Phytoplankton Bioassays: Technique Development for Assessing Bioavailability of Sediment Phosphorus in Aquatic Ecosystems

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I declare that this thesis does not contain any material which has been submitted by me previously for any degree or diploma at any university, and to the best of my knowledge, it does not contain any material published or written by another person except where due reference is made in the text.

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Abstract

Non-point source sediments with associated nutrients can enter aquatic ecosystems from agricultural runoff. *In situ* biogeochemical processes modify these nutrient and sediment inputs, altering the fraction of nutrients available for biotic uptake (ie. the bioavailable pool). Phytoplankton bioassays can assess the bioavailable pool of nutrients in aquatic ecosystems while chemical assays can determine nutrient conditions within agricultural soils. To assess bioavailable nutrients associated with sediments derived from agricultural soils, a comparison of biological assays and chemical assays was conducted. A phytoplankton bioassay was conducted to compare the effectiveness of *in vivo* chlorophyll *a* fluorescence, *in vitro* chlorophyll *a,* ^{14}carbon (^{14}C) uptake and cell concentration as measures of bioassay response. These were conducted in Eastern Moreton Bay and the Brisbane River Mouth. The nutrient additions were NO_3^- (200µM), NH_4^+ (30µM), PO_4^{-3} (20µM), SiO_4^{-4} (66µM) and a combination of all treatments. Correlations of *in vivo* chlorophyll *a* fluorescence and cell concentrations were high at both sites (r^2=0.89; r^2=0.72; $p<0.05$). ^{14}C uptake and *in vitro* chlorophyll *a* produced variable results possibly due to errors introduced with filtration. Bioavailable P was then determined for aquatic sediments extracted from 3 soil types collected from the Maroochy River catchment, South East Queensland, (Podzolic, Yellow Earth and Humic Gley) that were treated with four applications of P fertiliser (KH_2PO_4 at 0, 50, 100 and 200 kgP ha^{-1}). Sediments with a particle size less than 2µm were extracted by sedimentation of bulk soils. A preliminary sediment bioassay using Humic Gley sediments and *Skeletonema costatum*, as a bioassay organism, determined the effects of varying sediment additions (0, 50, 100, 200 and 500 mg L^{-1}). Examination of *in situ* soil bacteria was also conducted by sterilisation of extracted sediment with chloroform. Bioassay responses were assessed as the difference between the sediment *S. costatum* response and a no sediment addition. Bioassay responses were positively correlated with higher sediment additions and indicated no effect of soil bacteria. Phytoplankton bioassays were then conducted with unsterilised extracted sediments from the 3 soils using *S. costatum* supplied with excess NO_3^- (200µm) and SiO_4^- (66µm) with no P additions. Sediment Colwell P and $CaCl_2$ extractable P measurements were correlated (r^2=0.88 and r^2=0.71; $p<0.05$ respectively) with phytoplankton bioassay response. These results indicate that the protocols developed in the present study, can be applied to the assessment of bioavailable nutrients in aquatic ecosystems with chemical assays.

Introduction

Agricultural runoff has been implicated as a major source of non-point source of phosphorus (P) discharged into aquatic environments (Tafe, 1992). Erosion of agricultural lands produces sediments that are transported to marine and estuarine environments (Arakel et al., 1989). A substantial amount of adsorbed P is transported with these sediments. Within the aquatic environment sediment P and dissolved P are in a dynamic equilibrium (Froelich, 1988). The dissolved fraction contains a variety of compounds (mostly organic) with the inorganic P fraction being the most bioavailable (Cembella et al., 1984).

Bioassays are a methodological technique that utilises organisms to test a variety of ecological questions. Bioassays with ambient phytoplankton assemblages have been used to assess nutrient limitation of primary production and biomass (Fisher et al., 1994; Rudek et al., 1991; Paerl et al., 1990). Nutrient limitation is an established concept adopted from Liebig's Law of the Minimum which states that the nutrient in least supply is that which is limiting to production (De Baar, 1994). In marine and estuarine environments phytoplankton bioassays have shown that typically nitrogen (N) and P limits the growth and production of ambient phytoplankton populations (Ryther and Dunstan, 1971; Fisher et. al., 1992). Bioassays which use a selected test organism to assay the growth potential of a water mass are less common (Smayda, 1974).

Although numerous studies on phytoplankton bioassay experiments have been carried out in the past, the objectives, methodologies and applications of these studies have not been consistent (Elser et al., 1990; Fisher and Butt, 1994). This diversity of approaches makes the comparison of past studies difficult. In particular, there are several methodologies available to assess phytoplankton responses within bioassays. These include the radiocarbon tracer method (^{14}C uptake), enumeration of cell concentration, and chlorophyll *a* measurements. Little comparative work on these methods exists. Each method has advantages and disadvantages, and the appropriate technique needs to be selected for each particular application.

