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ASSEMBLE +

Association of European Marine Biological Laboratories Expanded

WP JRA2 CRYOPRESERVATION OF MARINE ORGANISMS CRYOMAR

SAMS

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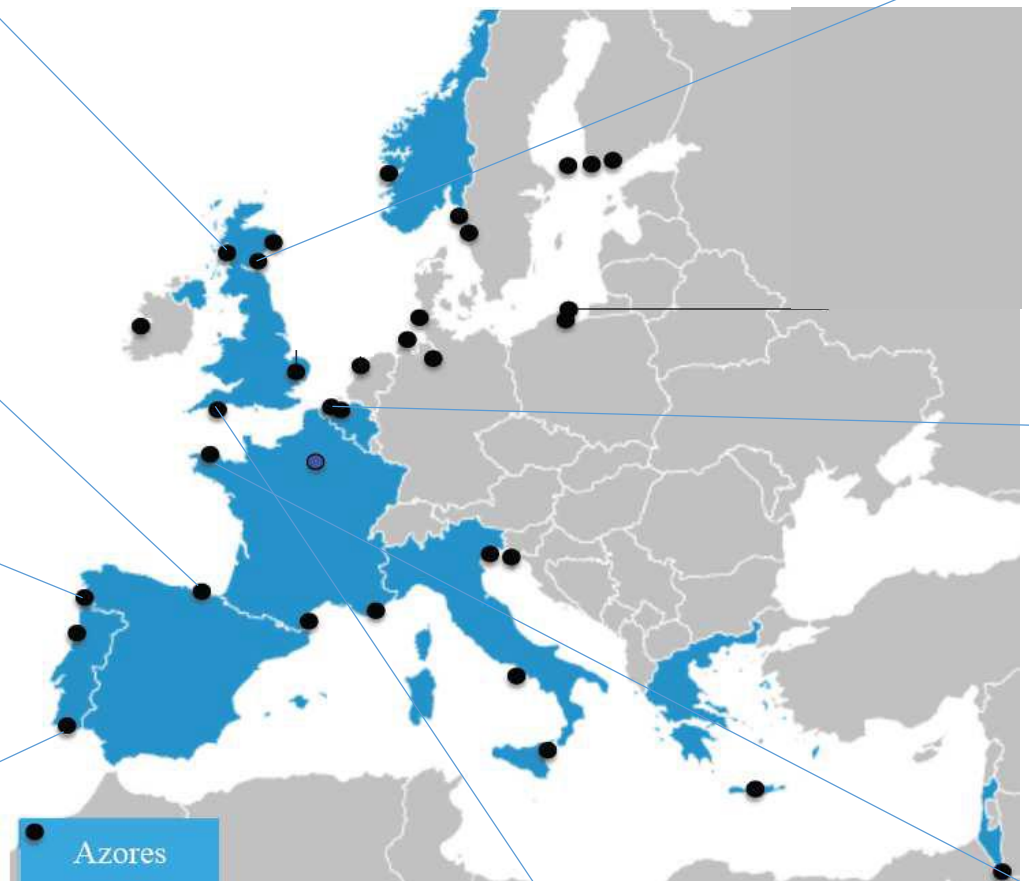


