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Publications

The Nanostructural Network Analysis Organisation (NANO) Major National Research Facilty Annual Report. p12.

Paterson, H. L. and B. Knott (in prep.). The role of mixotrophy/autotrophy ratio in contrasting inshore/offshore environments: in the Indian Ocean off south Western Australia. (Intended for *Journal of Plankton Research*)

Paterson, H. L., B. Knott and A. Waite (submitted). Microzooplankton community structure, and herbivory on phytoplankton, in an eddy pair in the Indian Ocean off Western Australia. (Resubmitted after review to *Deep-Sea Research II*)

Paterson, H. L. and J.A. Koslow (in prep.). Microzooplankton: Biomass, abundance and composition covering lagoon, shelf and shelf break (1000 m deep) waters of temperate south western Australia, 2002 to 2004. (Intended for *Journal of Plankton Research*)

Paterson, H. L., S. Pesant, P. Clode, B. Knott and A. Waite (submitted). Systematics of a rare radiolarian - *Coelodiceras spinosum* Haecker (Sarcodina: Actinopoda: Phaeodaria: Coelodendridae). (Resubmitted after review to *Deep-Sea Research II*)

2.2.10 Diurnal variations in physical processes & phytoplankton response

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Executive Summary

Oligotrophy, high irradiance and consistent diurnal cycles in water column stratification and mixing in the coastal waters offshore Perth, Western Australia presented an opportunity to study natural phytoplankton community responses to an interesting combination of physical processes. This research aimed to determine the phytoplankton response for varying strengths of water column stratification and irradiance at sites with different exposures. We estimated the phytoplankton community response through examination of vertical chlorophyll *a* concentration variability and distribution on an hourly timescale, in vivo fluorescence fluctuations, *in situ* primary production experiments and measurements of photosystem II efficiency (yield). A combination of air–sea heat fluxes and wind stress control the vertical stratification strength, measured through the potential energy anomaly. In general, the water column is well mixed in the morning because of night-time cooling and convection and stratifies during the day in response to solar heating. Our field study showed that chlorophyll *a* concentration and in vivo fluorescence (which were 48 % correlated) were both lowest in the surface (1-5 m) during prolonged (hours) periods of stratification when the irradiance experienced was highest and phytoplankton were retained in the surface layer due to an absence of vertical mixing (generally around midday). Short-term *in situ* primary production rates (measured through 1-hour carbon uptake incubations and normalised to chlorophyll *a*

concentration) showed a diurnal trend with the highest production at midday in the surface, even though instantaneous measures of photosystem II efficiency (through yield) and relative electron transport rate (rETR) were depressed at the surface during this time. Chlorophyll *a* concentration and in vivo fluorescence were both highest at the sea bed (10 m) in summer and highest near the middle of the water column (5 m) in winter, showing an increase from morning to late afternoon at all sites. At the more exposed sites, or when the water column was well-mixed and did not stratify as strongly, chlorophyll *a* concentration and in vivo fluorescence were more constant throughout the day. We propose that the increase in chlorophyll *a* concentration at depth (at the least exposed sites) was a result of favourable light conditions there and possibly in combination with nutrient enrichment from the sea bed. This research also aimed to characterise the effect on phytoplankton community response of the strong summer sea breeze regime, where wind mixing results in a breakdown of stratification in the early afternoon and likely exposes phytoplankton to a range of light levels.

Introduction

Vertical mixing effects on phytoplankton biomass and productivity have been studied extensively and modelled for at least two decades, generally in the ocean surface mixed layer of the open ocean (e.g. Cullen and Lewis, 1988, Denman and Marra, 1986). When the rate of vertical mixing is greater than that of photoadaptation, phytoplankton will experience a range of light levels and their productivity will be of an intermediate nature (Falkowski, 1983). If, however, the mixing rate is slower than photoadaptation, productivity will reflect the phytoplankton's immediate light history. Diurnal vertical mixing and stratification therefore impact on net primary productivity since the phytoplankton production will not be the same for a mixed regime as for a stratified water column, often due to the non-homogeneous nature of nutrient distributions. Also, if the water column is shallow or highly stratified and the irradiance that phytoplankton consequently experience is sufficiently great for sustained periods, photoinhibition can occur, resulting in reduced productivity and lower biomass (Neale and Richerson, 1987). Nutrient limitation enhances the effects of photoinhibition (Prézelin et al., 1986)—a situation commonly found in nutrient-depleted surface waters and oligotrophic waters. Prézelin (1982) showed how low light phytoplankton populations survived better than high light populations when subjected to nutrient-limited conditions. Unfortunately, there have been few field studies conducted in oligotrophic, high light coastal waters to support these laboratory experiments. To investigate the diurnal density cycle and irradiance effects on productivity and chlorophyll *a* concentration in an oligotrophic environment, sites along the south-western Australian coastline were chosen, where diurnal variations in chlorophyll *a* were almost of the same order as seasonal scale variations (0.1 – 1 mg/m^3).

