# Blue Earth Global Expedition (BEAGLE - 2003)

# Leg 4

**Santos (Brazil) – Cape Town (South Africa)** 

November 6<sup>th</sup> - December 5<sup>th</sup>, 2003

# **Bio-optics Group**

Principal Investigator : Shubha Sathyendranath (Canada)

**Specialist in charge on board :** Vivian Lutz (Argentina)

POGO Trainees : Valeria Segura (Argentina)

Jaqueline Leal Madruga (Brasil)

**Observer for South Africa**: Nonkqubela Silulwane (South Africa)

#### 1. Introduction

The Blue EArth GLobal Expedition 2003, 'BEAGLE 2003', is an oceanographic research program developed by Japan Marine Science and Technology Centre (JAMSTEC). The principal objective of this project is to enhance oceanographic research activities in the Southern Hemisphere, in accordance with the Sao Paulo Declaration (POGO, 2000). These are pointed out as follows: 1) To detect and quantify temporal changes in the Antarctic Overturn System corresponding to the global ocean and the Southern Ocean warming during this century through high quality and spatially dense observation along old WHP (World Ocean Circulation Experiment Hydrographic Program 1991-2002) lines. 2.). To estimate the amount of anthropogenic carbon uptaken by the Antarctic Ocean. 3.) To provide a training environment in which trainees could get a hand-on experience in collecting biological, optical samples and optical data.

# 2. Bio-optical Objectives

The general objectives of the bio-optical project on this expedition are:

➤ To generate an important database of bio-optical measurements and primary production from the under-sampled Southern Ocean.

To reach this objective, measurements of radiation (seawater reflectance) are being measured with a variety of radiometers (Simbad, Simbada, Ocean Optics), samples are taken for the analysis of chlorophyll a concentration, and for the determination of absorption properties of particulate (phytoplankton and detritus) and coloured-dissolved-organic-matter (CDOM); P&I experiments are also performed for the estimation of primary production parameters. Samples for the determination of phytoplankton pigment composition by HPLC, as well as for the quantification and identification of the small-sized phytoplankton by flow-cytometry are also being collected. Results from these analysis are expected to contribute to the validation and calibration, and probably to develop regional algorithms, for satellite-derived products (eg., chlorophyll a) by sensors such as SeaWiFS, MODIS, and MERIS.

➤ To provide a training environment in which trainees could get a hands-on experience in collecting phytoplankton related samples and bio-optical data. To get a first knowledge about some of the analysis and processing of bio-optical data.

# 3. Sampling and Methods

Protocols for the sampling and methods being used for the optical measurements and analysis of biological samples can be consulted in the URL of IOCCG (http://www.ioccg.org/training/pogo\_ioccg/protocols/protocols.html).

Most of the samples are taken at the surface, or near surface, of the ocean, except for a second set of samples for chlorophyll analysis which are taken at the depth of the fluorescence maximum. Analysis of chlorophyll concentrations, particulate and CDOM absorption, and P&I incubations are performed on board, while HPLC, flow cytometry and <sup>13</sup>C (for the calculations of P&I parameters), as well as a duplicate of particulate absorption samples are going to be processed in different laboratories (in Canada, Chile, South Africa, and Australia) after the end of the cruise. A preliminary processing of some of the data available is being developed onboard.

# 4. Peculiarities of Leg 4

During this leg the main focus of the expedition was on chemical and physical oceanography. A total of 111 CTD casts were performed. For all the bio-optical sampling two stations a day were occupied, one close to noon and another about 4 hs earlier or later. Whenever possible seawater samples were taken from Niskin bottles at 5 m depth, or 10 m (when the weather was too windy). In a few occasions, when there were not enough Niskin bottles for all the required sampling, samples were taken from the surface using a bucket. An extra sample for the analysis of chlorophyll concentration, was taken from the depth of the fluorescence maximum (indicated by the *in situ* fluorometer attached to the CTD).

Radiation measurements (seawater reflectance) were performed about one hour before the rosette sampling. Unfortunately, after the first week of the cruise one of the radiometers, SIMBAD-03, stopped working.

#### I. BIOLOGICAL SAMPLING

#### Photosynthesis v/s Irradiance (PI) Experiments

Everyday 1 or 2 experiments were carried out onboard. 42 bottles (+ 3 dark) were incubated with <sup>13</sup>C in a Larsen box for 3 hours, then filtered and dried.