NIOZ

CCMAR

THE MARINE BIOLOGICAL ASSOCIATION

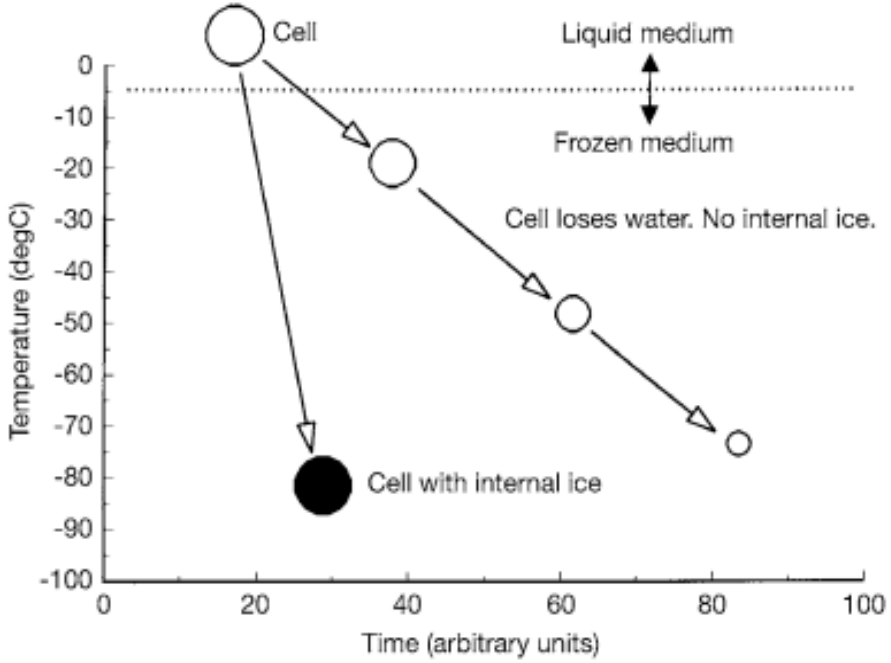
UPMC SORBONNE UNIVERSITÉS

Work package number	JRA 2	Lead Beneficiary				UPV/EHU	
Work package title	Cryobanking of marine organisms						
Participant number	19	1	3	18	14	19	20
Short name of participant	UPV/EHU	UPMC	NIOZ	SAMS	CCMAR	USTAN	MBA

WP JRA2 Cryopreservation of Marine Organisms will address a constraint in the exploitation of marine genetic and biological resources, namely current paucity of capability to conserve these resources ex situ with guaranteed genetic, phenotypic and functional stability. The JRA will develop robust, reproducible cryopreservation methodologies for various life-stages of a range of marine macro-organisms and currently cryo-recalcitrant microorganisms. The results will improve and expand the availability of biological resources for TA at significantly reduced costs.

WHAT IS CRYOPRESERVATION?

Cryopreservation consists on freezing, storing and thawing living organisms, cells or tissues in the presence of Cryoprotecting Agents (CPAs). A Well developed and worldwide recognized technique for achieving long-term storage of biological material at low temperatures.



Schematic representation of rapid and slow cooling of cells. From **Pegg 2007**

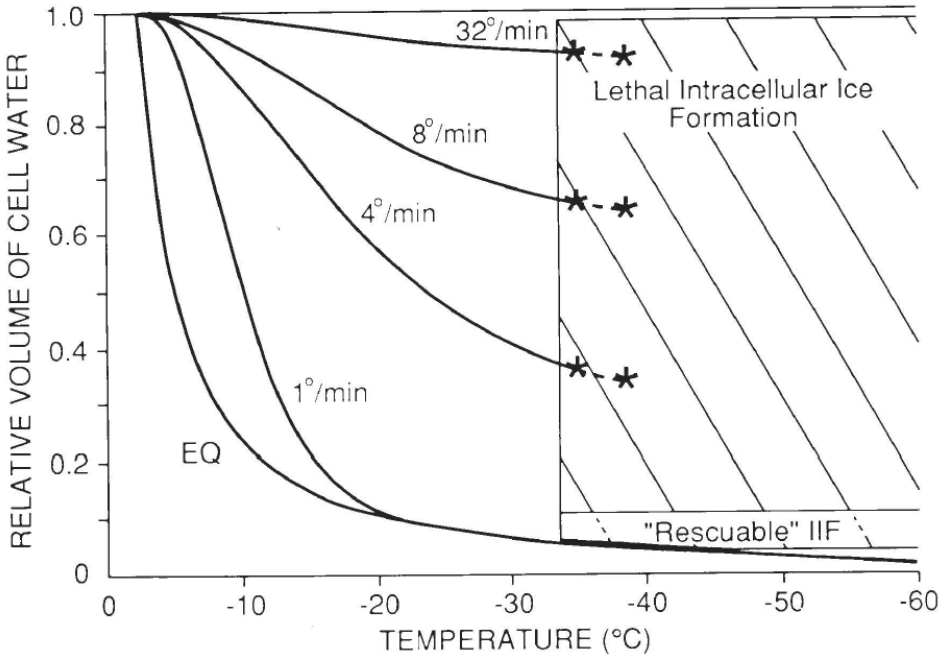
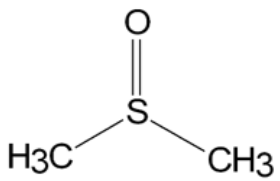


