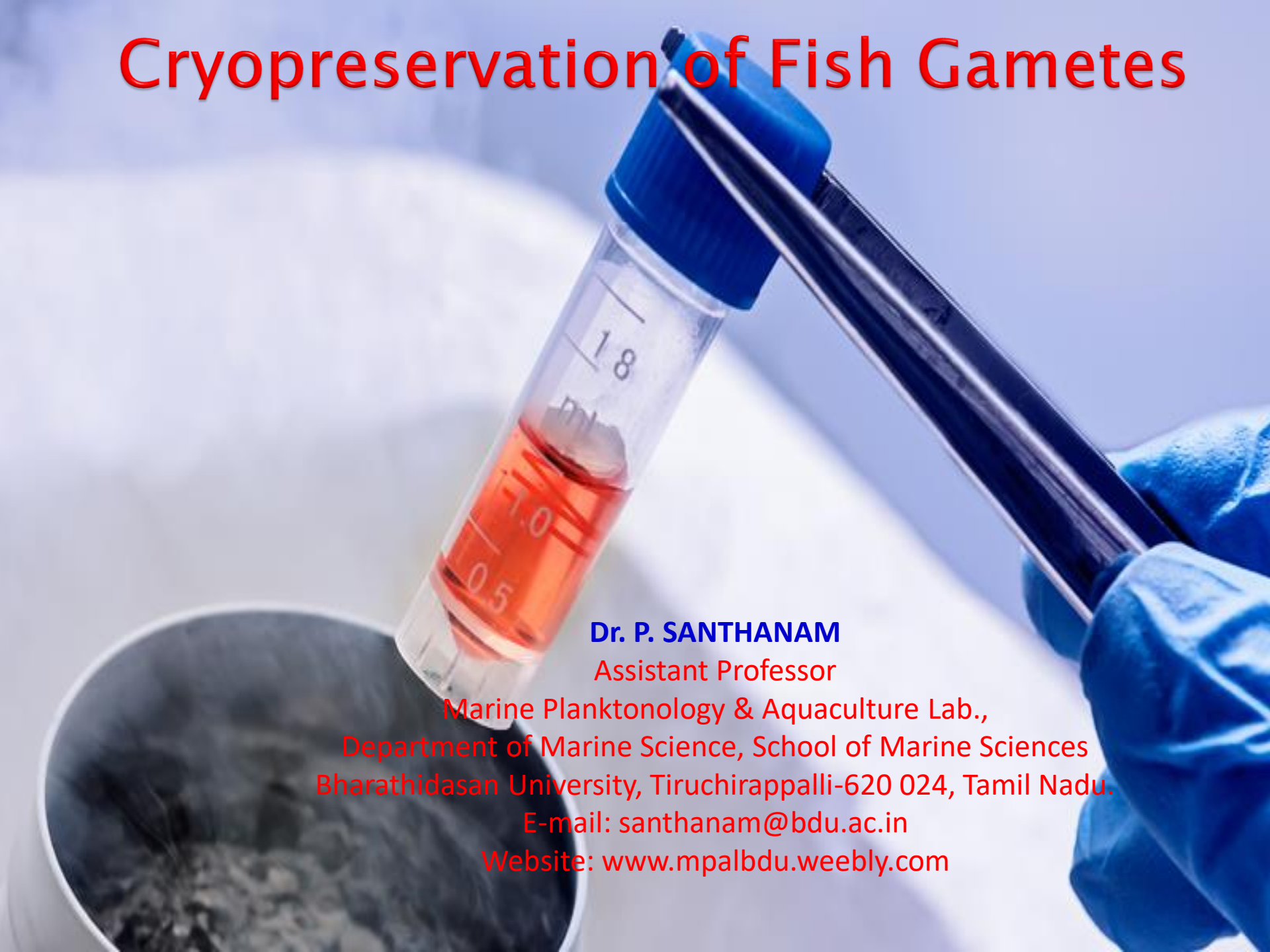
Cryopreservation of Fish Gametes



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Cryopreservation of Fish Gametes

- Preservation of living tissues/gametes/embryos in ultra cold storage temperature (-196°C).

- Freezing
- Storage
- Thawing

Cryoprotectant: protective chemicals

Extender: diluting solution

Types of Cryoprotectant

- Permeating: Glycerol, Dimethyl sulfoxide (DMSO), ethylene glycol and methanol.

- Non permitting: polyvinyl pyrollidone (PVP), glucose, sucrose, egg yolk, serum and skimmed milk.

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- Preparation of cryodiluent:

  5% sucrose with 20% glycerol or 10% DMSO or Modified Fish Ringer with 20% glycerol.