Storing: filters were labelled and stored in sets of 15 envelopes.

#### **CDOM**

Water for the determination of coloured-dissolved-organic-matter were filtered through 0.2  $\mu$ m membranes, and immediately scanned in a 10 cm quartz cuvette in a CARY spectrophotometer.

Storing: no samples were stored. Results are in folder JAMSTEC/CDOM/Leg4/dailyfolder

# **Chlorophyll Concentration**

Chlorophyll-a and phaeopigments concentrations were measured onboard using a digital Turner Designs fluorometer.

Storing: no samples were stored. Results are in folder JAMSTEC/Leg4/Chl/daily files

# **Particulate Absorption**

Two samples were collected and filtered through GF/F glass fiber filters for the determination of particulate absorption. One sample was immediately scanned on board in a CARY spectrophotometer, and the other will be analysed at the Bedford Institute of Oceanography (Att: Dr. Venetia Stuart).

**Storing**: Results of samples analysed on board are in folder JAMSTEC/Absorption/Leg4/dailyfolder. Duplicate samples were frozen in liquid nitrogen into a labelled cryogenic vial and then stored in a deep freezer (-80°C).

# **High Performance Liquid Chromatography**

Two samples were collected and filtered through GF/F glass fiber filters for the determination of phytoplankton pigment composition by HPLC. These samples will be analysed in 2 different laboratories: Cape Town (South Africa) and Hobart (Australia).

**Storing**: Both samples were frozen in liquid nitrogen and then stored in 2-separated labelled aluminium foil envelopes into a deep freezer (-80°C).

#### II. OPTICAL SAMPLING

#### **SIMBAD**

The hand-held battery operated radiometer collects data in five spectral bands that are centred at 443, 490, 560, 670, 870 nm. This instrument has an external GPS antenna and measures direct sunlight intensity and water leaving radiance. The GPS must first find the instruments position before readings can be made. The sequence of measurements are 1 Dark, 3 Sun, 6 Sea, 3 Sun, and 1 Dark.

*Storing*: The files are in the folder JAMSTEC/Leg4/simbad03/dailyfolder.

#### **SIMBADA**

This instrument is an above-water radiometer and it measures water-leaving radiance and aerosol optical thickness in 11 spectral bands. The bands are centred at 350, 380, 412, 443, 490, 510, 565, 620, 670, 750 and 870 nm. The instrument has an internal GPS antenna that must home in on 3 or more satellites before readings can be taken. The sequence of measurements are 1 Dark, 3 Sun, 6 Sea, 3 Sun, and 1 Dark.

*Storing*: The files are in the folder JAMSTEC/Leg4/simbada21/dailyfolder.

# Hyperspectral radiometer

This instrument measures irradiance from 350 to 1000 nm at 0.5 nm intervals and has a special fibre optic that collects the irradiance from the sky and the sea surface. The downwelling irradiance is measured using a spectralon that diffuses the incident irradiance.

*Storing*: Files are in folder JAMSTEC/Leg4/HyperSp/dailyfolder.

# **Photosynthetic Active Radiation (PAR)**

The PAR sensor is mounted outside, above the Atmospheric Observation laboratory. The Licor 1400 data logger connected to the sensor reads measurements every 60 seconds and records hourly average on the hour. Data are downloaded at the end of the leg to be later processed at BIO in Canada.

Storing: Files are in folder JAMSTEC/Leg4/PAR\_sensor\_data/PAR\_Leg4.txt

# 5. Pogo Trainees Activities

In this leg apart from the two official POGO trainees, Valeria Segura and Jaqueline Leal Madruga, we have to thank the participation of Nonkqubela Silulwane (observer for South Africa), who joined the bio-optical team. Valeria and Nonkqubela worked very hardly and enthusiastically learning all the bio-optical techniques. Jaqueline, concentrated on light measurements and some light-data processing.

#### **5.1** Trainees Remarks

### Nonkqubela Silulwane

It was an exciting challenge and experience to be onboard R/V Mirai and to be part of a bio-optic research team. Although I have been to a couple of research cruises before, I believe that this cruise is a major highlight of my sea-going experiences.

I got hands-on training in different studies such as P&I experiments, particulate absorption, CDOM, light measurements, as well as extensive CTD sampling. Such an excellent training came at a crucial time of my career when I am faced with a major challenge of starting my own research project in South Africa. With so much that I have learnt, now it is even difficult to choose which experiments to consider for my research project from the set of experiments that I conducted onboard as they are all important and of interest to me.

