

University of St Andrews



WP 8 CRYOPRESERVATION OF MARINE ORGANISMS CRYOMAR



Task 1

De-fragmentation of existing cryobiological knowledge relevant to the marine sector, establishment of a JRA specific discussion forum and depository of methods

Task 2

Exploring the potential to cryopreserve marine invertebrate larvae, embryos and/or gametes and to develop appropriate biobanks and procedures

Task 3

R&D on cryopreservation, protocol development and cell recovery in teleost germ cells

Task 4

R&D on cryopreservation and biobanking of macroalgae

Task 5

Cryopreservation research on Amphioxus

Task 6

Development and application of novel cryopreservation approaches to cryopreserve a wide range of protists, microbial consortia and mutant libraries

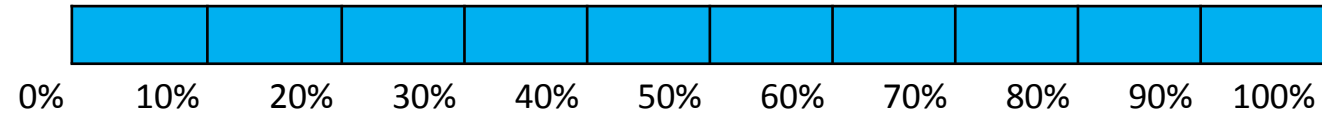


Task 1

De-fragmentation of existing cryobiological knowledge relevant to the marine sector, establishment of a JRA specific discussion forum and depository of methods

Status: **FINISHED**

State of progress:



Task 1: Future developments

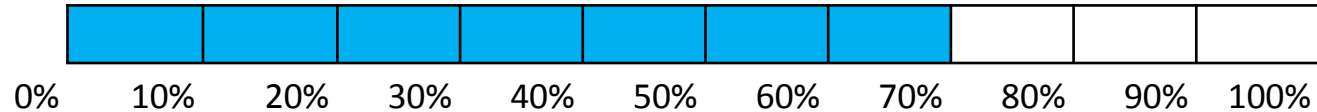
Planning on writing a review manuscript all together (all WP8)

Task 2

Exploring the potential to cryopreserve marine invertebrate larvae, embryos and/or gametes and to develop appropriate biobanks and procedures

Status: on schedule

State of progress:



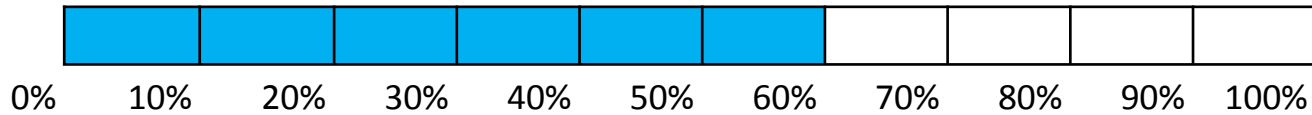
- First steps have been taken towards cryopreservation of mussel sperm for Cryo-banking and posterior functional analysis of motility. Two meetings have taken place to run a ring-test exercise Faro (Algarve), Vigo (Galicia) and Plentzia (Basque Country) for environmental monitoring application. Sperm sampling and Cryo-preservation protocol has been established for mussels, although first attempts during 2018 spawning season were not satisfactory in the 3 locations.
- Two manuscripts are being produced with results of cryopreservation experiments for the *M. galloprovincialis* larvae that had been designed by Universidade de Vigo for Aquaculture applications.
- CCMAR team has been working in the development of cryopreservation protocols for *Chamelea gallina* and *Crassostrea angula* sperm and larvae. For *C. angulada* sperm, optimization of previous protocols incorporating antioxidants were made in collaboration with the University of Vigo.