Aims

Our research aims to test the hypotheses that the diurnal cycle of density stratification and irradiance is directly related to chlorophyll *a* concentration structure and *in vivo* fluorescence in the water column, and that a response to these physical processes on a short (hours) time-scale is also seen in photosystem II efficiency (yield) and *in situ* primary production. We propose that the supra optimal light conditions and nutrient-limitation stress when phytoplankton are retained for prolonged (hours) at the surface, favours chlorophyll *a* to be synthesized at depth relative to the surface. Possible enrichment of nutrients continuously released from the sediments and taken up by phytoplankton immediately upon stratification (and therefore retention) at depth may also additionally contribute to the enhanced chlorophyll *a* concentrations particularly by the late afternoon, at depth.

Methodology

Field experiments were conducted in the shallow oligotrophic coastal waters of the Indian Ocean's eastern boundary. The region exhibits a low tidal range (maximum of 0.8 m), and currents are predominantly wind-driven (Pattiaratchi *et al.*, 1997). During winter, the coast experiences onshore winds and frequent (about 30 a year) storm events, whilst during summer there are offshore winds and strong (up to 15 ms^{-1}) sea breezes, commencing at approximately noon and weakening during the night (Pattiaratchi *et al*., 1997). Study sites were chosen for their degree of exposure (in terms of exposure to wave climate as well as coastal currents), where Mangles Bay was the most enclosed of the three sites, Warnbro Sound was semi-exposed, and Two Rocks was the most exposed site.

A mooring was deployed at each site for six to ten days to measure small-scale temporal changes in water properties. The mooring consisted of an acoustic Doppler current profiler (ADCP), conductivity-temperature-depth and dissolved oxygen sensor (CTD-DO), *in situ* fluorometer, and integrating light sensor. While the mooring was deployed, sampling was conducted aboard a boat, adjacent to the mooring, during daylight hours for two consecutive days. A CTD and *in vivo* fluorometer were deployed to obtain vertical profiles every 20 minutes. Water samples for chlorophyll *a* extraction from the surface, 5, and 10 m depths were taken hourly and filtered on board—2 L through a Nitex filter to capture the size fraction greater than 5 µm and 1 L through a glass fiber filter (GF/F) for all size fractions greater than 0.7 µm. Nutrient samples (for nitrate + nitrite, silicate, orthophosphate and ammonium) were taken hourly from the surface, 2, 5, 7, and 10 m and frozen for later analysis. *In situ* carbon uptake experiments were conducted four times during the second day of sampling at each site. Samples from the surface, 5, and 10 m were inoculated with 1 ml (40 µCi) ^{14}C and duplicates, plus a shaded dark bottle, were incubated *in situ* at the depth from which they were obtained. Incubations were ended (with formalin) after one hour and stored in the dark until the end of the day when they were transported to the laboratory to be filtered and analysed. Light sensors were fastened to the rope onto which the bottles were clipped to record the ambient light at each depth. A pulse amplitude-modulated (PAM) fluorometer was used to estimate maximum photosystem II quantum yield (Φ_e or F_v/F_m) and this was used to calculate the relative electron transport rate (rETR) and to create rapid light curves for dark-adapted samples from the surface, 2, 5, 7, and 9 m depths each hour. The curve-fit of Platt *et al*. (1980) was used to find maximum rETR and α (the initial slope of the curve).

