"Freezebass"

Setting up of a sperm cryobank for sea bass

Q5CT-2002-71209

Final report

(01 January 2003 – 30 June 2005)

SUMMARY

Section 1: PROJECT IDENTIFICATION		**NOT CONFIDENTIAL**
Title of the project: Setting up of a sperm cryobank for sea bass		
Acronym of the project: Freezebass		
Type of contract CRAFT		**Total project cost** 421 100 €
Contract number Q5CT-2002-71209	**Duration** 30 **Months**	**EU contribution** 209 550 €
Commencement date **1 January 2003**		**Period covered by the progress report** 1 January 2003 – 30 June 2005

Project coordinator

Name:	**Title:**	**Address:**
Olivier POLINE	Doctor Engineer	Écloserie Marine de Gravelines, ZI des Huttes, 59820 Gravelines
Telephone: +33 328238123	**Telefax**: +33 328238133	**E-mail address:** olivier.poline@aquanord.com

Scientific coordinator

Name:	**Title:**	**Address:**
Béatrice CHATAIN	Unit director	Ifremer, Chemin de Maguelone 34250 Palavas-Les-Flots, France
Telephone: +33 467504109	**Telefax**: +33 467682885	**E-mail address:** bchatain@Ifremer.fr

Key words
Sea bass, cryopreservation, sperm, genetic origin, pathogen free

World wide web address

List of participants

A1: Écloserie Marine de Gravelines
Address: Z.I. Les Huttes 59820 Gravelines, France
Contact name: Olivier POLINE **Status:** SME **Participant role:** Coordinator
Telephone: +33 328 238123 **Telefax:** +33 328 238133 **E-mail address:** olivier.poline @aquanord.com

A2: Panittica
Address: Strada Comunale del Procaccio, 72010 Torre Canne di Fasano, Italy
Contact name: Hervé CHAVANNE **Status:** SME **Participant role:** Contractor
Telephone: +39 080 4829888 **Telefax:** +39 080 4829922 **E-mail address:** panamel@libero.it

B1: Ifremer
Address: Chemin de Maguelone, 34250 Palavas-les-Flots, France
Contact name: Béatrice CHATAIN **Status:** RTD **Participant role:** Scientific coordinator
Telephone: +33 467504109 **Telefax:** +33 467682885 **E-mail address:** bchatain@Ifremer.fr

B2: Universita'degli studi di Lecce
Address: Viale Gallipoli, 73100 Lecce, Italy
Contact name: Carlo STORELLI **Status:** RTD **Participant role:** Contractor
Telephone: +39 083 2320670 **Telefax:** +39 083 2324220 **E-mail address:** storelli@ilenic.unile.it

Section 2: PROJECT Final Progress Report	NOT CONFIDENTIAL

Objectives

Presently, most of marine fish farmers around the Mediterranean basin grow genetically wild fish strains, which have not been genetically improved for culture performance (growth, appearance, disease resistance etc.). It is inevitable that the present gap for genetically improved strains of marine fish, and their reliable supply, will be filled. It will ultimately dominate the competitive component of marine culture industries once they evolve into commodity-style agribusiness (with fish prices being set at the lowest possible cost of production and the best quality). Those countries, which can competitively develop and supply the improved strains, will enjoy the benefit (perhaps survival) for their local industries. They will avoid introduction of disease and may also generate new export industries based on the sale of low volume high value "seeds". The countries, which do not, will be obliged to import the improved "seeds" for their industries to remain competitive.

FREEZEBASS deals with the setting up of a bank of sperm for the sea bass *Dicentrarchus labrax*. The needed cryopreservation methods will be transferred from RTDs to SMEs. This bank will gather, in strictly controlled sanitary conditions, the whole genetic variability known to date for the species. Three geographic and genetically differentiated populations exist and will be collected from the Atlantic Ocean (AT), the Western (WM) and Eastern Mediterranean Sea (EM). The bank will enable to close rearing populations with enough variability to ensure the sustainability of the farms from a sanitary point of view but also if future domestication or selection programs are opened.
The SMEs involved in this project are presently rearing several species among which they have chosen the sea bass for its great economical importance (European production x 15 fold in 10 years).