Task 3

R&D on cryopreservation, protocol development and cell recovery in teleost germ cells

Status: **on schedule**

State of progress:



- UPV in Plentzia studies thicklip grey mullets *Chelon labrosus* as sentinels of environmental health in pollution monitoring campaigns in sites of the Basque coast with high prevalence of xenoestrogenic chemical compounds (produce fish feminization). Within the scope of this JRA attempts have been done to assess sperm mobility upon sperm cryopreservation instead of in fresh sperm. Cryopreservation should allow carrying out the analyses in the laboratory, after sample collection in the field. Protocols for mullet sperm cryopreservation have been applied, although procedures have been hampered by the difficulties of obtaining “flowing” males in estuaries (spawning occurs in the sea, where sperm hydration takes place).
- In this task CCMAR team has been **optimizing cryopreservation protocols in zebrafish sperm strains**. For that the selection of donor fish samples based on fish age and striping frequency was established and new protocols for sperm cryopreservation developed.

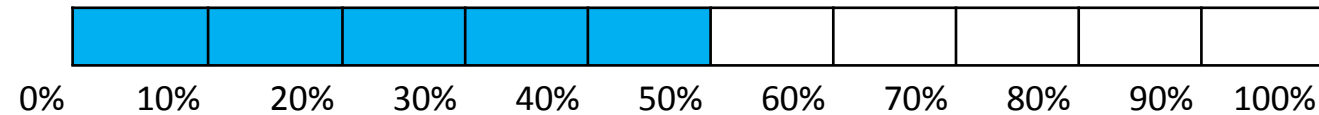
Task 4

R&D on cryopreservation and biobanking of macroalgae



Status: on schedule

State of progress:



- At SAMS, the team have investigated a method for cryopreserving gametophytes of the brown seaweed, *Saccharina latissima* and this has resulted in a paper Under Review: Visch W, Rad Menendez C, Nylund GM, Pavia H, Ryan MJ, Day JG. (2018) Underpinning the development of seaweed biotechnology: Cryopreservation of brown algae (*Saccharina latissima*) gametophytes
- Roscoff is interested on other species like Laminaria, Prophyra ...

Task 5

Cryopreservation research on Amphioxus



Status: postponed (until next spring)

State of progress:



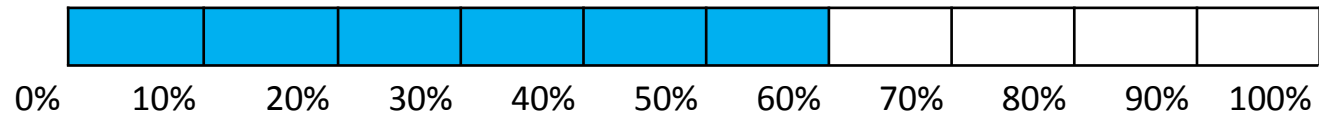
Amphioxus are seasonal spawners, and unfortunately the timing of their spawning in the late spring didn't allow time to start the experiments in 2018. Experiments are therefore scheduled for next spawning season to test a number of cryopreservation techniques on embryos, and then to evaluate using markers and/or sequencing techniques.

Task 6

Development and application of novel cryopreservation approaches to cryopreserve a wide range of protists, microbial consortia and mutant libraries

Status: on schedule

State of progress:



- Dinoflagellates are recalcitrant to conventional cryopreservation, SAMS is developing research on the cryopreservation of 2-3 species of dinoflagellate cysts. MBA carried out a screening of strains in the MBA culture Collection using a standard method to determine which, if any, survived. Only *Amphidinium* retained viability, morphology and motility, producing healthy cultures. *Procentrum* and *Scripsiella* showed 'normal' looking cells with no motility but no cell division.
- Universidade de Vigo is working on the application of vitrification for marine microalgae testing all the methodologies currently available.
- UPV has initiated the application of protocols of cryopreservation of microalgae as applied in the Station Biologique de Roscoff (UPMC) handling, keeping and transfer of its collection of 500 microalgae strains.
- NIOZ has successfully developed a cryopreservation protocol for hypersaline microbial mats that can now be preserved at $-150\text{ }^{\circ}\text{C}$ and can be grown again for 120 days. NIOZ is extracting DNA for the determination of the bacterial community composition post cryopreservation.