The hard work, perseverance and dedication from Dr Vivian Lutz making sure that I followed the sampling protocols and her clear explanations when I could not understand deserve a mention. Furthermore, it was such a pleasure and a privilege for me to conduct the experiments and be able to learn so much because of the other enthusiastic bio-optic team member (Valeria Segura) with high team spirit.

Special thanks goes to JAMSTEC for their financial support and giving me the opportunity to be onboard R/V Mirai. The training provided under POGO programme is highly appreciated.

# Valeria Segura

My experience on R/V Mirai was incredible and unique not only because I got professional training as a scientist but because I was also exposed and obtained a personal experience in living in an environment with people from different cultures, and this I will never forget.

I learnt a lot about biological and optical measurements because I worked with Dr. Vivian Lutz who has a lot of experience in this discipline and she is good as a teacher. In addition, I learnt how to take water samples from a CTD for different measurement such as dissolved Oxygen, CFCs, Salinity, PH, Total Dissolved Carbon, Alkalinity, Nutrients, Total Organic Carbon, etc.

I would like to thank POGO for giving me the opportunity to be trained onboard this fantastic research vessel because I have learnt a lot of things about routine sea measurements. I am also grateful to JAMSTEC for their wonderful research collaboration.

# **Jaqueline Leal Madruga**

My participation in BEAGLE 2003 was very important for my training in new technologies and methodologies about light measurements. This opportunity permits a integration with researchers of other countries of South America and South Africa that is very important to improve our relationship of work.

The PAR and ocean optics data set could be very useful in the validation of BRAZILSR Radiative Transference Model that estimates the PAR in Brazilian coast. This model was adapted for Brazilian coast by National Institute of Space Research, but it isn't validated yet.

I intend to use the light measurements background in the ANTARES Project that is a pionner project to install fixed oceanographic stations in the coast of South America.

# 6. Data Processing

A series of Fortran routines developed during Leg 3, were used to process the absorption data. The only modification to the routines is step (7), which applies a running average to try to smooth even more the data, since phytoplankton concentrations were extremely low during this Leg. They can be found in the directory /JAMSTEC/Leg4/data-process. These data can be later reprocessed to make any necessary adjustments, for example choosing a more appropriate Beta factor (once HPLC pigments would be available; see Stuart et al., 1998). This modification could be even more relevant for the data of Leg 4, where we could expect to find abundance of small cells, including perhaps some Prochlorophytes.

In the case of the particulate absorption, the first routine 'absorption\_n.for' retrieves the optical density values (OD) from the ASCII files produced by the Cary spectrophotometer in a format which can be read by Fortran. The second routine 'absorption\_f.for' processes the OD values following the steps described in the protocols. Basically:

- 1) it subtracts the value of absorption at 750 nm from the whole spectrum;
- 2) it averages the 10 replicates of each type of measurement;
- 3) it subtracts the averaged blank from the averaged sample absorption;
- 4) it organises the spectrum from the lower to the higher wavelength;
- 5) it corrects the spectrum for the Beta factor (using the equation proposed by Hoepffner & Sathyendranath, 1992; see reference in protocols);
- 6) it transforms OD into absorption (passing from log<sub>10</sub> to log<sub>e</sub> and considering the area and volume of filtration);
- 7) it smoothes the data by doing a running average every 5 nm;
- 8) it subtracts the detritus from the total to retrieve the phytoplankton absorption;
- 9) it calculates the specific absorption coefficient of phytoplankton (dividing the phytoplankton absorption by the chlorophyll *a* concentration of the sample).

The program generates four output files with the results of the processing:

SampleID+ABT.txt SampleID+ABD.txt SampleID+ABPHY.txt SampleID+ABSPHY.txt

CDOM data was not corrected on board. It will be processed after the cruise using the routines developed during Leg3.

# 7. Preliminary Results

This is just a preliminary analysis of some of the results obtained on Leg 4. The cruise track is shown in figure 1.

