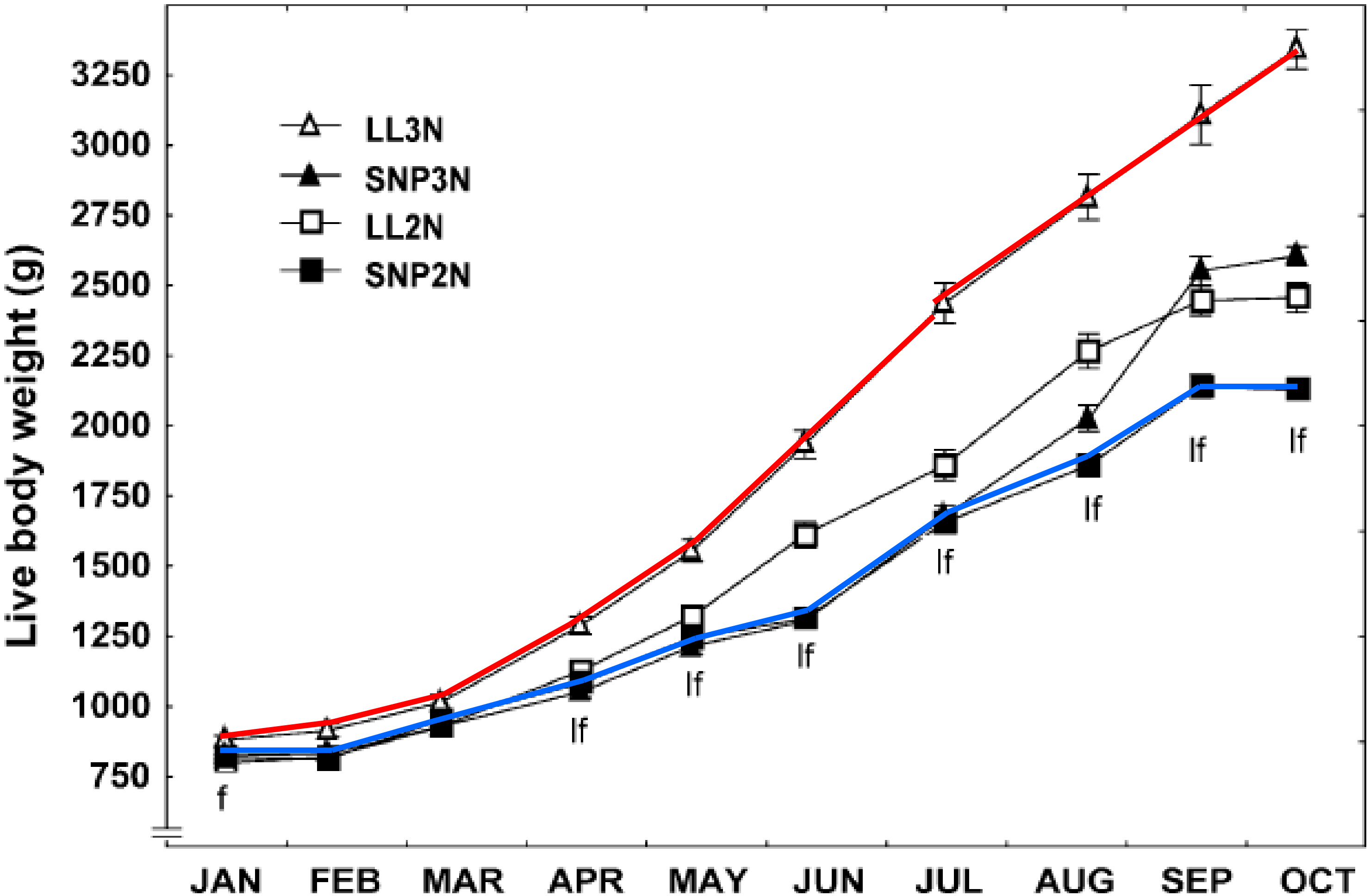
Parental Contribution to Growth



ect

UNIVERSITY OF STIRLING

Growth performances



Oppedal, Taranger and Hansen, 2003

Consumer/market perception?

Can triploid salmon be marketed? What price?