Workshop on CRYOBANKING IN AQUATIC SPECIES: from sample cryopreservation to collection management. OCTOBER 2nd 2018

This workshop is jointly organized by

- ASSEMBLE plus <http://www.assembleplus.eu/>, a consortium of marine biological stations and installations dedicated to marine biology and ecology

- AQUAEXCEL2020 Project <https://aquaexcel2020.eu/> that works towards advanced integration and standardization of tools for aquaculture research

MORNING (with coffee break) 9h – 13h

Assemble plus introduction

Antonio Villanueva, Universidade de Vigo, Spain (15' talk)

Cryopreservation for Aquaculture: mussels

Estefania Paredes, Universidade de Vigo, Spain (15' talk + 10' discussion)

Fish and mussel sperm and their use in ecotoxicology

Ibon Cancio, Universidad del Pais Vasco, Spain (15' talk + 10' discussion)

Cryopreservation for conservation

Elsa Cabrita, CCMAR, Portugal (15' talk + 10' discussion)

Algae cryopreservation SAMS

Christine Campbell, SAMS UK (15' talk + 10' discussion)

Cryopreservation of algae at the Roscoff Culture Collection

Ian Probert, Station Biologique de Roscoff, France (15' talk + 10' discussion)

Cryopreservation at the Marine Biological Association

Angela Ward, MBA, UK (15' talk + 10 discussion)

NAGOYA Protocol

Ibon Cancio, Universidad Pais Vasco, Spain. (15' talk + 10' discussion)

Buffet for all participants 13h – 14h

AFTERNOON (with coffee break) 14h-18h

AQUAEXCEL2020 introduction

Marc Vandeputte (INRA) (15' talk + 10' discussion)

Management of the French National Cryobank for domestic resources

Catherine Labbé (INRA) (20' talk + 10' discussion)

Case study on the use of cryopreserved resources after a massive loss in aquaculture

Lionel Goardon (INRA) (20' talk + 10' discussion)

Designing a software for low-scale bank management

Elsa Cabrita (CCMAR) ((15' talk + 10' discussion)

Presentation of the cryobanks in AQUAEXCEL2002 and their managements

Alain Vergnet (IFREMER), Marek Rodina (JU), Elsa Cabrita (CCMAR), Lionel Goardon (INRA), UoS ?, IEO ?, NAIK ? 5' each cryobank (3-5 slides) + 20' discussion.

Round table about the uses and needs of cryobanking activity

- What is user demand within each facility
- Do we disseminate the collections (what for)? Discussion about ownership, sanitary regulation, stock, advertising on genetic resources ...
- Do we standardize cryobanking practices (what for)? Discussion about traceability (software), quality assessment, procedures ...
- Plans for a cryobank network in Europe (what for)?

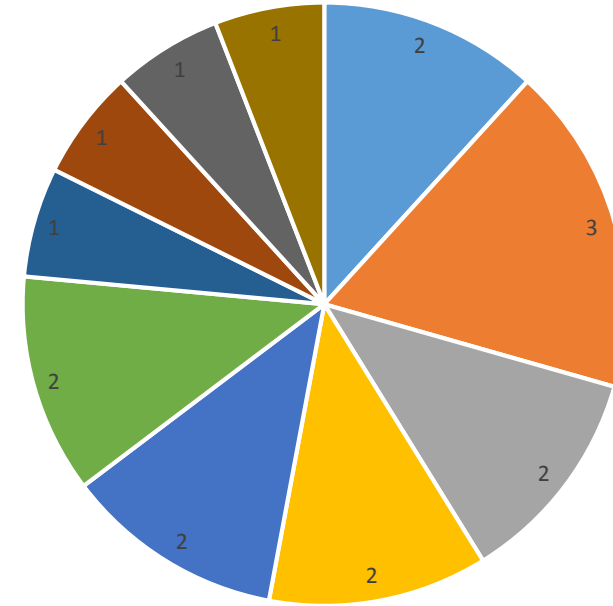


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**Workshop on CRYOBANKING IN AQUATIC SPECIES: from sample cryopreservation
to collection management.**





■ Uvigo ■ INRA ■ UPMC ■ CCMAR ■ IEO ■ IFREMER ■ SAMS ■ UPV ■ MBA ■ USB

ASSEMBLE

ASSOCIATION OF EUROPEAN MARINE BIOLOGICAL LABORATORIES EXPANDED