The bioassay technique used to assess phytoplankton nutrient limitation can be modified to assay the bioavailable nutrient fraction of sediments derived from

agricultural soils. Extraction of simulated sediments from soils enables a direct assessment of the impact of sediment nutrients in agricultural runoff. Simulation of runoff sediments requires the sorting of soil particles by size, to recreate the deposition of larger particles before input into aquatic ecosystems (Arakel et al., 1989). Few studies have been conducted with simulated agricultural runoff and bioassays (Sharpley et al., 1991; Huettl et al., 1979).

Bacterial interactions with phytoplankton have been suggested to have significant effects on the bioassay response. Underestimates of photosynthesis are thought to arise from variations in chemolithotrophic bacterial uptake in light and dark incubations (Carpenter and Lively, 1980). Bacterial immobilisation of N and P in bioassays has also been suggested to occur with the presence of nutrient poor organic matter (Thayer, 1974). Sediments derived from agricultural soils are likely to have varying concentrations of organic matter and *in situ* soil bacteria. The importance of bacteria in sediment bioassays is unknown however.

Sediments may act as a source of P if placed in nutrient poor seawater (Chase and Sayles, 1980). The amount of P desorbed will depend on the sediment type, in terms of surface charge and potential, and the ambient P concentration of the receiving water (Pailles and Moody, 1992). For some sediments, sorption may occur removing dissolved P from the water column. Without sorption-desorption experiments the sediment concentration range needed to elicit a bioassay response is unknown. Consequently, a certain sediment addition may either saturate or alternatively fail to provide a bioassay response.

The principle aim of the study was the development of phytoplankton bioassay methodologies to determine bioavailable P on aquatic sediments. This was accomplished via:

- A comparison study of four techniques frequently used to assess changes in phytoplankton bioassays.
- Determination of the amount of sediment required for a bioassay response.
- An assessment on the influence of bacterial activity in sediment bioassays.
- An assessment of the use of the estuarine diatom *Skeletonema costatum* as a bioassay organism to test bioavailable sediment P.

Materials and Methods

Methodology Comparison

A phytoplankton bioassay was performed to assess the appropriateness of 4 techniques as measures of bioassay response. The bioassay was conducted with ambient phytoplankton assemblages from the Brisbane River Mouth and Eastern Moreton Bay. These two sites were selected for comparative purposes as they differ significantly with higher values of chlorophyll *a*, total suspended sediments, nutrient concentrations and secchi depth at the Brisbane River Mouth site compared with the Eastern Moreton Bay site (Moss et. al., 1992). Sub-surface water samples (30 L) were collected from each site, returned to the laboratory within 3 h and subdivided into 6 4 L aliquots. Each sub-sample was filtered (200 µm) to remove large zooplankton and placed in a clear 6 L polycarbonate container. Nutrient additions consisted of NO_3^- (200 µM), NH_4^+ (30 µM), PO_4^{-3} (20 µM), SiO_4^- (66 µM) and a plus all treatments. An unamended sample was used as control. Nutrients were supplied once at the start of the experiment. All treatments were maintained in a flow through water bath to maintain a constant temperature of 24±1 °C and received 50% ambient irradiance with the application of neutral density screening. Bioassay responses were assessed daily for all treatments with *in vivo* chlorophyll *a* fluorescence, *in vitro* chlorophyll *a* concentration, cell concentrations and ^{14}C uptake.

Daily sub-samples were collected after gently mixing each treatment. Approximately 40 mL was collected in a test tube and placed in darkness for 30 minutes to allow for dark adaptation. *In vivo* chlorophyll *a* fluorescence of each sub-sample was measured using a Turner Design fluorometer (Model 10 series).

In vitro chlorophyll *a* concentration was measured in a 120 mL sub-sample for the first 2 d of the experiment. For the remaining incubation period duplicate 120 ml samples were collected. Each sub-sample was immediately filtered through Whatman GF/F filters, placed in foil and frozen. Chlorophyll *a* was extracted with 90% acetone and measured spectrophotometrically as described in Parsons et al., (1984).

^{14}C uptake was measured within a 480 mL sub-sample of each treatment daily. These sub-samples were divided into 4 120 mL polycarbonate bottles and inoculated with

$^{14}HCO_3$ (10 µCi). Two of these bottles were wrapped in foil and all 4 bottles were incubated with 100% natural light in a water bath kept at a constant temperature. Samples were filtered (0.45 µm) and placed in scintillation vials. Filters were analysed using a scintillation counter and ^{14}C uptake rates were calculated as in Parsons et al., (1984).

Cell concentrations was measured in a 120 mL sub-sample. Each sub-sample was immediately preserved with Lugol's solution. Cell concentrations were estimated using an Olympus CH2 microscope and counting chambers appropriate for the cell concentration observed (Palmer Maloney, Haemocytometer and Sedgwick Rafter).