During this leg chlorophyll concentrations were extremely low (Table 1). As a result, although ~ 2 litters of seawater were filtered, some of the particulate absorption data lay close to the limit of detection of the spectrophotometer. This cause that spectra look somehow noisy (Figure 2). It is expected that duplicate samples, to be ran at the Bedford Institute (Canada) using a more sophisticated spectrophotometer (double beam and with an integrating sphere), may show a better resolution. Nevertheless, the data processed on board show some conspicuous features. This data set, consistent of 45 samples, showed no huge differences in their main characteristics. It was noticeable that total absorption coefficients, at their maximum in the blue, were one order of magnitude lower than spectra on Leg 3. The maximum value of total particulate absorption (ABT ~ 0.022 at 440 nm) occurred at stations A10-626 and A10-631 (close to the Brazilian coast), were similar to the lowest values found on Leg 3. Most stations showed low detritus absorption, indicating that total particulate absorption was dominated by phytoplankton absorption, typical of case 1 waters.

The specific absorption coefficients of phytoplankton ( $a_{ph}^*(\lambda)$ , ABPHY/Chla), were higher than those estimated for Leg 3. The values of  $a_{ph}^*(\lambda)$  (Figure 3), oscillated between ~ 0.1 and 0.2 for the maximum at the blue. This, would indicate the predominance of small cells, less affected by the packaging effect, in the phytoplanktonic community. Flow cytometric data of the samples collected will provide and excellent tool to identify and quantify this small fraction of the plankton.

# 8. Acknowledgements

We would like to thank people at POGO, IOCCG and JAMSTEC for giving us the opportunity of being able to participate in this exciting BEAGLE-2003 expedition, especially to Prof. Masao Fukasawa for his support and overall leadership of the whole expedition. The experience will be most useful to us in our future work. The good will and effort of Ana Claudia de Paula (official Brazilian observer) allowed us to obtain the necessary permit to sample in Brazilian waters. The 'engineering' support received from Luiz Nonnato, was invaluable: Muito obrigado. We want to thank Kathleen Peard and Bronwen Currie from Namibia, for their support on board. Special thanks go to Shubbha Sathyendranath, Venetia Stuart, Tony Paysant, Marie Helene Forget, Robert Frouin and Brian Irwin for their long distance support. We sincerely

appreciate the collaboration received on board from Yasushi Yoshikawa, chief scientist, Shuichi Watanabe, the whole sampling-team of JAMSTEC and Marine Works Japan, as well as the captain and crew of the R/V Mirai.

**Table 1.** Chlorophyll a concentrations in the samples of Leg 4.

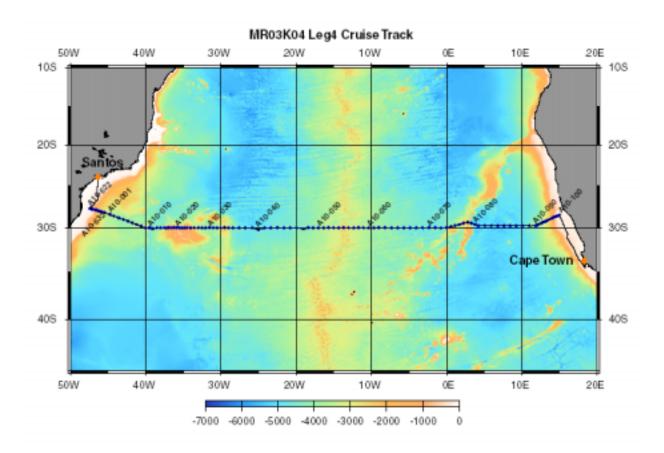
Number (Fig.2)	Station #	Sample ID	Depth (m)	Chla (mg m <sup>-3</sup> )
1	A10-623	264314	0	0.094
			5	0.120
2	A10-626	264315	0	0.148
			75	0.292
3	A10-631	264316	0	0.141
			70	0.179
4	A10-632	264317	5	0.112
5	A10-003	264318	5	0.075
			100	0.181
6	A10-004	264319	5	0.074
			130	0.130
7	A10-007	264320	0	0.071
			95	0.284
8	A10-008	264321	0	0.057
			105	0.201
9	A10X17-C	264322	5	0.067
			100	0.307
10	A10-013	264323	0	0.065
			100	0.215
11	A10 X23C	264325	5	0.067

