MEASURING AND MODELLING THE DYNAMIC RESPONSE
OF REMOTE MOUNTAIN LAKE ECOSYSTEMS TO
ENVIRONMENTAL CHANGE

A programme of **MO**untain **LA**ke **R**esearch

MOLAR

PIGMENT AND CNS IN SEDIMENTS

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Pigments and CNS in sediments

Work Package 3

1. Field and shipping instructions

During the field work, the sediment should be protected against direct sun light and excessive warming. After slicing the core, the sediment samples should be deep frozen (-20°C) as soon as possible and shipped frozen to Pallanza.

As regards pigment stability, the frozen samples are stable for ca. 6 months. Sediment samples could be sent in an "ice-cream" like box with dry ice. Warning: Be sure that the ink or label on the bags are resistant to very low temperature such as with dry ice.

We need some basic data from site operator on each sample: Water content and Loss On Ignition at 550C.

The amount of sediment needed to perform pigment and CNS determination is ca. 3 g of fresh sediment.

2. Laboratory Methods

Algal and bacterial pigments will be extracted (overnight, at 10°C, in the dark and under nitrogen) from ca. 1 - 2 g wet sediments using 90% acetone and then centrifuged at 3000 rpm for 10 minutes in 15 ml glass centrifuge tubes. Duplicate samples will be extracted with HPLC-grade Acetone 90%.

Chlorophyll derivatives (CD) and total carotenoids (TC) will be extracted with 90% acetone and expressed as in Guilizzoni et al. (1983) and Züllig (1982), respectively.

Specific pigments will be determined by ion-pairing, reverse-phase HPLC (modified from Mauntoura & Llewellyn, 1983, and Hurley, 1988) and expressed as nanomoles per gram of organic matter. The ion pairing (tetrabutyl ammonium phosphate 10^{-3} M) allows for greater resolution of the dephytolated acidic chloropigments (Chl c, chlorophyllide a, and pheophorbide a). The equipment employed consisted of a gradient pumping system and dual channel variable wavelength UV-VIS detector (set at 460 nm and 656 nm for carotenoids and chloropigments, respectively) controlled by a computer (Beckman System Gold). An auto-sampler for sample injection was connected through a precolumn to a reverse-phase C18 ODS column (5 µm particle size; 250 mm a 4.6 mm i.d.). After sample injection (330 µl of acetone extract not dried in a rotary evaporator), a gradient program that ramped from 85% mobile-phase A (80:20, by vol. methanol:aqueous solution of 0.001 M ion-pairing and 0.001 M propionic acid) to 100% mobile-phase B (60:40, acetone:methanol) in 30 minutes with a hold for 20 minutes provided sufficient resolution of all pigments of interest. Flow rate from 1 ml min-1 to 2 ml min-1. The column was re-equilibrated between samples by linear ramping to 85% mobile-phase A for 5 minutes and maintenance for 10 minutes before sample injection. With this procedure, we are able to separate zeaxanthin from lutein and ϑ-carotene from phaeophytin *a.* Analysis of replicates sediment samples yielded at C.V. of 4.5% - 11.5%, depending on pigments.

Identification of all pigments was confirmed by comparing the absorption spectral characteristics and chromatographic mobility of pigments isolated from sediments with those obtained from TLC analysis (Züllig, 1982; Guilizzoni et al., 1986), commercial standards

(Sigma Chemical Co.), standards kindly donated by Hoffmann-La Roche of Basle, water samples of known phytoplankton composition, and published values (Foppen, 1971; Davies, 1976; Züllig, 1982; Mantoura & Llewellyn, 1983). Standard of okenone was obtained from culture of Chromatium okenii kindly provided by Dr. H. Züllig. Spectra will be obtained with a Perkin-Elmer Lambda 6 spectrophoto-meter.

Concentrations of pigments will be determined, following Mantoura & Llewellyn (1983), on the basis of molar extinction coefficients at the detection wavelengths. The molar extinction coefficient El%460 and E1%656 is derived from the E1% max reported in Davis (1976) and Mantoura & Llewellyn (1983).

Total carbon and nitrogen will be determined on dry sediment using a CNS analyzer (Carlo Erba). The inorganic C will be measured with the CNS analyser on a sub-sub.sample previously ignited at 550^{o}C. Total nitrogen is essentially organic N, since inorganic N is usually negligible (<2% dry weight).

3. References

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