Results

Irradiance

Irradiance on the Western Australian coast often peaks around 1500 µmol/m^{2}s during the day in summer and can reach 1000 µmol/m^{2}s at the surface in winter. Typical summer and winter extinction coefficients (k) were 0.153 ± 0.0145 s^{-1} and 0.317 ± 0.0590 s^{-1}, respectively. Irradiance for which photoinhibition is likely to start differs for various species of phytoplankton as a result of the variations in the size of light harvesting complexes associated with photosystem II (Juneau and Harrison, 2005). Primary production by natural populations of phytoplankton is reported to plateau around 200 µmol/m^{2}s and some are photoinhibited at greater irradiances (Harris, 1978). The phytoplankton community found in this coastal environment is likely to be adapted and acclimated to these high light conditions and could be expected to have well developed capabilities for photoprotection with limited photoinhibition manifest during the most extreme exposures to supra optimal irradiances.

Stratification & mixing

Three distinct stratification phases may be identified from the field data during the diel summer cycle in Western Australia (Imberger, 1985, Pattiaratchi et al., 1997, Zaker *et al*., 2002): (1) a relatively calm (wind) period of solar heating from about 0800 to 1200; (2) a period of wind mixing, due to the afternoon sea breeze, from about 1200 to 2300; and, (3) a period of penetrative convection from about 2300 to 0800. Time series of the potential energy anomaly (Φ) demonstrate the effect of this wind and solar radiation cycle on the water column density structure (Figure 2.39). The water column's progressive stratification during the morning and the start of de-stratification due to wind mixing in the afternoon is illustrated in this figure. The degree of stratification was found to be negatively correlated to site exposure. Previous studies have shown the eroding of stratification continues until the sea breeze has completely subsided (about midnight). There was a distinct gradient in the potential energy anomaly, from lower values (0.2) at the more exposed site (TR) to higher values (6.1) at the enclosed site (MB). Sampling at all sites was undertaken on calm days during winter. Again, a progressive stratification was observed at sites that experienced no wind mixing during the day (data not shown). Values of Φ ranged from 0.1 to 2.6 in winter—much lower than the degree of summer stratification (0.2–6.1). Unlike summer, no significant trend existed between Φ and site exposure.

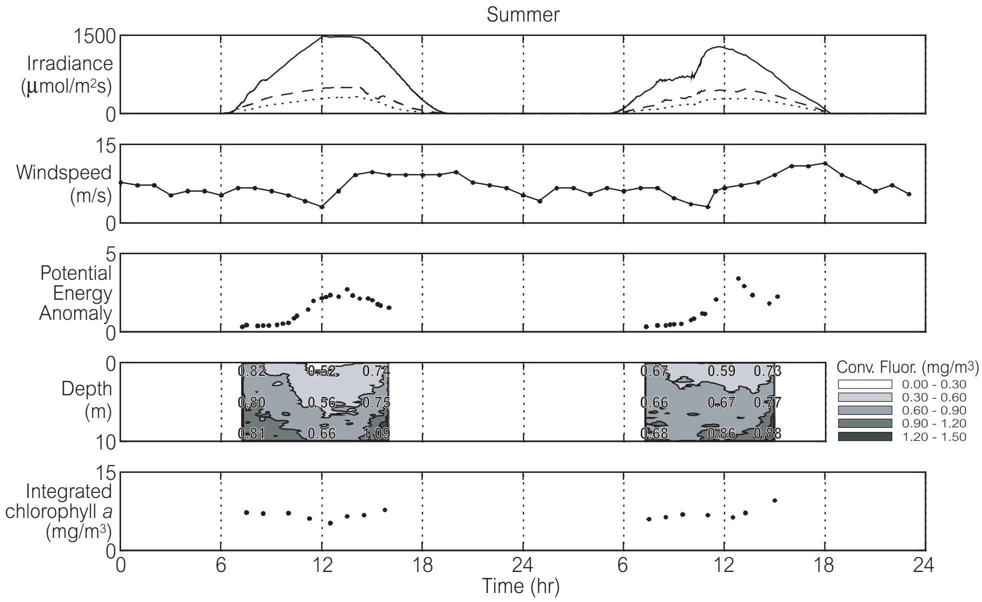

Figure 2.39: Summer (a) solar irradiance, (b) wind speed, and (c) potential energy anomaly, (d) chlorophyll *a* distribution with depth and (e) integrated chlorophyll *a*.