			110	0.164
12	A10-18-C	264326	10	0.059
			125	0.204
13	A10-25-C	264327	5	0.076
			100	0.324
14	A10-26-N	264328	5	0.061
			130	0.192
15	A10-30-N	264329	0	0.066
			120	0.204
16	A10-31-C	264330	10	0.078
			130	0.157
17	A10-35-C	264331	5	0.060
			150	0.163
18	A10-36-N	264332	0	0.058
			150	0.159
19	A10-39-N	264333	5	0.053
			120	0.133
20	A10-X16-C	264334	0	0.056
			120	0.183
21	A10-42-N	264335	5	0.057
			130	0.233
22	A10-43-C	264336	5	0.044
			135	0.265
23	A0-46-N	264338	5	0.037

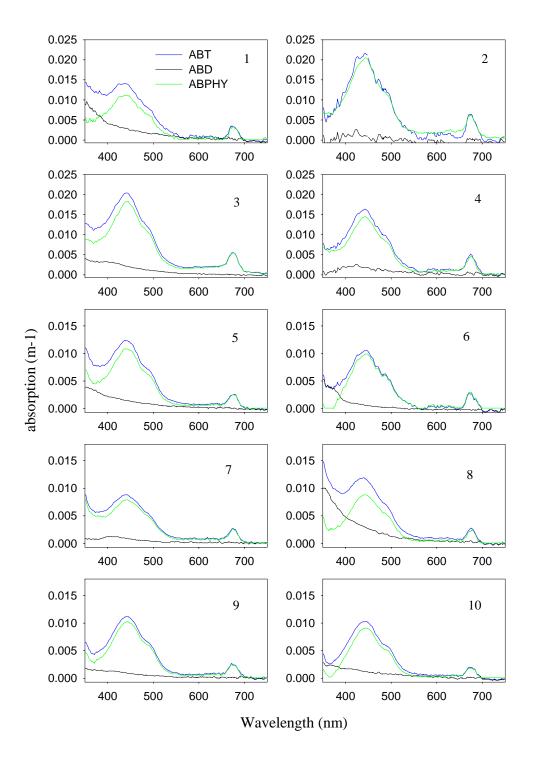
			150	0.171
24	A10-X15-C	264339	5	0.032
			160	0.213
25	A10-50-N	264340	5	0.034
			160	0.217
26	A10-51-C	264341	5	0.031
			150	0.163
27	A10-55-C	264342	5	0.033
			150	0.207
28	A10-56-N	264343	5	0.037
			155	0.215
29	A10-59-C	264344	5	0.033
			150	0.187
30	A10-60-N	264345	0	0.028
			122	0.196
31	A10-63-N	264346	5	0.038
			105	0.315
32	A10-64-N	264347	5	0.028
			90	0.328
33	A10-67-C	264348	5	0.035
			120	0.192
34	A10-68-N	264349	5	0.034
			100	0.265
35	A10-72-N	264350	5	0.103

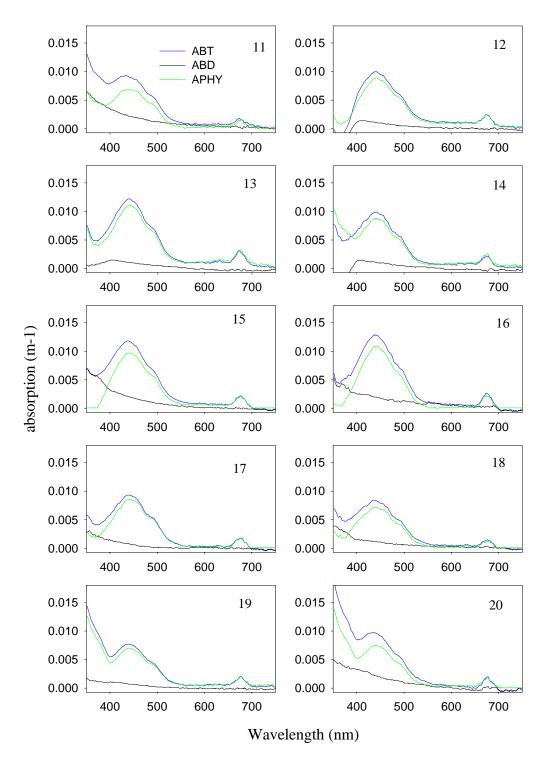
			50	0.241
			75	0.237
36	A10-71-C	264351	0	0.052
			50	0.116
37	A10-76-N	264352	5	0.040
			100	0.274
38	A10-77-C	264353	5	0.053
			115	0.222
39	A10-80-N	264354	0	0.048
			50	0.155
40	A10-81-C	264355	5	0.053
			80	0.210
41	A10-84-N	264356	0	0.059
			100	0.274
42	A10-85-C	264357	0	0.118
			90	0.251
43	A10-X13-C	264358	10	0.082
			75	0.229
44	A10-89-N	264359	10	0.085
			55	0.164
45	A10-91-N	264360	0	0.133
			50	0.256