Microbial and Sediment Load Effects

Simulated aquatic sediments were created from a Humic Gley soil collected from the Yandina area of the Maroochy River Catchment, South East Queensland. Sedimentation of bulk soil based on settling times calculated from Stoke's Law were used to extract clay particles (diameter<2µm) (Tanner and Jackson 1947). The soil was treated with 50 kgP ha^{-1} and 300 g of the soil was added to water to make a 1:4 ratio (total volume 1.2 L). This mixture was placed in a perspex cylinder and shaken vigorously. The cylinder was then allowed to stand without agitation for 4.5 min, after which the top 10 cm of the mixture was removed. After a further 4.5 min without agitation the second top 10 cm of the mixture was collected. This process was repeated four times and collected suspensions were centrifuged (20 min at 4000g). The supernatant was removed and the sediments were oven dried at 50°C for 2 d. Approximately half of the sediment yield was placed in a vacuum desiccator with a separate tray filled with chloroform for 24 h to sterilise the sediment. After exposure the vacuum was left on for a further 48 h to expel any residual chloroform.

Sediment bioassays were conducted in 1 L Erlenmeyer flasks with 500 mL of modified F/2 medium (Guillard and Ryther, 1962) which lacked P. Seawater used for medium was collected from Amity Point, North Stradbroke Island on an incoming tide and stored in 20 L opaque containers. Sediment additions were used as the sole source of P in the bioassays. Sediments were added to final concentrations of 0, 50, 100, 200 and 500 mg L^{-1}. *Skeletonema costatum* (clone CS167, Culture Collection of

CSIRO Division Fisheries Tasmania) was used as a bioassay organism. Stock cultures were maintained at 21°C in F/2 medium with continuous fluorescent light. When the stock culture was determined to be in exponential growth a sub-sample was inoculated into each treatment to give an initial density of $1*10^6$ cells L^{-1}. Changes in culture biomass were measured by cell enumeration over a period of 7 d and ^{14}C uptake was determined after 15 hours of incubation at low light intensities (~95µM quanta) as described previously.

Sediment Phosphorus Bioassay

Podzolic, Yellow Earth and Humic Gley soils were collected from the Yandina area of the Maroochy River Catchment, South East Queensland. These soils were chosen to assess the effectiveness of the sediment bioassay technique. 300 g of each soil type was fertilised with 0, 50, 100 and 200 kgP ha^{-1} using KH_2PO_4 to provide a range of P concentrations for each sediment type. To allow adsorption of P all sub-samples were brought up to field capacity, through addition of distilled water and incubated at 45 °C for 1 week.

Incubations were conducted in 6 L polycarbonate incubation vessels with 4 L of seawater enriched with NO_3^- (200µM) and SiO_4^- (66µM) and lacking P. The cultures were incubated with natural light attenuated by 50% shading using neutral density screening in a flow through water bath at 24±1 °C. Extracted sediments were used in the bioassays as the sole P source added to achieve a final concentration of 200 mg L^{-1}. A sample received no sediment addition and was used as a control. All treatments were inoculated with *S. costatum*, cultured as above, to an initial density of $1*10^6$ cells mL^{-1}. Changes in phytoplankton biomass were measured using *in vivo* chlorophyll *a* fluorescence as described above. Nutrient stimulated bloom potential for each treated soil was determined by the difference between the maximum biomass of the sediment bioassay and the control.

Chemical assays of Colwell P (P_C), using a $NaHCO_3$ extractant (Colwell, 1963) and $CaCl_2$ extractable P (P_D) (Warrel and Moody, 1984) were conducted on the simulated sediments collected from the treated soils. These were then plotted against the nutrient stimulated bloom potential of the sediment bioassays. P_C has been used to

determine bioavailable P in agricultural science (Moody et. al., 1997) and measures the quantity of bioavailable P. P_D a measure of P in the soil solution has also been correlated to bioavailable P (Moody et. al., 1990). P_D has also been used to assess sediment P desorption (Pailles and Moody, 1992).

Results

Methodology Comparison

Nutrient limitation was evident at both sites with cell concentrations. Phytoplankton concentrations in the Brisbane River Mouth bioassay (Fig. 2a) were two orders of magnitude greater than the Eastern Moreton Bay bioassay (Fig. 2b) in all treatments. Light limitation was evident in the Brisbane River site as substantial growth occurred in the controls after 3 d. After 4 d the NO_3^- addition elucidated a response although this was less than the plus all treatment. This indicates that the phytoplankton community of the Brisbane River Mouth site was primarily limited by NO_3^-, with a synergistic interaction with the addition of NH_4^+, SiO_4^- or PO_4^{-3}. In the Eastern Moreton Bay site responses with single nutrient additions did not differ from the control. A response was evident in the plus all treatments with phytoplankton cells concentrations three times higher than the control. This indicates that there was a combined nutrient limitation of NO_3^-, NH_4^+, SiO_4^- or PO_4^{-3} in this site. Similar bioassay responses were evident when assessed with *in vivo* chlorophyll *a* fluorescence (Fig. 2c and 2d).

Appreciable variability was evident in the bioassay responses using *in vitro* chlorophyll *a* measurements. A NO_3^- response was in the Brisbane River Mouth site (Fig. 2e), however no trend was evident in the Eastern Moreton Bay Site (Fig. 2f). This may be due to the low phytoplankton biomass in this site and the limitation on the amount of water available for filtration. The lack of replicates for the first 2 d may also have contributed to the variability with this method.

