3. PREPARATION OF DIATOMS FOR MICROSCOPY

3.1 Introduction

This protocol describes how to prepare permanent slides from benthic diatom samples collected from rivers. For ease of identification, all internal contents of the frustule (chloroplast, cytoplasm etc.) need to be removed, along with extraneous organic materials that were included in the sample. The "cleaned" valves (digestion usually separates the two valves of the frustule) are then mounted in a special mountant with a high refractive index in order to make it easier to see surface ornamentation such as striae. The outcome is a permanent slide which can be stored for future reference.

There is not a "right" way to clean diatom frustules and several methods are in common use. For this reason, a single protocol will not be defined for the DARES project. Any method will be acceptable so long as the resulting slide meets the following criteria:

- Organic matter completely removed from valves
- Mountant properly cured
- No air bubbles in mountant
- Mountant spread right to edge of coverslip
- Distribution of valves on coverslip not significantly clumped and no significant edge effects (i.e. the density of some diatom taxa is greater at the edge of the strew than in the centre).
- Foreign matter in sample either absent or insufficient to cause problems in identification or enumeration of the specimens.
- Ideally 5-15 valves per field of view, but not less than 1 valve and not more than 20 valves per field of view.

Two methods are described in the revised *TDI Manual*. (Kelly *et al*., 2000) but other methods based on strong oxidising agents are also suitable. References for the two methods included in the *TDI Manual* are Hendey (1974) and Battarbee (1986), whilst Barber and Howarth (1981), Round (1993) and BS EN 13946 describe alternative approaches.

Samples should be labelled with the following information: DARES sample number, stream name, reach name, date of sample collection, substratum and the initials of the person responsible for slide preparation. Avery labels J8651 (38.1 × 21.2 mm) are recommended as they can be printed using an inkjet or laser printer. If this is not possible, use a fine-tipped pen on a gummed slide label),

3.2 Health and Safety Note

All methods for cleaning diatom frustules use one or more oxidizing agents, which are, by their very nature, highly reactive and/or explosive. **For this reason those preparing diatoms for microscopy must be fully conversant with the appropriate health and safety guidelines before they start and Good Laboratory Practice must be followed at all times.** Methods are not inherently dangerous if proper precautions are followed.

3.3 Archiving diatom slides and samples

Diatom slides provide a permanent historical record of conditions at a site and should be stored in order to ensure that they can be accessed for future analyses. For the DARES project, two slides should be prepared from each sample. One of these should be retained in the laboratory where the original analysis was performed and the other submitted to Bristol initially for harmonisation and subsequently for lodgement in an appropriate herbarium.

Storage of suspensions permits additional slides to be prepared in the future, and for further analyses (e.g. by scanning electron microscope) to be performed. For long-term storage, diatom material should be allowed to settle, the supernatant decanted and the material resuspended in methanol or ethanol. Suspensions should be stored in airtight glass vials and retained in the laboratory where the original analysis was performed until further notice.