Chlorophyll *a* concentration and in vivo fluorescence

Chlorophyll *a* concentration (measured by filtering and extracting the pigments in samples) was used as an estimate (or proxy) of phytoplankton biomass. Although the chlorophyll *a* only explained 52 % of the variation in in vivo fluorescence, the latter was used to give an indication of how fluorescence fluctuated during the diurnal cycle with depth, and how this related to the chlorophyll *a* concentration variations. In summer, three stages of development of vertical chlorophyll *a* concentration structure and in vivo fluorescence signal were observed in concurrence with the diurnal stratification and the mixing cycle previously described (1) Overnight when the water column was vertically mixed, the chlorophyll *a* concentration was also homogenously distributed through the water column and in vivo fluorescence was constant throughout (Figure 2.39d). This feature was observed at all sites; (2) Progressive stratification of the water column through solar radiation was also common to all sites and during this time (morning to mid-afternoon) the chlorophyll *a* concentration gradually depleted vertically throughout and the in vivo fluorescence signal was very low, particularly in the surface waters, and this is clearly demonstrated in Figure 2.39e. The extent to which the integrated chlorophyll *a* concentration was depleted was directly related to the degree of stratification due to the intensity and quantity of light phytoplankton experienced at particular depths in the water column; and (3) During sea breeze events, experienced at only some sampling periods, the chlorophyll *a* was mixed through the water column, with the depth of mixing depending on the strength of the sea breeze. At sites that did not experience a sea breeze, the chlorophyll *a* concentration near the seabed continued to increase, and the water column showed vertical differences in chlorophyll *a* concentration and in vivo fluorescence by late afternoon. Sites that had a higher potential energy anomaly during the early part of the day had a higher integrated chlorophyll *a* concentration by late afternoon.

Winter chlorophyll *a* concentration was approximately double that of summer at all sites, as reported in other work (Lourey et al., 2006, Thompson and Waite, 2003). This is in response to the higher nutrient status in winter (Lourey et al., 2006). Spatial and temporal chlorophyll *a* and in vivo fluorescence trends were weaker than during summer, yet still showed definite periods that correlated to the diurnal stratification and mixing regime (data not shown). Overnight, the water column chlorophyll *a* concentration was homogenously mixed; resulting in a high integrated biomass and the in vivo fluorescence signal was also constant with depth. As the water column stratified (more weakly than in summer) from early morning to late afternoon, the same trends were observed as in summer, where chlorophyll *a* concentration was depleted in the surface waters, but stayed constant at the seabed and the in vivo fluorescence signal showed low values in the surface and higher values at depth. Generally, there was no mixing period during the afternoon due to the absence of a sea breeze. As was observed in summer, winter chlorophyll *a* concentration also increased in the water column by early afternoon and continued developing well into the night. The distribution of chlorophyll *a* concentration in the afternoon was not as localised near the seabed as in summer, but was higher near the middle of the water column.

Primary production and photosystem II quantum yield

Two techniques were employed to investigate photosynthesis rates and efficiency (1) *in situ* carbon uptake rate experiments through 1 hour incubations and (2) pulse amplitude-modulated (PAM) fluorometry. The former is a measure of the rate that carbon is taken up into the cell whilst the latter measures how well photosystem II transports electrons through the electron transport chain. Summer results showed that primary production (carbon uptake) that had been normalised to chlorophyll *a* concentration was highest at midday in the surface samples for the least exposed sites (Figure 2.40) and the trend was less pronounced at the most exposed site. Results from the PAM however showed yield was depressed around midday, especially at the surface. Maximum electron transport rate ($rETR_{max}$) was seen to be homogenous in the morning, highest at the sea bed around noon (and lowest at the surface) and increases at all depths to homogeneity by the end of the day (Figure 2.41). During winter, carbon uptake per unit chlorophyll *a* was highest in the surface and mid-water for the least exposed sites and again showed no clear trend at the most exposed site. Trends in winter for the PAM measurements mirrored those of summer but due to the lower irradiances, the depression in yield was only observed in the surface waters, and maximum yield was seen around mid-water rather than near the sea bed (winter data not shown). In summary, although the short-term measurements from the PAM showed a depression in yield when irradiance was highest and phytoplankton were retained at their respective light levels, production experiments revealed that carbon uptake per unit chlorophyll *a* was still greatest at the surface around midday. When the water column was well mixed in the morning, yield was vertically homogenous and was relatively high. As the water column progressively stratified and irradiance increased, yield decreased and was higher at depth relative to the surface. In the afternoon, when the irradiance again decreased and there was a sea breeze, the yield increased to a vertically homogenous high value.