Considerable variability was evident with the ^{14}C uptake method (Fig. 2g and 2h). In the Eastern Moreton Bay site, several negative values were found suggesting that this method was also at the lower end of its detection limit. The Brisbane River Mouth site yielded similar results to those found with cell concentrations, however the primary limitation of NO_3^- appeared to be higher. Another anomaly was the considerably high measurements obtained after 2 d incubation. This variability may be due daily fluctuations of incident irradiance.

Significant correlations were found with *in vivo* chlorophyll *a* and the other methods within the Brisbane River Mouth site (Table 1). A significant correlation was also found with *in vitro* chlorophyll *a* and phytoplankton concentrations. A poor correlation was found with ^{14}C uptake and phytoplankton concentrations. This may have been due to the relatively high photosynthesis estimates after 2 d incubation. In the Eastern Moreton Bay site, *in vivo* chlorophyll *a* was significantly correlated with cell concentrations (Table 2). Poor correlations were found with comparisons of the other methodologies.

These results indicate that cell concentrations and *in vivo* chlorophyll *a* fluorescence are the good indicators of phytoplankton bioassay responses. Methods that utilise filtration introduce errors in bioassays with low phytoplankton biomass. These errors are avoided with cell concentration and *in vivo* chlorophyll *a* fluorescence since only a limited volume is required for the estimate. Poor correlations with ^{14}C uptake and cell counts in the Brisbane River Mouth may have been due changes in the rate of photosynthesis on a per cell basis between treatments or over time.

Microbial and Sediment Load Effects

After 1 week incubation phytoplankton cell concentrations with all treatments were significantly higher than the control ($p<0.05$). The bioassay response was positively correlated with sediment concentrations (Fig. 3). High sediment concentration responses were an order of magnitude greater than the controls. This response increased at a lesser rate as more sediment was added. This indicates a saturation of *S. costatum* with higher amounts of sediment P.

Sterilisation had no effect on phytoplankton cell concentration responses (Fig. 3). This indicates that the bacterial effect was negligible over the 1 week incubation. It also indicates that treatment of sediments by chloroform did not affect the long term growth of *S. costatum*.

The greatest stimulation of ^{14}C uptake for both sterilised and unsterilised sediments was evident at 50 mg L^{-1}. Higher additions of sediments resulted in either lower stimulations or repression of photosynthesis. Compared with the sterilised sediments, unsterilised sediments gave a greater stimulation for all sediment loads. Higher

additions of sterilised sediments reduced the bioassay response by a greater degree than was seen in the unsterilised sediments. This indicates that sterilisation of sediments had an inhibitory effect on photosynthesis of *S. costatum*.

Sediment Phosphorus Bioassay

Exposure to Humic Gley sediments resulted in the lowest response for all treatments (Fig 4a). Exposure to sediments collected from the Podzolic (Fig. 4c) and the Yellow Earth soils (Fig. 4c) resulted in a higher stimulation. A time lag difference was evident with the Yellow Earth sediments, with maximum populations being achieved after 8 days. Maximum populations of the other soils were observed after 5 days of incubation. The control with no P did not show any significant increase in biomass.

The bioassay response for each soil type was significantly correlated with P_C (r^2=0.88) (Fig. 5a) and P_D (r^2=0.71) (Fig. 5b). P_C and P_D were highly correlated to each other (r^2=0.92) however the measured values of P_C were similar to predicted values, while P_D had higher measured values for the samples amended with 200 kgP ha^{-1} (Fig. 6). This discrepancy in P_D measured values may be responsible for the lower correlations with the bioassay response.

Discussion

This study determined that *in vivo* chlorophyll *a* fluorescence was a good estimate of changes in cell concentration. Previous studies have related the fluorometric method of measuring chlorophyll *a* to changes in phytoplankton photosynthesis and biomass (Falkowski and Kiefer, 1986). However the high correlation seen with *initial* fluorescence and cell concentration in this study was unexpected. Initial fluorescence measurements have been suggested to be an inconsistent indicator of chlorophyll *a* resulting from variations in fluorescence yield (Roy and Legendre, 1979). The high correlation of cell concentration with *in vivo* chlorophyll *a* fluorescence in the present study may be explained by the experimental control of light. Variations with *in vivo* chlorophyll *a* fluorescence have been found associated with different light histories (Kiefer, 1973a; Vincent et. al., 1983). Phytoplankton bioassays maintained a constant light regime across all the treatments. Consequently, variation in cellular chlorophyll *a* content and fluorescence due to light was minimised, perhaps

accounting for the constant *in vivo* chlorophyll *a* fluorescence per cell for all treatments.