FREEZEBASS will be the first bank of reference gathering the present wild genetic resources for the sea bass. It will allow:

- to increase the genetic variability of farmed populations by adequate mixing of genes,
- to avoid all problem related to the displacement of alive wild broodstock,
- to realize sophisticated and large crossing scheme necessary to breeding programs,
- to utilize seasonal desynchronized males,
- to protect the genetic progress when breeding programs are set up.

Results and Milestones

The different steps that have marked the project are the following:

- The setting up of a common straw nomenclature for all the partners.
- The training of all partners to the same cryopreservation methods thanks a practical training in Ifremer and the realization of a video film on cryopreservation methods ("Mediaqua" company sub-contracted by Ifremer).
- The setting up of cryopreservation units by each partners and the constitution of a planning for sperm collection.
- The collection of the breeders and the conditioning of the males for maturation.
- The stripping the fluent individuals and the cryopreservation of the motile sperms using the methods mentioned above.

Two reproduction seasons (winter 2004-2005 and winter 2005-2006) allowed to set up a sperm bank of almost 3,000 straws gathering the whole sea bass genetic variability and comprising 138 different sperms cryopreserved of which 59, 27 and 52 individuals have originated from the Atlantic, West Mediterranean and East Mediterranean populations respectively. The plans of the project were therefore largely respected since a minimum of 20 different sperms per population was expected.

Deviation from the initial project

- A 6 months extension of the project was requested because the signature of the contract has occurred at the end of the first reproduction season which would have not allow to cover two spawning as it was foreseen in the initial project.
- A double amount of breeders had to be fished because a majority of females were caught.
- The coordinator Aquastream never redistributed to the partners the budget allocated by the EU Commission and stops its activities for the project one year after it has started.
- Due to this defection, the scientific coordinator (Ifremer) took upon its shoulders the coordination task not realized by Aquastream until the nomination of a new coordinator (EMG) and extra work was done by two other partners (Panittica and Univ. of Lecce) to provide extra Atlantic and East Mediterranean populations respectively.
- Because of the total lack of funding, the two thirds of WP3 could not be realized *i.e.* the genetic origin and sanitary control of straws. Only the last third (fertility control) was done but with simplified procedures (recommended by this task responsible partner, Univ. Lecce) and each partner had to support the extra work of checking its own sperm motility before and after cryopreservation.

Benefits and Beneficiaries

Besides of the direct objective of the project, the cryopreservation units built in the private partners companies give them the immediate possibility to preserve the sperm of specific fish they will consider important for the future of their production. It can be fish having specific phenotypic characteristics or selected fish. For the partners already applying selective breeding programs, it is a security of saving half their genetic progress in case of loosing a part of their broodstock.

The training of all partners directly by the scientific partners that have setting the cryopreservation protocol up is the best guarantee of recovering the entire know-how of the proposed method.

The production of the video in the early stages of the project allows the training of other members of the companies or of the research organisms that had no possibility to participate to the training and that will be able to participate to the cryopreservation process on the fields.

Future Actions (if applicable)

REPORT

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Olivier POLINE	Doctor Engineer	Écloserie Marine de Gravelines, ZI des Huttes, 59820 Gravelines
Telephone: +33 328238123	**Telefax**: +33 328238133	**E-mail address:** olivier.poline@aquanord.com

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Béatrice CHATAIN	Unit director	Ifremer, Chemin de Maguelone 34250 Palavas-Les-Flots, France
Telephone: +33 467504109	**Telefax**: +33 467682885	**E-mail address:** bchatain@Ifremer.fr

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A1: Écloserie Marine de Gravelines
Address: Z.I. Les Huttes 59820 Gravelines, France
Contact name: Olivier POLINE **Status:** SME **Participant role:** Coordinator
Telephone: +33 328 238123 **Telefax:** +33 328 238133 **E-mail address:** olivier.poline @aquanord.com

