AQUAEXCEL

Aquaculture Infrastructures for Excellence in European Fish Research

Project number: 262336

Combination of CP & CSA
Seventh Framework Programme
Capacities

Deliverable D9.4

Established isogenic lines in salmon, carp and seabass

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Start date of the project: March 1st, 2011 **Duration**: 48 months

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Revision: V1

Project co-funded by the European Commission within the Seventh Framework Programme (2007-2013)	
Dissemination Level	
PU Public	**X**
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	
CO Confidential, only for members of the consortium (including the Commission Services)	

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Glossary

AQUAEXCEL:	Aquaculture Infrastructures for Excellence in European Fish Research
ddRADseq:	double-digest restriction site-associated DNA sequencing
SNP:	single nucleotide polymorphism

Definitions

Androgenesis: a form of induced parthenogenesis in which the nuclear DNA of the organism is of paternal origin only. The unfertilised egg is irradiated to inactivate the maternal nuclear genome. Diploidy may be restored by suppression of mitosis in the haploid zygote (leading to dihaploid, completely homozygous). Note that the mitochondrial DNA (mtDNA) in an androgenetic individual will still be of maternal origin (the sperm does not pass on mtDNA to the zygote; egg mtDNA appears to be unaffected by radiation used to inactivate the maternal nuclear genome).

Gynogenesis: a form of induced parthenogenesis in which the nuclear DNA of the organism is of maternal origin only. The sperm is irradiated before fertilising the egg, to inactivate the paternal nuclear genome. Diploidy may be restored by suppression of the second meiotic division (leading to “meiotic” gynogenetic, partially heterozygous due to recombination at meiosis) or of mitosis in the haploid zygote (leading to “mitotic” gynogenetic, dihaploid, completely homozygous).

Homozygous clone founder: a completely homozygous individual produced by androgenesis [A1] or mitotic gynogenesis [G1] from an outbred clone founder. Genetically identical progeny produced from such an individual (by androgenesis [A2] or gynogenesis [G2]; in subsequent generations, hormonal sex reversal may be used to produce individuals of both sexes to allow propagation by normal crosses) will constitute an **isogenic line**.

Isogenic line: a group of genetically identical, completely homozygous individuals. Also called **fully inbred clonal line.** In this context, **homozygous clone founders** are produced by induced androgenesis or mitotic gynogenesis from **outbred clone founders**, then these founders are propagated by androgenesis or gynogenesis. In subsequent generations, hormonal sex reversal may be used to produce individuals of both sexes to allow propagation by normal crosses.

Outbred clone founder: an individual used as a source of eggs or sperm to produce a homozygous clone founder by mitotic gynogenesis or androgenesis. Such an individual may come from an outbred population, a defined strain or a line selected for particular traits. Such individuals would be expected to be heterozygous at a proportion of loci.

Appendix 1 illustrates the production of an isogenic line using androgenesis.

Summary

Objectives
The objective of this deliverable was to describe the establishment of isogenic lines (through androgenesis or gynogenesis) in salmon, carp and seabass by the end of the AQUAEXCEL project.

Main findings
Five putative isogenic lines have been produced in Atlantic salmon. At the end of the project, these were embryos approaching hatching, so had not yet been verified using genetic markers. These five lines were from the 2011/12 year class. In total >2100 G1 fish are being held by IMR (240 from 2011/12 year class; 800 from 2012/13 year class; 1100 from 2013/14 year class).

In sea bass, 39 G1 fish are being held by IFREMER. These have not yet matured, so the production of clonal lines has not been possible. These are not expected to mature until 2016: if (female) gamete quality is good enough, it would then be possible to produce isogenic lines in 2016.

In common carp, 35 G1 and 32 A1 fish were being held by HAKI and VURH. These have not yet matured, so the production of clonal lines has not been possible. It is possible that some of these fish will mature in spring 2015: if gamete quality is good enough, it would then be possible to produce isogenic lines in 2015.

Teams involved
Atlantic salmon: IMR, UoS
Sea bass: IFREMER, INRA, Ugent, UoS
Common carp: VURH (JCU), HAKI (NARIC-HAKI), INRA, UoS

Geographical areas covered
Norway, France, Czech Republic, Hungary, UK; isogenic lines are intended to be made available more broadly.