The results of the present study provided conflicting bioassay responses to sediment additions with ^{14}C uptake and cell concentration measures. This may be due to fundamental differences in the two techniques. ^{14}C uptake measures the rate of light induced carbon fixation as an indication of photosynthesis (Peterson 1980) while cell concentration is a measure of biomass. Variability between biomass and photosynthesis has been observed with the addition of oil, an energy source for heterotrophic bacteria (Roy et. al. 1991). Input of organic matter in the present study may have occurred with the addition of Humic Gley sediments. Greater concentrations of sediment in the present study could account for higher rates of bacterial activity, which had an inhibitory effect on light C fixation. The sterilisation of sediments would not have eliminated bacterial activity in the sediment bioassay since the *S. costatum* inoculum and nutrient media was non-axenic. Consequently, bacterial competition for nutrients (Thayer 1974) may be responsible for the response observed in the short term bioassay. Thus, the present study has demonstrated that ^{14}C uptake is not an effective technique for measuring changes in non-axenic sediment bioassays.

The observed decrease in ^{14}C uptake with sediment concentration may also be explained by light limitation. The particle size of the sediment addition was less than 2 μm. Based on Stoke's Law some of these smaller particles can stay in suspension for several hours (Tanner and Jackson 1947). Greater additions of simulated sediments will lead to higher concentrations of suspended sediments that can inhibit photosynthesis, and consequently light induced C fixation.

Higher concentrations of sediment produced higher bioassay responses, in long term experiments, presumably due to high P availability. This result demonstrates that the concentration of sediment is critical to the measurement of bioavailable P. Previous sediment bioassays have used values ranging from 2 g L^{-1} (Sharpley, 1993) to 200 mg L^{-1} (Williams et. al., 1980). The present study has demonstrated that comparative sediment studies need to standardise the amount of sediment addition otherwise

bioassay responses to sediment P concentrations will be confounded with sediment concentration effects.

Concentrations of sediment P as measured by Colwell P (P_C) and $CaCl_2$-extractable P (P_D) were significantly correlated to the bioassay response. This result was consistent with previous studies conducted with freshwater algae (Huettl et al., 1979; Williams et al., 1980; Robinson and Sharpley 1994). Resin-extractable P has been shown to be highly correlated to the bioassay response of *Selenastrum capricornutum* and *S. quadricauda* (Huettl et al., 1979; Williams et al. 1980). However application of this extraction method to marine and estuarine environments is difficult due to the high anion concentrations of seawater, which can affect the P extraction efficiency of the method. Other freshwater studies have shown that the P measured with Fe-oxide strip extractions (P_F) are also highly correlated to algal bioassays with the freshwater genera *Anabaena, Euglena* and *Selenastrum* (Sharpley, 1993a; Robinson et al. 1994). Application of P_F to marine environments has also shown a qualitative correlation to bioavailable P with seagrasses (Udy and Dennison 1996).

The measurement of P_C has been suggested to vary with the presence of carbonate sediments (Udy and Dennison, 1996). Variation in the P extraction efficiency with sediment type may limit the usefulness of P_C to assess the bioavailable fraction of sediments. P_D has been used to estimate rapidly desorbable P in sediments (Pailles and Moody, 1992), however its variability with sediment type is unknown. If P_D can be demonstrated to extract P without variation in sediment type it may be appropriate for assessing bioavailable P in marine and estuarine sediments. P_F has been found to be unaffected by sediment type however further tests are needed to assess its effectiveness in estimating bioavailable P (Udy and Dennison, 1996). Measurement of P_F on the sediments used in the present study can be completed to test this effectiveness.

The chemical characteristics of sediments are determined by the parent soil (Arakel et al., 1989). Humic Gley soils are characterised by the reduction of Fe under anaerobic waterlogged conditions. Brown and black mottles are present with ferric and magniferous concretions (Buol et al. 1989). Podzolic soils are characterised by chemical migration of Al, Fe or organic matter to the eluviated layer (Buol et. al.

1989). Presumably, sediments derived from these soils will be rich in cations that can strongly bind P. The Yellow Earth soil is characterised by low levels of iron and organic matter (Buol et. al. 1989). Sediments extracted from this soil type will presumably be low in Fe and Al. These differences in the chemical characteristics of sediments are likely to affect their ability to bind P.

The 3 soil types in the present study produced sediments with a concentration of adsorbed P that was commensurate with the P application response and dependent on soil type. The amount of sediment P in agricultural runoff has been found to vary with the chemical and textural composition of the parent soil, and the P fertiliser application rate (Romkens and Nelson 1974). Variation of inorganic P content of sediments has been closely associated with the iron (Fe) oxyhydroxide content (Strom and Biggs 1982). Other cations such as calcium, aluminium (Al) and magnesium (Mg) can also affect inorganic P by forming cation complexes affecting P bioavailability (Cembella et al. 1984). These complexation reactions are affected by the redox state of the sediments. Under oxic conditions, Fe oxyhydroxides and other cations have a strong affinity for P. In anoxic sediments reduction Fe oxyhydroxides and cations reduces this affinity (Krom and Berner 1980). The sediment bioassays in the present study were conducted under oxic conditions, thus any P associated with Fe would have not have been bioavailable. Consequently, the observed bioassay responses were due to the desorbable P fraction of the sediments.