Figure from **Mazur et al. 2005**

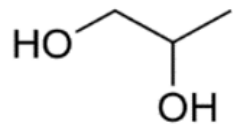
Cryoprotecting Agents (CPAs)

Cryoprotecting agents (CPA) are chemical compounds which need to have some special qualities: highly soluble in water even at low temperatures, have low toxicity and depress the freezing point of a solution .

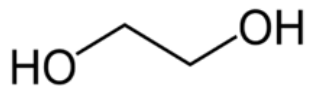
Dimethyl sulfoxide (Me_2SO)



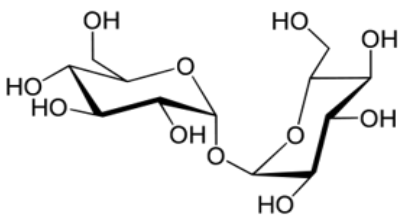
Propylene glycol (PG)



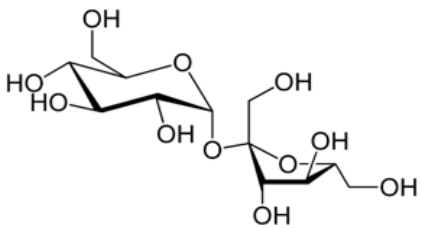
Ethylene glycol (EG)



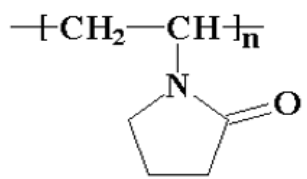
Trehalose (TRE)



Sucrose (SUC)

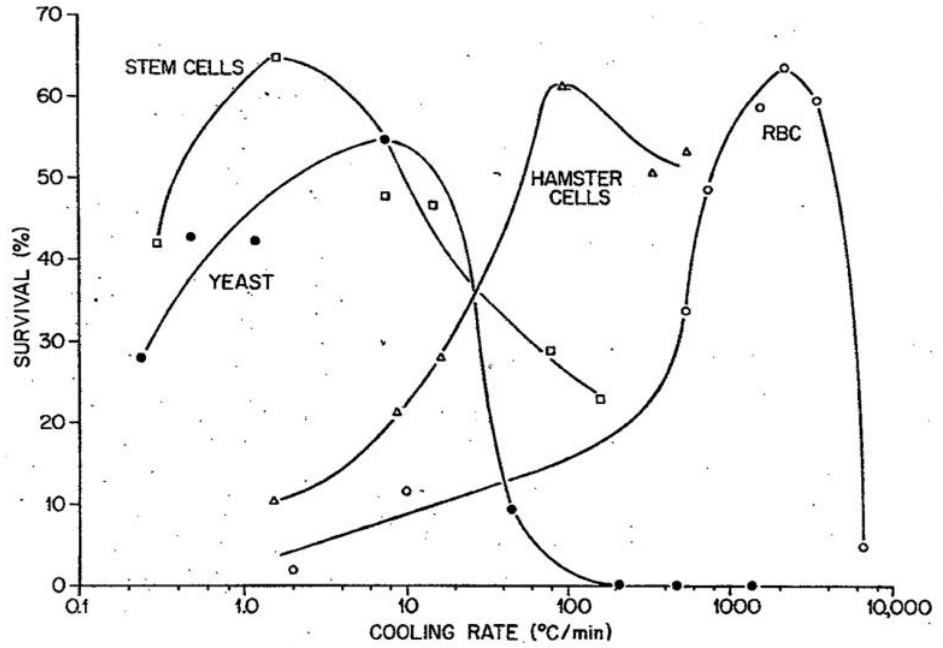


Polyvinyl pyrrolidone (PVP)



Inverted “U”

Finding the optimal cooling rate



Mazur P. Cryobiology (**1976**)