1. Atlantic salmon (*Salmo salar*)

The 2011/12 year class of G1 fish was produced from a single female and verified using a set of 18 microsatellites (see D 9.3 for this and phenotypic description of G1 fish). From these, six females matured and produced eggs in late 2014, from which five gave surviving embryos following the induction of meiotic gynogenesis (putative isogenic clonal lines). These putative isogenic lines will need to be verified using genetic markers.

The remainder of the 2011/12 G1s and the subsequent year classes of G1's are being grown for the production of further clonal lines. A facility has been established at IMR for these fish.

2. European sea bass (*Dicentrarchus labrax*)

Trials to establish androgenesis were unsuccessful – it appears that there are protective and/or repair mechanisms in sea bass eggs that prevent successful inactivation of the female genome through UV irradiation (Colleter et al, 2014; D 9.3). This was the preferred route for the production of isogenic clonal lines in the sea bass due to the reduced generation time (males mature earlier than females in this species), but in the light of this problem mitotic gynogenesis was used instead.

Mitotic gynogenesis was induced in eggs from 11 females in March 2013, from which 39 G1 fish were obtained (from 6 different females). In addition to the mitotic gynogenetics (G1s), meiotic gynogenetics, biparental diploids and fish with partial paternal contribution were detected using 12 microsatellite markers, highlighting the need for screening such fish (G1/A1) using a set of DNA markers with adequate resolution. Rearing and analysis of these fish is described in depth in D9.3. Analysis of a family of meiotic gynogenetic fish using ddRADseq produced 340 paternal-specific SNP markers and 804 female heterozygous markers, allowing a greater depth of screening in the development of isogenic clonal lines.

It is expected that at least some of these fish will mature in early 2016.

3. Common carp (*Cyprinus carpio*)

Table 1 summarises the numbers and strains of origin of A1 and G1 common carp that were being held at VURH (JCU) and NARIC-HAKI in early 2015. The screening of these fish with 14 microsatellite markers (including elimination of biparental and meiotic gynogenetic fish) and phenotypic description are dealt with in detail in D9.3.

Table 1. Summary of common carp A1 and G1 fish being held by AQUAEXCEL partner instititutes in early 2015.

Type	**VURH (JCU)**	**NARIC-HAKI**	**Total**
A1 (Androgenetic)	**30** (14 ROP, 16 M72)	**2** (SZARVAS 15)	**32**
G1 (mitotic gynogenetic)	**16** (ROP)	**19** (SZARVAS RED)	**35**

It is expected that at least some of these fish will mature in spring 2015.