Sorption or desorption of sediment P has been suggested to be mediated by the sediment equilibrium P concentrations and the dissolved P concentration of the water column (Pailles and Moody 1992). In the present study, the significant correlation of the bioassay response to the sediment P concentration indicates that the addition of sediments resulted in net P desorption.

SiO_4^- has been found to compete with P for sediment sorption sites in turbid rivers (Mayer and Gloss 1980). In the present study, SiO_4^- was added so that the siliceous diatom *S. costatum* was not limited by silica (Si) and this may have favoured P desorption. Conversely, uptake of Si may have inhibited P desorption. Variations in $[PO_4^{-3}]$ have not been found to affect $[SiO_4^-]$ sorption reactions in fluvial systems (Mayer and Gloss 1980), however the effect in estuarine and marine systems is

unknown. This is particularly significant for sediment bioassays that use diatoms, such as the present study, since Si can also limit the bioassay response.

Soluble inorganic P is generally assimilated faster than organic P (Paerl and Downes 1978). Variations in the inorganic and organic P fractions within the sediment bioassays may have occurred over time. Chase and Sayles (1980) found that successive sediment rinses with fresh seawater led to smaller amounts of P release. In the present study, biotic uptake of P may have a similar effect to rinsing. However estuarine sediments have been suggested to act as a buffer maintaining a constant soluble inorganic P concentration (Pomeroy et al., 1965; Froelich, 1988). Thus the variations in the inorganic P fractions are unknown. This may affect the bioassay response since organic P compounds can be utilised by phytoplankton (Cembella et al. 1984). To assess the use of organic P, another sediment bioassay using an alkaline phosphatase assay is needed.

This first step in demonstrating that sediment bound P can be assayed with phytoplankton needs to be extended further into the concept of non-point source nutrient loads. Standard phytoplankton bioassays use the addition of PO_4^{-3} however most P loadings occur as sediment P (Reddy et. al. 1978). The addition of sediments in phytoplankton bioassays would provide a more realistic simulation of nutrient loading.

Non-point source nutrient loads are delivered to marine and estuarine environments through hydrological means (Mallin et. al. 1993). Variations in salinity are likely to arise when these nutrient inputs enter estuarine systems (Christian et. al. 1991; Dennison et. al. 1997). An accurate simulation of non-point source nutrient loads would require sediment bioassay additions with different salinities. Variations in the NO_3^- supply have also been correlated with rainfall events (Mallin et. al., 1993; Thompson and Hosja, 1996). Future sediment assays with a variation in the ratio of added nutrients can be used to ascertain the extent of multiple nutrient limitations. This can be achieved simply by varying the NO_3^- concentration in the sediment assays.

Modification of the phytoplankton test species will show if the different algal species respond to different P compartments, as has been found in agricultural systems (Moody et. al. 1990; Moody et. al. 1997). Differences in growth patterns and physiological adaptations are likely to occur from one test organism to another. These studies will form the basis for the use of ambient phytoplankton populations in sediment bioassays.

In summary, simulated sediments elicit a bioassay response that can be related to chemical assays. Long term incubations which are unaffected by bacteria appear to be the most appropriate sediment assay technique, the sediment concentration needs to be standardised for sediment comparisons. *In vivo* chlorophyll *a* fluorescence can accurately measure changes in cell concentrations in sediment bioassays. Sorption-desorption properties of different sediments may elicit different bioassay responses to sediment concentrations. The P extraction technique also affects the P measurement based on sediment type. The present study provides the initial step in establishing the appropriate methodologies for soil derived sediment assays. This provides a crucial link between nutrients applied to soils and bioavailable nutrients in marine and estuarine ecosystems.

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Tables

Table 1. River Mouth Methodology Comparison

	Cell Counts	*in vivo* chlorophyll *a*	*in vitro* chlorophyll *a*	^{14}C Uptake
^{14}C Uptake	0.21	0.74*	0.86*	-
in vitro chlorophyll *a*	0.86*	0.82*	-	
in vivo chlorophyll *a*	0.72*	-		
Cell Counts	-			

* $p<0.05$

Table 2. Eastern Moreton Bay Methodology Comparison

	Cell Counts	*in vivo* chlorophyll *a*	*in vitro* chlorophyll *a*	^{14}C Uptake
^{14}C Uptake	0.66*	0.61*	0.41	-
in vitro chlorophyll *a*	0.31	0.34	-	
in vivo chlorophyll *a*	0.89*	-		
Cell Counts	-			

*p<0.05

Table 3. ^{14}C Uptake with sterilised and unsterilised sediments

Sediment Load	Nutrient Stimulation	
	Sterilised	Unsterilsed
0	1.00	1.00
50	2.42	9.01
100	0.62	4.65
200	0.16	1.79
500	-0.02	0.85

Figure legends

Figure 1. Diagrammatic representation of sediment extraction technique and sterilisation procedure.

Figure 2. Response of ambient phytoplankton assemblages from the Brisbane River Mouth (BRM) and Eastern Moreton Bay (EMB) to nutrient enrichments. (a) Cell concentrations of BRM; (b) Cell concentrations of EMB; (c) in vivo chlorophyll a fluorescence of BRM; (d) in vivo chlorophyll a fluorescence of EMB; (e) in vitro chlorophyll a of BRM; (f) in vitro chlorophyll a of EMB; (g) Photosynthesis of BRM and (h)) Photosynthesis of EMB.