  Extender 1: Modified Fish Ringer: Nacl: 0.65g; KCl:0.30g

  $NaHCO_3$: 0.02g
  CaCl2-0.03g
  DDW: 100ml
  pH: 8.0

  Extender 2: 5% sucrose in 100ml double distilled water

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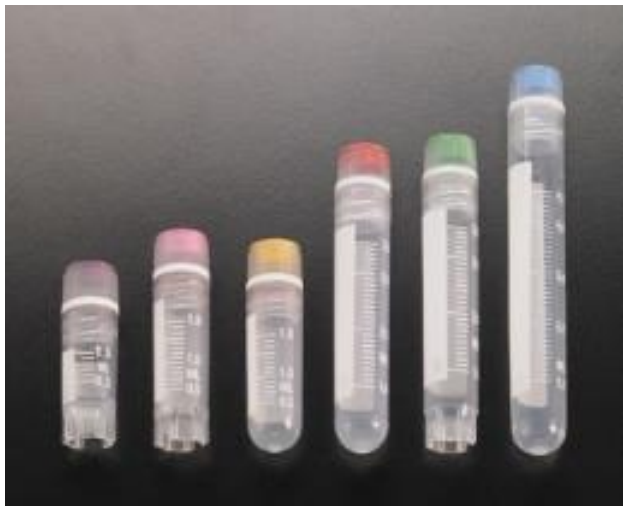
- The extender prepared and cryoprotectant added. The solution is called cryodiluent.
- Milt or egg will be collected. Collected milt or eggs will be stored in cryovials/tubes and kept in ice.
- The egg/milt will be mixed in the ratio of 1:3; one part of milt/egg and 3 parts of cryodiluent will be added.
- The known volume of milt/egg will be pipeted out and to that cryodiluent will be added and mixed well. Filing of the diluted milt in cryovials called ampouling .
- The time interval between mixing of milt and dipping into liquid nitrogen is called equilibration time.



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- **Freezing:**

A two stage freezing protocol will be followed.

1. **Pre-chilling:** the samples which will initially be kept in ice are first exposed to liquid nitrogen vapors at a height of 4-8cm from the level of liquid nitrogen in a ice box. The holding temperature will be about -60 to -70^{o}C. Pre chilling will be done for 10 minutes to prevent cold shock to the cells.

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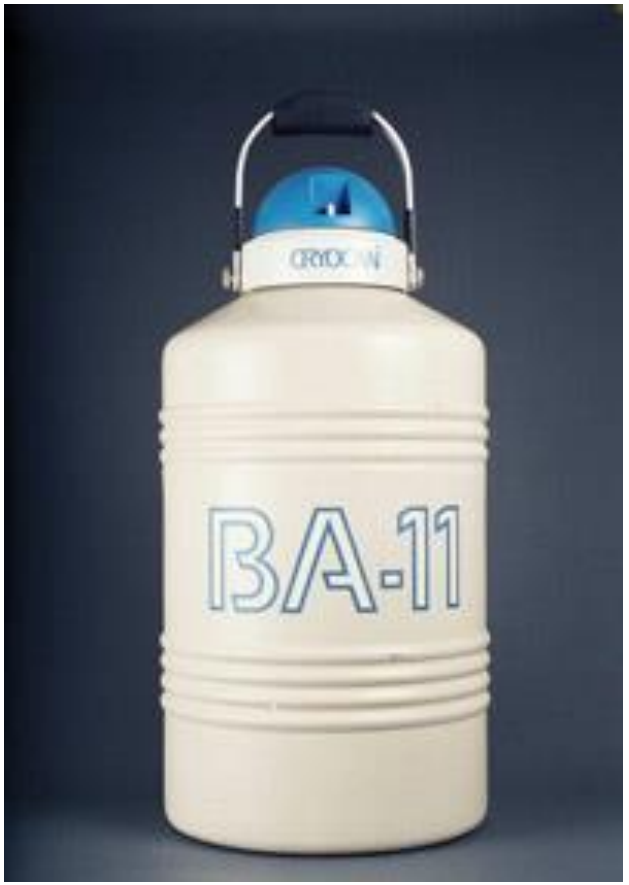
2. Dipping the samples in liquid nitrogen: After pre-chilling, the samples will be dipped into liquid nitrogen by placing them in labeled canisters.



One fourth of the canister is first filled with cotton, on which cryovial will be placed. The canisters again plugged with cotton and immersed in the liquid nitrogen can. Liquid nitrogen levels will be maintained by constantly replenishing, to compensate for loss through evaporation. Liquid nitrogen cans can be kept in cold room to minimize the loss through evaporation.

Thawing

- The cryovials will be taken out of the liquid nitrogen and thawed by dipping in Luke warm water (35-40^{o}C) for a few seconds. Seawater will be used for activation.