1. **Triploid is not a GMO**
2. **Sensory analyses/flesh quality studies did not reveal any differences between 2n/3n**
3. **Triploid oyster are already on the market (sold as “sterile” or “Four seasons oysters”, rainbow trout (80% of the French production), brown trout for restocking and salmon already in Canada.**
4. **Certification for organic triploid already in place in France**
5. **Country specific?**
6. **Need for consumer / market perception studies in the EU prior to any implementation**

Confusion between triploid and GMO

In this issue »
Editor's Notebook »
Inside Story »
Reverb-erations »
Explorer »
À la Carte »
Mosaic »
On the horizon »

Online exclusives »
Subscribe! »
Archives »
Geography Resources »
Site Map »
CG Home »

MAGAZINE CG In-depth

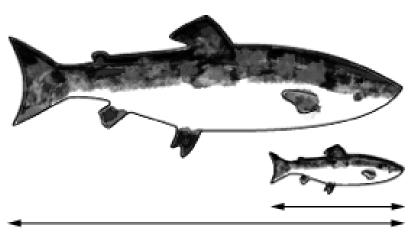
Salmon farming

Home | Feature | Frankenfish | Land-based aquaculture | St. George, N.B. | Sport fishing | International lessons | Timeline | How to cook great salmon | Games | Map | Quiz | Books | CG vault | Re:sources

Frankenfish: Just another monster?

Genetic technology has entered aquaculture, but not many are catching the wave

Story by Dan Blouin



At a 30 percent growth rate faster than wild salmon (bottom), "frankenfish" (top) are bigger and stronger at a younger age, raising the question as to whether or not non-genetically modified fish would have a chance in the wild

Often dubbed "Frankenfish," transgenic salmon have had DNA segments spliced into their cells — for instance, genes coded for anti-freezing proteins or growth hormones. Absorbed by the fish's eggs, these stowaway genes enable the salmon to grow faster and to survive in colder water. But the resulting transgenic fish haven't been approved as food by Health Canada or the FDA.

Transgenic salmon may appear to be the best thing since tartar sauce for the business of aquaculture, but so far it seems fish farmers and consumers alike just aren't taking the bait.

Fullført

SALMOTRIP

FEASIBILITY STUDY OF TRIPLOID ATLANTIC SALMON PRODUCTION
2008 - 2010

LEAD CO-ORDINATOR Dr Herve Migaud, Dr. John Taylor

RTD Partner: IMR, Tom Hansen, Norway

WUR, Dr Adriaan Kole, Netherlands

SME Partner: UK, Ireland and Norway

DELIVERABLES: (experimental)

- **Transfer of triploid induction technology to SMEs**
- **Strengthen knowledge on triploid biological and culture requirements**
- **Advance knowledge of the smolt process and monitoring**
- **Provision of triploid specific smoltification regimes**
- **Develop a welfare scheme for triploid fish**
- **Define parentage contribution to performance based on ploidy**
- **Identify perceived risk-benefit of triploidy and define marketing strategy**

UNIVERSITY OF STIRLING

Key questions addressed by SALMOTRIP

Salmotrip focuses on four key areas of innovation:

- **Smoltification**

Are there specific requirements of triploid fish to successfully undergo smoltification? **Can out of season S0 triploid be produced?**

- **Triploid Welfare**

What is the sensitivity of triploid fish to suboptimal environmental conditions throughout the production cycle? **Overall, in which conditions triploid fish should not be produced commercially?**

- **Selection Programs**

What is the family effect on triploid performance? i.e. **is there a need to select fish for triploidisation?**

- **Market Perception**

How is triploid salmon production perceived within Europe and how can market acceptance be optimised? i.e. **how to potentially market such a fish as compared to diploids?**

UNIVERSITY OF STIRLING

Take home messages

- **Triploid is not a GMO**

  Confusion between <u>genetic modification /</u> genetic <u>manipulation</u> (can be found in wild stocks)

- **Triploid is the only available “commercially acceptable“ means of sterility**

  Can help to address the environmental impact of escapees, detrimental effects of maturation on flesh quality, to protect IPR for breeding companies ….. and overall maintain a <u>sustainable and eco-friendly industry</u>

- **Triploidy is already commercially used**

  Fruit/vegetable, oyster, trout

- **Triploids can perform as well as diploids**

  When reared in optimal conditions from good quality eggs and selected stocks

- **Further research is needed**

SEVENTH FRAMEWORK PROGRAMME

INSTITUTE OF MARINE RESEARCH
HAVFORSKNINGSINSTITUTTET

WAGENINGEN UR
For quality of life

A number of partners from the EU salmon industry

THANK YOU

UNIVERSITY OF STIRLING