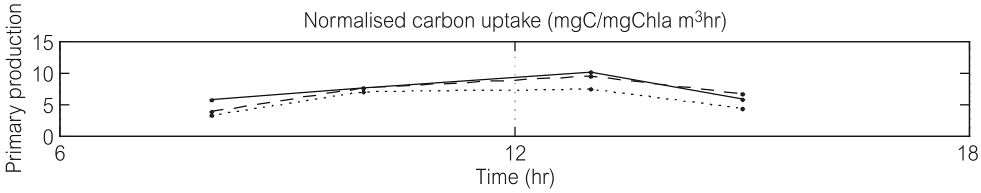

Figure 2.40: Summer carbon uptake per unit chlorophyll *a* (mgC/mgCha m^{3}hr) for surface 0 m (solid line), mid-water 5 m (dashed line) and sea bed 10 m (dotted line).

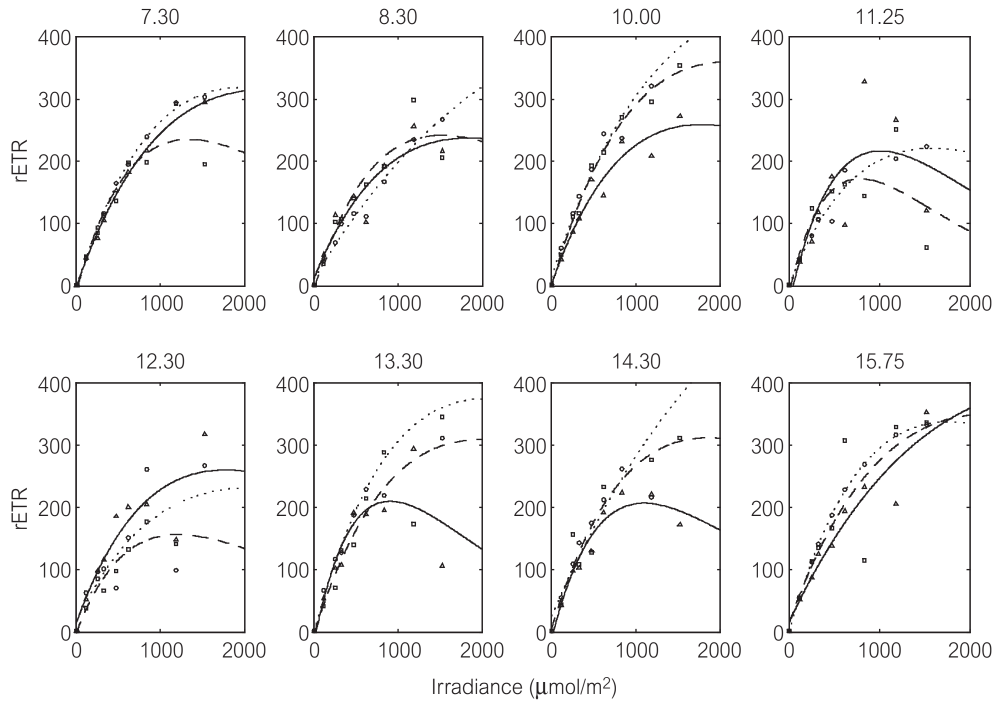

Figure 2.41: Summer relative electron transport rate (rETR) with irradiance for consecutive samples at surface 0 m (solid line), mid-water 5 m (dashed line) and sea bed 10 m (dotted line).