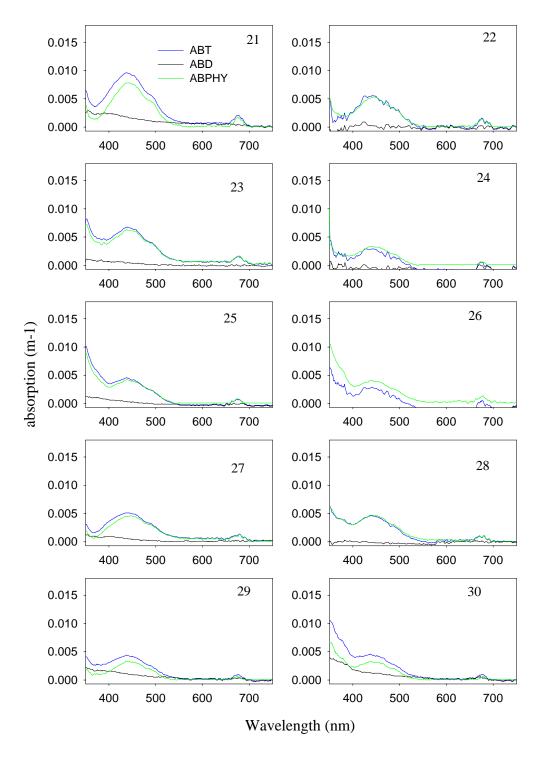
Figure 1. Cruise track, and stations positions in Leg 4.

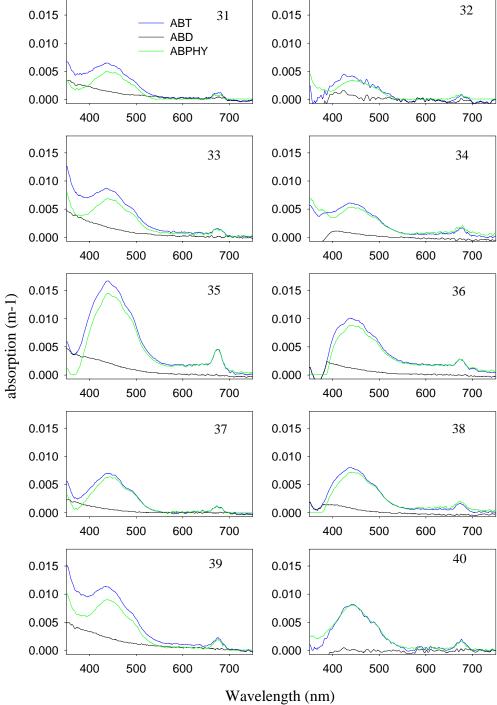


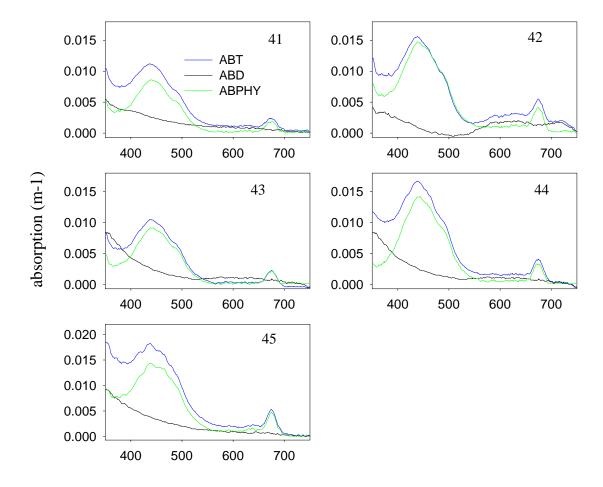
**Figure 2**. Absorption coefficients of total particulate material, ABT, detritus, ABD, and phytoplankton, ABPHY, in the samples of Leg 4.











Wavelength (nm)

**Figure 3.** Specific absorption coefficient of phytoplankton,  $a_{ph}^*(\lambda)$  in the samples.