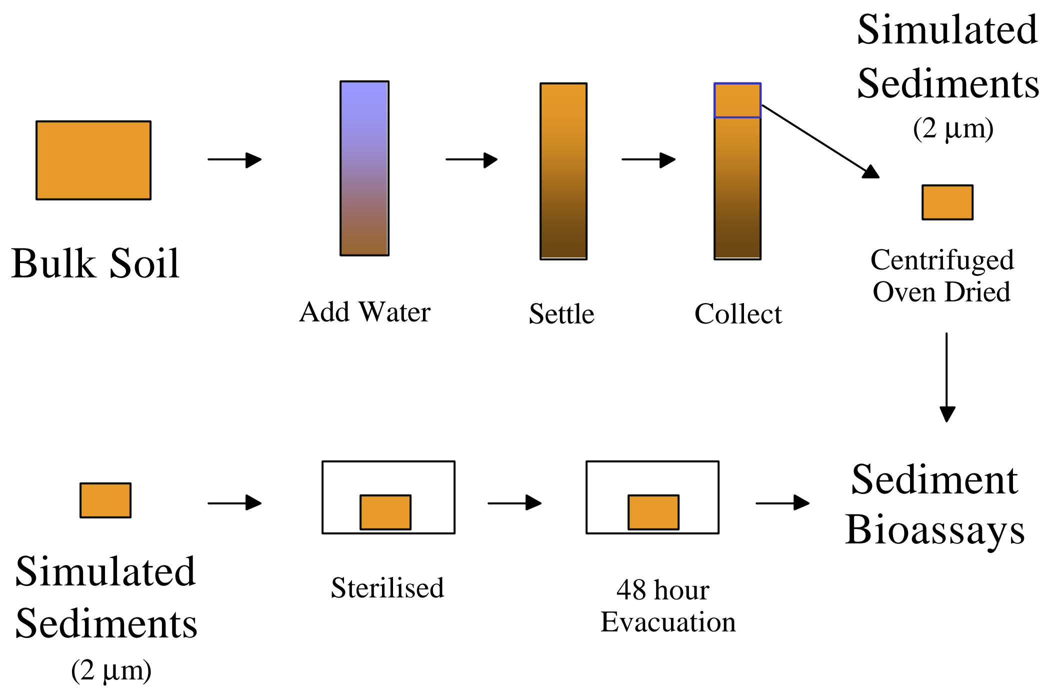
Figure 3. Response of S. costatum to sterilised and unsterilised sediment additions with. Response is expressed as the difference between maximum cell concentrations of the treatment and the control after 1 week incubation.

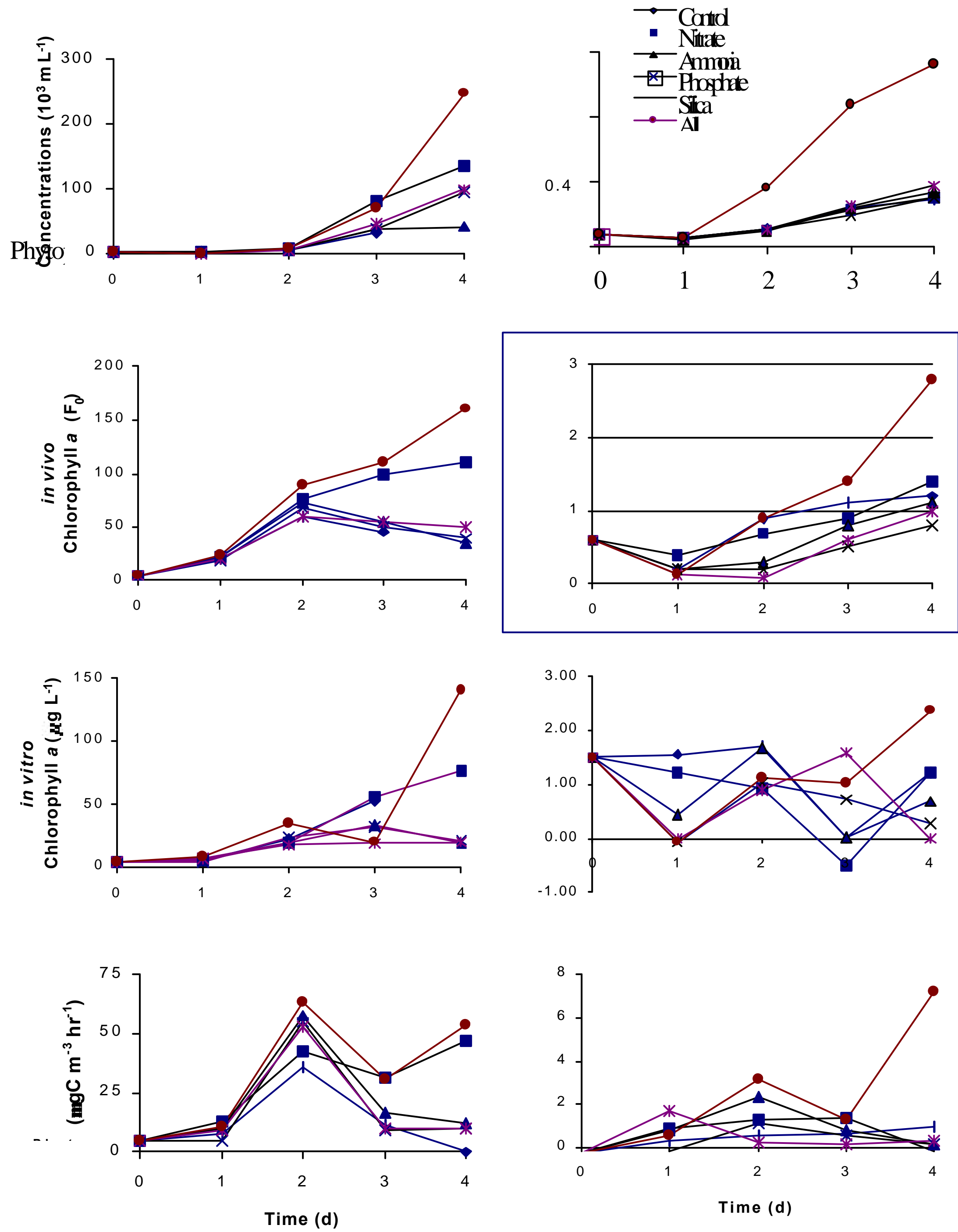
Figure 4. Response of S. costatum to sediment additions of (a) Humic Gley sediments; (b) Yellow Earth sediments and (c) Podzolic sediments. Response was measured by in vivo chlorophyll a fluorescence.