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Address: Strada Comunale del Procaccio, 72010 Torre Canne di Fasano, Italy
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Address: Chemin de Maguelone, 34250 Palavas-les-Flots, France
Contact name: Béatrice CHATAIN **Status:** RTD **Participant role:** Scientific coordinator
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SYNOPSIS

1. Introduction

Presently, most of marine fish farmers around the Mediterranean basin grow genetically wild fish strains, which have not been genetically improved for culture performance (growth, appearance, disease resistance etc.). It is inevitable that the present gap for genetically improved strains of marine fish, and their reliable supply, will be filled. It will ultimately dominate the competitive component of marine culture industries once they evolve into commodity-style agribusiness (with fish prices being set at the lowest possible cost of production and the best quality). Those countries, which c an competitively develop and supply the improved strains, will enjoy the benefit (perhaps survival) for their local industries. They will avoid introduction of disease and may also generate new export industries based on the sale of low volume high value "seeds". The countries, which do not, will be obliged to import the improved "seeds" for their industries to remain competitive.

Conceptually, genetic improvement by selection can be made simply by setting aside the "best" individuals for breeding the next generation. Already, for strictly marine fish, simple selection programs (mass or individual selection) based on this approach have commenced, for turbot *Scophtalmus maximus* (France, Spain, Greece), for sea bream *Sparus aurata* (France, Israel) and for sea bass *Dicentrarchus labrax* (France, Italy, Israel, Spain, and Greece).

Although this simple approach may luckily yield interesting results, the long-term efficiency of a breeding program will require to start with the maximum of genetic variability before the stock closure, its domestication or its selection. It will also require the mastering of mating. Indeed, it was demonstrated in marine fish, when spawning naturally occurs in captivity, that only a few of the breeders participate to reproduction (*e.g.* 10% in sea bream). Moreover, in sea bass, the descendants of the parents that have reproduced do not survive equally. In other words, in captivity, natural spawning leads to quick loose of genetic variability and favorizes inbreeding.
These observations led to the recent development of artificial fertilization in several marine fish species to allow specific crossing plans. In sea bass, the trials of large-scale crossing schemes showed the necessity of the use of cryopreserved sperm. Indeed, during such long operations the sea bass sperm cannot be kept in the refrigerator without quickly deteriorated.

The aim of the work planned in the *Freezebass* project was the setting up of a bank of sperm for the sea bass *Dicentrarchus labrax* and the transfer of the cryopreservation methods set up by research institutes to SMEs. This bank will gather the whole genetic variability known to date for the species from 3 genetically differentiated populations: Atlantic Ocean (AT), the Western (WM) and Eastern Mediterranean Sea (EM). It will ensure the sustainability of the farms by giving the possibility of:

- closure, domestication or selection of rearing stocks with enough genetic variability
- to design optimized breeding programs with specific pair mating techniques
- of long-term protection of genetic progress thanks the possible saving of the last male generation
- to generate the progeny of a selected male

To perform these different tasks, a consortium of engineers, scientists and technicians has been set up. They belong to 5 different and independent organisms: 3 industrials (Aquastream, Panittica and EMG) and 2 laboratories (Ifremer and Univ. Lecce). They cover all necessary and complementary fields needed for the project *i.e.* physiology of reproduction, cryopreservation, zootechny, pathology, molecular biology. Overall the manpower devoted to the project represents an equivalent of an equivalent of 2.5 full-time persons (59 person. months) over the 2-year duration of the project.

2. Material and methods

Cryopreservation procedures, training and planning

Straw nomenclature

A common straw nomenclature (annex 1) was set up for all the partners according to the following nomenclature with 3 elements: 2 letters and one number.

The first letter indicate the fishing origin:

- Atlantic (A)
- West Mediterranean sea (W)
- East Mediterranean sea (E)

The second letter indicate the partner (through his geographic location):

- Panittica: B (for Brindisi)
- Lecce: L
- Aquastream: A
- EMG: G (for Gravelines)
- Palavas (P)

The number indicates the number of the stripped male (from 1 to n).
e.g.: WP12 indicate the 12th fish captured in the West area and stripped by Palavas.

