# Methodology

## • Data Acquisition

**Data acquisition** includes all methodologies involved in the collection, processing and analysis of raw material or raw data up to the point where data are generated.

#### A. Data Collection

 Describe the gear/instrumentation used giving name, gear code and details of its deployment. Please include references, e.g. Spade box corer (SBC) modified 0.25m<sup>2</sup> USNEL box corer, Hessler & Jumars, 1974.

See InstrumPVM3 file for calibration date and instrument references.

The moored system The Underwater Video Profiler (UVP) was constructed in the Laboratoire d'Océanographie Biologique et d'Ecologie du Plancton Marin in Villefranche sur mer, France (UPMC/CNRS) with the support of the CNRS (Centre National de la Recherche Scientifique) and the European MAST II and III programs. The UVP has been developed for the acquisition of large-particle (> 240  $\mu$ m) abundance and size distribution data from 0 to 1000 m. Three models have been constructed since 1990 (Gorsky et al. 1992). They were designed to minimize the disturbance of the illuminated volume in order to reduce a possible disruption of imaged particles. All models are autonomous and can be lowered on any hydrological cable. The third model is described here.

The UVP model III is a vertically lowered instrument mounted on a galvanized steel frame  $(1.1 \times 0.9 \times 1.25 \text{ m})$ . The lighting is based on two 54W Chadwick Helmuth stroboscopes. Two stainless steel mirrors spread the beams into a structured 10 cm thick slab. The strobes are synchronized with two Exavision XC 644 blackand-white CCD video cameras with 12 and 6 mm C-mount lenses. The illuminated particles in a volume of respectively 1.3 and 6.5 liters are recorded simultaneously onto two Hi8 recorders. The cameras are positioned perpendicular to the light slab and only illuminated particles in dark background are recorded. The short flash duration (pulse duration =  $30 \ \mu$ s) allows a fast lowering speed (up to 1,5 m/s) without the deterioration of image quality. Four 100 W spotlights can be used instead of the stroboscopes for continuous observations of a larger non structured water volume. In this case the lowering speed is slower. Depth, temperature and conductivity data are acquired using a Seabird Seacat 19 CTD probe (S/N 1539) with fluorometer and nephelometer (both from Chelsea Instruments Ltd.). The system is powered by four 24V batteries and is piloted by a Texas 370 microprocessor. The data acquisition can be time or depth related and programmed prior to the immersion.. The UVP is well adapted to count and measure fragile aggregates such as marine snow as well as delicate zooplankton.

### 2. Describe the techniques used for positioning the instrumentation.

The depth of the images is obtained with the SBE19 probe fixed in the main frame and geographical position by the ships instruments (mainly GPS).

### 3. Please comment on any limitations associated with using this sampling strategy.

Daylight can modify the optical properties of particles in the upper 10-80 meters. The depth range of this layer depends on the characteristics of the light penetration. Therefore, data analysis starts at depth where the measured background value of daylight remains identical to that of night profiles or to that of deep layers, not influenced by changing light regimes.

### **B. Sample Processing**

1. State whether sample was processed in-situ, shipboard or laboratory. Give the name of the Laboratory where samples are consistently processed if other than your own.

Samples consist of Hi8 video tapes and CTD data, see data processing below.

## • Data Processing

**Data processing, modelling and analysis** includes the further manipulation, processing or enhancement of this generated data.

### C. Data Processing, Modelling/Analysis

1. Describe clearly the different stages in processing and analysing the data.

The UVP has two important features: a) it does not disturb the recorded particles or organisms and b) it allows quick data retrieval and processing. Processing of images obtained by the UVP in the structured light beam is automated. Onboard, a rapid image analysis is carried out at a rate of 5 or more images per second (depending on the computer specifications). The recorded profile is digitised without compression using a Matrox frame grabber (512 x 512 x 256 pixel matrix). The images are analysed and treated automatically by custom-made software. The objects (> than 3 pixels) in each image are detected and enumerated. The area and maximum length of every individual object is measured. Data are stored in an ASCII file and can be combined with the associated CTD, fluorometer and nephelometer data (Seasoft Software) using a spreadsheet software. Vertical profiles can be printed out onboard, approximately 30 min. after the recovery of the UVP.

The complete profile, consisting of approximately 25 000 images (0-1000 m profile at a 1m/s lowering speed and at the acquisition rate of 25 images/s) is treated in the laboratory by two custom built programs. The first, written in Visual C++ (Microsoft), digitises the images from a Hi8 player at normal speed and without compression and performs the image analysis. Data concerning the number of particles per image and their attributes are stocked in an ASCII file. The second program (MATLAB, Scientific Software) is used for data treatment and presentation.

The results of the calibrations indicate that the tested configuration can detect 240  $\mu$ m-sized particles and can reliably measure particles larger than 460  $\mu$ m in diameter. The metric surface as a function of the pixel surface for the 12 mm and 6 mm lens cameras can be expressed by the following equations:

#### 12 mm : Y= 0.02 X 1.137, r2= 0.873; 6 mm : Y= 0.008 X 1.556, r2=0.828.

The calibrations were carried out in a dark test tank filled with 3 m3 filtered (20  $\mu$ m) sea water. The brightness measured in the test tank was similar to that in the aphotic layers. A calibration grid, placed at different depths of the light slab, was used to estimate the recorded water volume. The dimensions and volume of the parallel light beam recorded by the 12 mm and the 6 mm cameras are 131.6 x 125 x 95 mm representing 1.33 litre, and 288.5 x 264.7 x 99.25 mm representing 6.51 litres respectively. The pixel/mm relationship was calibrated in a test tank by injection of biological particles (range 90  $\mu$ m - 20 mm) measured prior to their use with a stereomicroscope (Gorsky et al., Estuarine, Coastal and Shelf Science, in press).

Bodies of zooplankton might be recorded and considered as particles. We analysed and compared zooplankton profiles with profiles of particles at sea (Stemmann et al., Deep-Sea Research, in press). The number of living organisms was found to be one or two orders of magnitude lower than that of large non-living particles.