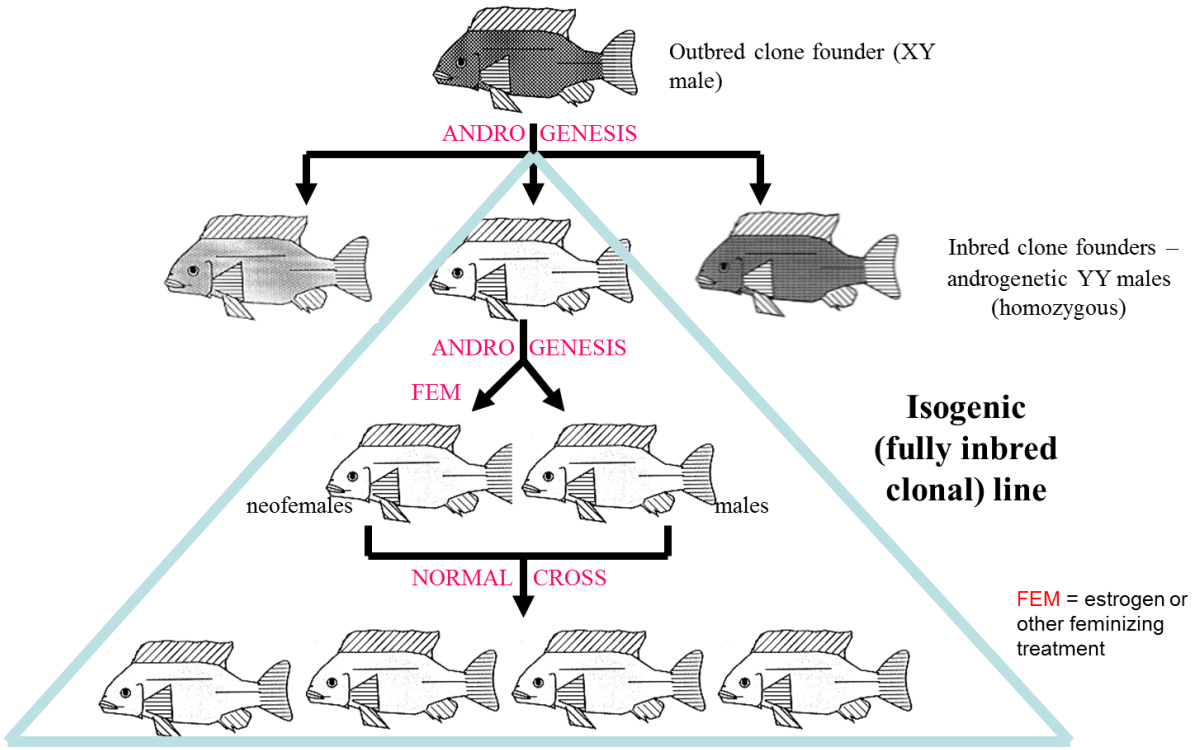
Conclusions

- Five putative isogenic clonal lines have been developed in the Atlantic salmon (nearing hatching stage, awaiting verification).
- G1 (mitotic gynogenetic) fish are available in all three target species: Atlantic salmon (n >2100), European sea bass (n = 39) and common carp (n = 35). These were verified using molecular markers (primarily microsatellites).
- A1 (androgenetic) fish are available for only the common carp (n = 32).
- At least some of these G1 and A1 fish are expected to mature and give rise to further isogenic clonal lines over the next breeding seasons.

Appendix 1:

Example of development of an isogenic line, in an XX/XY species via androgenesis

The diagram below illustrated the development of a YY isogenic line in a species with XX/XY sex determination, using androgenesis (and hormonal feminization in later generations). A single outbred clone founder will generate inbred clone founders that differ from each other, reflecting heterozygosity in the outbred clone founder and recombination leading to gamete (sperm) production. Thus isogenic lines generated from sibling inbred clonal founders will vary from each other.



References

Colléter J, Penman DJ, Lallement S, Fauvel C, Hanebrekke T, Osvik RD, Eilertsen HC, D'Cotta H, Chatain B, Peruzzi S (2014). Genetic inactivation of European sea bass (*Dicentrarchus labrax* L.) eggs using UV irradiation: observations and perspectives. PLOS ONE 9:e109572.

Annex 1

Deliverable Check list (to be completed by Deliverable leader)

	Check list		Comments
BEFORE	I have checked the due date and have planned completion in due time		*Please inform Management Team of any foreseen delays*
	The title corresponds to the title in the DOW		*If not please inform the Management Team with justification*
	The dissemination level corresponds to that indicated in the DOW		
	The contributors (authors) correspond to those indicated in the DOW		
	The Table of Contents has been validated with the Activity Leader		*Please validate the Table of Content with your Activity Leader before drafting the deliverable*
	I am using the AQUAEXCEL deliverable template (title page, styles etc)		*Available in "Useful Documents" on the collaborative workspace*
The draft is ready			
AFTER	I have written a good summary at the beginning of the Deliverable		*A 1-2 pages maximum summary is mandatory (not formal but really informative on the content of the Deliverable)*
	The deliverable has been reviewed by all contributors (authors)		*Make sure all contributors have reviewed and approved the final version of the deliverable. You should leave sufficient time for this validation.*
	I have done a spell check and had the English verified		*Ask a colleague with a good level of English to review the language of the text and do a spell-check too.*
	I have sent the final version to the Activity Leader and to the 2nd Reviewer for approval		*Send the final draft to your Activity Leader and the 2nd Reviewer and leave 2 weeks for feedback and final changes before the due date. Once validated by the 2 reviewers, the draft is ready to be sent to the Management Team that will ask for the Coordinator validation and then transfer it to the EC.*