If a male was stripped twice, during two reproduction seasons, he got 2 numbers but was identified has the same individual thanks his tag number recorded on an Excel table.

Cryopreservation methods

All partners were trained and have used the same cryopreservation methods set up according to the Ifremer method adapted for sea bass. These methods are described in a video film, produced in the frame of the project, to standardize the different partner cryobanks.

Sperm collection planning

The planning for sperm collection covered 2 spawning seasons (winters 2004 and 2005) and took place as follows in the different sites:

- Aquastream : all around the year for captive fish and December in Aegean Sea.
- Panittica : December-February
- EMG : November-December
- Univ. of Lecce : December-February
- IFREMER : December-January

Each partner was responsible of a sampling zone. These zones (fig. 1) were attributed according to the farm or laboratory geographical situation and/or fishing possibilities/facilities (*e.g.* fisherman or aquaculturist partners on site). They covered the whole repartition area of sea bass.

Fig. 1 Map of collection areas.

3. Results

Sperm collection & cryopreservation

The 3 populations of wild males originating from the Atlantic Ocean (AT), the Western (WM) and Eastern Mediterranean (EM) have been collected. Samples covered the major repartition area of the species: North Atlantic, Brittany, Andalousia, Gulf of Lions, North of Crete and Sicilia-Sardinia.

The number of fish that were caught and the number of stripped males is summarized in table 1.

The total number of fish stocked and/or conditioned has reached 864. Among these 864 fish, 297 males and 567 females were identified (male-ratio = 34%).
From these 297 males, 138 sperms were cryopreserved after their motility has been verified. These sperms came from 59, 27 and 52 individuals from the Atlantic, West Mediterranean and East Mediterranean populations respectively.

Table 1. Number of males and females fish caught and number of stripped males.

Population	Fishing Sites	Partners	N males foreseen	N fish	N males	N females	N stripped males	N stripped males/population
East Mediterranean	Aegean sea	Aquastream	200	0	0	0	0	52
		Univ. Lecce	40	120	42	78	32	
		Panittica	0	15	15	0	15	
	Crete	Panittica	0	4	4	0	4	
	Croatia	Panittica	0	17	8	9	1	
	Adriatic sea	Panittica	120	0	0	0	0	
West Mediterranean	Alboran Sea	Ifremer	40	0	0	0	0	27
	Gulf of Lions	Ifremer	40	91	29	62	27	
Atlantic	Brittany	Aquastream	200	300	100	200	0	59
		Panittica	120	130	41	89	40	
	Sicilia-Sardinia	Panittica	0	15	9	6	2	
	Andalusia	Panittica	0	88	30	58	0	
	North Atlantic	EMG	80	84	19	65	17	
Total			**840**	**864**	**297**	**567**	**138**	**138**

As many straws as possible were made from each male. The mean perm volume per male was about 1ml that was diluted to the third. The straw volume was 125 µl. The fertility of each sperm was done by a simple check under microscope of sperm motility before and after cryopreservation using a drop of sea water to activate the spermatozoa.

The number of straws recovered per male has varied from 9 to 40 with a mean around 24.

4. Discussion-Conclusion

A sperm bank gathering the whole sea bass genetic variability is therefore presently available as it was foreseen in the project but the straws were not shared between partners because neither sanitary analysis nor genetic origin control could be realized due to the lack of funding (funds never distributed by the coordinator).

The remaining SME partners (EMG, Panittica) agreed in sharing the straws with Ifremer as foreseen in the initial plans but only when they will find the funds to realize at least the sanitary control as mentioned in the project.

Freezebass is the first bank of reference gathering the present wild genetic resources for the sea bass. It will allow:

- to increase the genetic variability of farmed populations by adequate mixing of genes,
- to avoid all problem related to the displacement of alive wild broodstock,
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ANNEX 1

Mask for the identification of the straws

N°	Nomenclature	Pit tag number	Volume of milt sampled (ml)	Number of straws	Sanitary status	Genetic origin	Fertility
1							
2							
3							
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