Nutrients

The nutrient analyser was not sensitive enough to measure the low nutrient concentrations, therefore we generally could not detect whether there were any significant relationships between nutrient concentrations and chlorophyll *a* concentration (especially in summer when concentrations were lowest). Our inability to measure these fluctuations did not mean that the concentrations that were present did not affect phytoplankton growth and variability; most likely the phytoplankton community that are present in this environment are well-adjusted to low nutrient availability and nanomolar concentrations are more likely the most significant scale to this community. For concentrations that were within the instrument measurement range (winter Si, PO_4, NO_x & NH_4; summer NO_x & NH_4 only), we performed ANOVA two-tailed t-tests between sampling depths (0 & 10 m, 0 & 5 m and 5 & 10 m) and between morning and afternoon samples to test for significant trends. Significant ($p < 0.05$) linear decreases were found in winter from morning to afternoon in integrated water column NO_x and NH_4 concentrations at the two least exposed sites. In summer, only at the least exposed site, surface NH_4 concentration was significantly ($p = 0.03$) higher at the surface relative to the sea bed. All other t-tests for comparisons between depths and times of day were found to be insignificant ($p > 0.05$). Although we could not measure higher concentrations of nutrients that may have been emanating from the sea bed, recent research has shown that there is a discharge here that may be of importance (Forehead 2006, PhD thesis).

Discussion

In this research we studied the temporal response of the phytoplankton community in a system dominated by diurnal physical processes. Limited research has been conducted in coastal regions such as this that exhibit extreme irradiances and oligotrophy. The results we have presented indicate that significant variations in chlorophyll *a* concentration and in vivo fluorescence both in the vertical and temporal scales are driven by diurnal cycles of irradiance, stratification and mixing, and possibly nutrient enrichment at the sea bed. Although no significant correlation was found between nutrient concentration fluctuations and short term chlorophyll *a* variation, nanomolar scale nutrients may be of importance to phytoplankton here, a scale of measurement that we could not capture with our instrumentation. Our results also provided evidence that photosystem II quantum yield and carbon uptake rate was directly correlated to the diurnal physical processes. Differences existed in the outcomes of this biophysical 'model' according to site exposure, degree of mixing and stratification, season, and possibly the phytoplankton community structure, but the underlying mechanisms remained constant. Although the most enclosed sites during summer generally experienced the highest degree of stratification and the most exposed sites during winter generally experienced the highest degree of mixing, there were atypical cases where stratification was observed at the more exposed site in winter and mixing occurred at the more enclosed site all day in summer. In these cases, where the typical seasonal conditions did not prevail, the biological response to the physical driving processes was more complex to define, but still followed the same general rules as the base case situations.

An interesting observation was the decrease of chlorophyll *a* concentration and lower in vivo fluorescence in the top part of the water column at most sites during the middle of the day, especially the most enclosed site during summer. It was realised the light intensity the surface phytoplankton must have experienced during these midday times was extremely high (often greater than 1500 μmol/m^{2}s) and prolonged for several hours as they were retained in the surface layer whilst the water column was stratified. Experiments conducted in freshwater Lake Titicaca (Peru/Bolivia) by Neale and Richerson (1987) revealed similar patterns in diurnal density stratification and mixing, high irradiances (surface ~ 2000 μmol/m^{2}s) and a depressed midday fluorescence response. After examining our PAM results, which showed depressed photosynthesis through lower yield and lower $rETR_{max}$, and considering the low background concentration of nutrients in the water column, it was deduced that, overall, the phytoplankton may have been photo-protecting themselves during these times. Primary production results (through carbon uptake experiments) however showed that during the highest light and most stratified times, phytoplankton from the surface were taking up the greatest carbon per unit chlorophyll *a* relative to the rest of the water column. This may mean that on a short-term (minutes) timescale, the community was contesting the high light through photo-protecting mechanisms (as seen in the PAM data) but on the longer (hourly) timescale they were not actually photo-damaged. Natural populations of phytoplankton are generally photoinhibited around 200 μmol/m^{2}s (Harris, 1978) but these studies are often from higher latitudes that have an overall lower irradiance profile.

Chlorophyll *a* concentration and in vivo fluorescence were greater near the seabed where there was more moderate light and possibly nutrient enrichment from the sea bed and there was a relative increase in chlorophyll *a* concentration and fluorescence towards the afternoon at most sites. This enhanced chlorophyll appeared to originate near the sea bed and increase progressively throughout the day, to then be distributed throughout the water column when there was a sea breeze. On days that did not exhibit a sea breeze, the higher chlorophyll *a* was retained at the depth of synthesis. Our study has shown the importance for phytoplankton community response of daily physical processes such as the irradiance and density cycle in retaining and mixing phytoplankton throughout the water column in shallow coastal waters.

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Conference attendance & presentations

Indian Ocean Marine Environmental Conference (IOMEC) 2005

Biotechnological and Environmental Applications of Microalgae (BEAM) 2005

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