Cooling rates

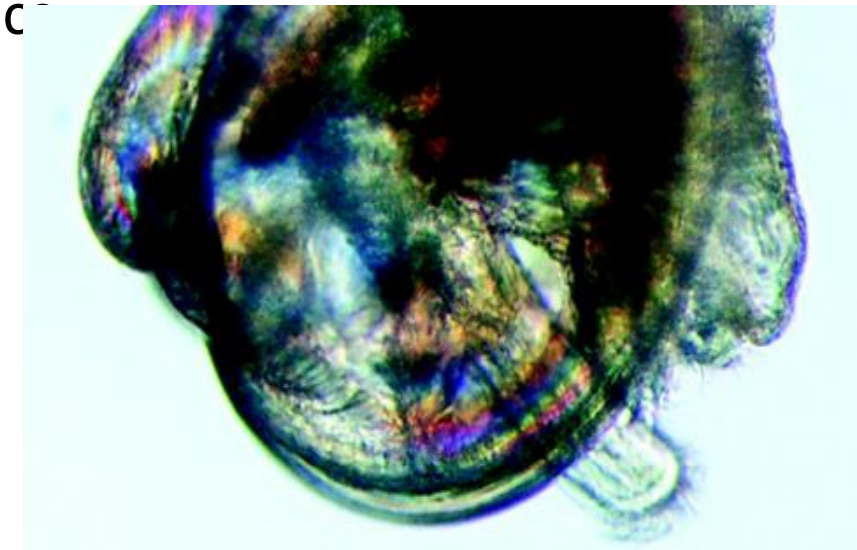
Too slow cooling damage due to solution effects

Reach equilibrium and freeze without IIF

Too fast cooling damage due to IIF

Cryobiology & Marine Environment

- Unique situation:
  - Salt content
  - Organisms sensitivity to CPA´s
  - Hard structures
  - Lack of Knowledge about membrane characteristic
  - Fairly recent, uncharted territory
  - Reproduction seasonality



Advantages

Disadvantages

Less expensive/time consuming

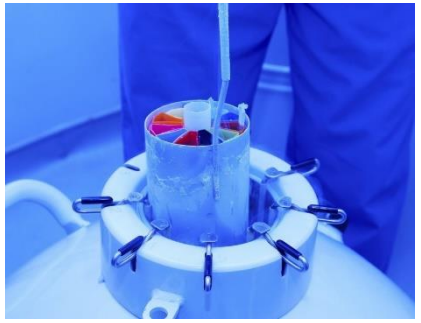
Marine R&D benefits

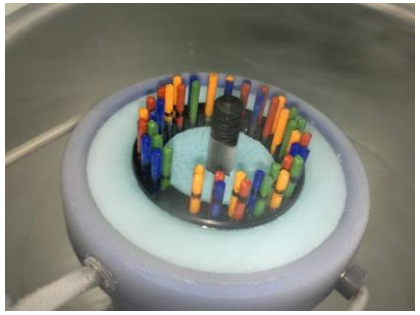
Flexibility of the access to gametes without restrictions

Long term storage, Genetic stability

Cold-chain and sample stability

Lack of cryo protocols for many species and cells





Task 1

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

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Task 2

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

Molluscs



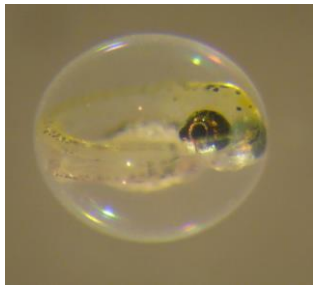


Echinoderms

Crustaceans


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

University of St Andrews

Task 6

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

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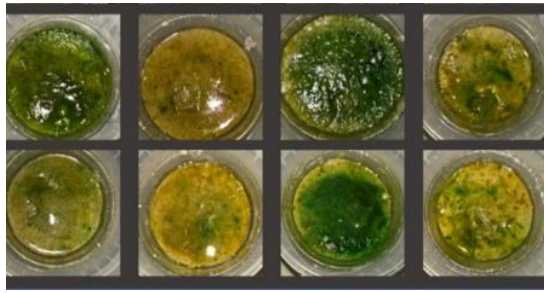

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