Report of the cryopreservation workshop (21 June 9-12h)

During the lectures, few questions were raised, as they would be asked during the round table discussion. A general conclusion for the diatoms was that the protocols needed to be optimized for each species, and this could probably be said for a part of the algal diversity.

The use of viability stains was discussed. JD uses neutral red, fluorescein diacetate (FDA) and carboxyfluorescein diacetate succinimidyl ester (CFSE) (latter stain has generally less background "flare" than FDA), CC uses 2-(4-Iodophenyl)- 3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT; light microscopy) with cyanobacteria, and SAG uses FDA. JD advises to wait 24h after retrieving the cryopreserved stocks before trying the viability stain. CC is quite negative on the use of INT as an early indicator of future survival of cyanobacteria.

The lack of reproducibility was addressed. Indeed, there is variability in the material to be preserved, in the equipment and cooling devices used, and then, the human errors. JD indicates that different straws and different cryovials from the same batch of samples could show variable results, not least because each may have experienced a slightly different thermal history.

A discussion on the use of AFLP indicated that the method required high quality, high molecular weight DNA and axenic cultures. With this method it was not easy to get reproducible results.

About the growth phase of the culture to be cryopreserved: the culture should be healthy. JD advises the late logarithmic to early stationary phase. He also suggests to acclimate the cultures to colder temperatures, in order for the membranes to modify their composition and become more resistant, old papers including those by John Morris et al. indicate that this has a significant influence on viability. However, this did not help when it was tested with marine species, but the reason might also be that there were fewer cells at 5°C.
Practically, when JD started at CCAP, his colleagues would let the cultures sediment at the bottom, and they would pipet the 'green paste' to start cryopreservation. Indeed, his experience is that it is better when people pipet dense cell suspensions, as one can obtain a healthy culture for dispatch to customers more quickly. However, at UTEX, they look for

optimal cell densities. Work undertaken there on *Chlamydomonas* suggests there is an optimal cell density. It may be worth paying attention for the Volvocales, where it seems possible that lytic enzymes are released by damaged cells during the cryopreservation process when the cell densities are high and this could be a negative factor for cryopreservation. The evidence is not fully conclusive, but there was an effect shown by Crutchfield et al. (1999, Eur J Phycol 34 : 43±52).
ML indicates that a colleague working in biotechnology told her to get the highest possible densities for cyanobacteria.

WV asks if there could be too high concentrations of CPA?
A question is raised: whether there could be small gradients of CPA when there is a thick algal paste? JD answers that a normal algal suspension would correspond to 0.2 g/L of dry weight, so quite little cell biomass in suspension. It this is concentrated and reach 200 g DW per liter, it is still quite fluid. Therefore, the existence of gradients is not likely.

WV asks about the production of dormant stages by some algae. JD recognizes that this induces an additional complexity in the cryopreservation protocol. For ex., the aplanospores of *Haematococcus* are bigger and less permeable than the vegetative cells, and thus, the treatment with CPA must be 15 minutes longer.

Another problem can be caused by intracellular structures, like vacuoles. However, there is not much published on this, as it is impossible to drill down on each problem. JD highlighted the problems associated with replicating methods when equipment procedures and cultivation regimes may have changed over the years. He tested methods designed long ago by Ellen Simon who worked at ATCC (50-70ies). JD tried to cryopreserve *Paramecium* and *Tetrahymena*, using the published methods. Though the cells first survived, due to damage to the contractile vacuole function, after some time, the *Tetrahymena* died.. ML thought that DMSO was more harmful than methanol when there were vacuoles.

WV asked more information on the use of antioxidants, on which John Day had talked in his lecture. JD answered that the addition of antioxidants in the recovery medium had been tested at the start of the COBRA project and was promising. However, there was no further study. JD thinks it could be used for plant cells, plantlets, algae. It has been shown to improve the survival and recovery for animal cell lines.

As the recovery phase is important, JD suggests to put the culture in the dark for 24 hours or more, then to ramp up the light intensity progressively and then, to try to improve. In the case of non pure cultures, it could be useful to add some antibiotics, to help the targeted algae not to be overgrown by bacteria.

A participant from Nigeria (CO) asked how to transport material collected if there was no cryopreservation facility available? JD answers that it is best to send live material. In summer or in hot conditions, it is good to use a cryobox for the expedition. Of course, take care of the regulations. For ex. for class 1 organisms, they should be in sealed container with enough absorbent paper in the case of a leakage.