ASSEMBLE +
Association of European Marine Biological Laboratories Expanded

WP JRA2 CRYOPRESERVATION OF MARINE ORGANISMS CRYOMAR
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.
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.
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 (Me₂SO)**
- **Propylene glycol (PG)**
- **Ethylene glycol (EG)**

- **Trehalose (TRE)**
- **Sucrose (SUC)**
- **Polyvinyl pyrrolidone (PVP)**
Finding the optimal cooling rate

- Too slow cooling: damage due to solution effects
- Reach equilibrium and freeze without IIF
- Too fast cooling: damage due to IIF

Mazur P. Cryobiology (1976)
Cryobiology & Marine Environment

• Unique situation:
  
  • Salt content
  • Organisms sensitivity to CPA’s
  • Hard structures
  • Lack of Knowledge about membrane characteristics
  • Fairly recent, uncharted territory
  • Reproduction seasonality
Advantages

- Less expensive/time consuming
- Marine R&D benefits
- Flexibility of the access to gametes without restrictions
- Long term storage, Genetic stability

Disadvantages

- 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.
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

Task 6

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