Figure 5. Correlation of (a) Colwell P and (b) $CaCl_2$ extractable P of sediments with S. costatum response. . Response is expressed as the difference between maximum biomass of the treatment and the control after 1 week incubation.

Figure 6. Correlation of Predicted sediment P concentrations and measured sediment P concentrations for the Colwell P and $CaCl_2$ extractable P. Predicted values were obtained by taking measured values for the 0 kgP ha^{-1} and adding the appropriate fertiliser treatment.

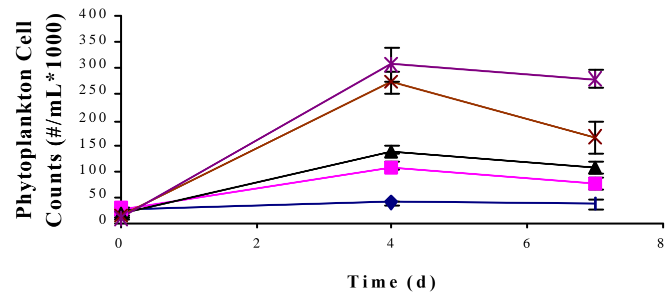
Figures


Simulated Sediments
(2 µm)
Bulk Soil
Add Water
Settle
Collect
Centrifuged
Oven Dried
Sediment Bioassays
Simulated Sediments
(2 µm)
Sterilised
48 hour
Evacuation

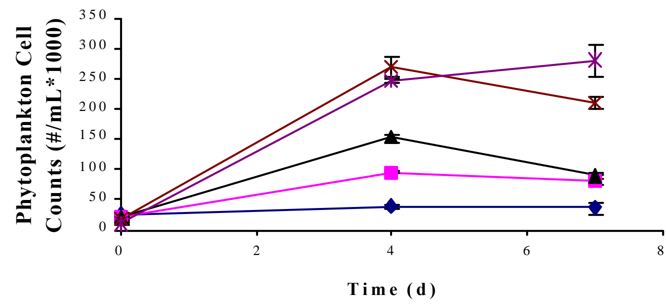


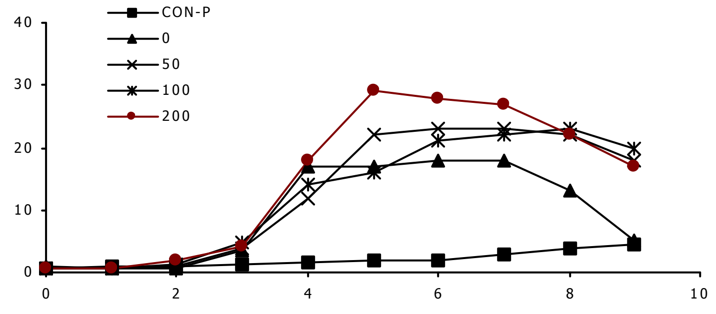
Control
Nitrate
Ammonia
Phosphate
Silica
All
Phyto Concentrations (10³ m L⁻¹)
in vivo Chlorophyll a (F₀)
in vitro Chlorophyll a (µg L⁻¹)
(mgC m⁻³ hr⁻¹)
Time (d)
Time (d)

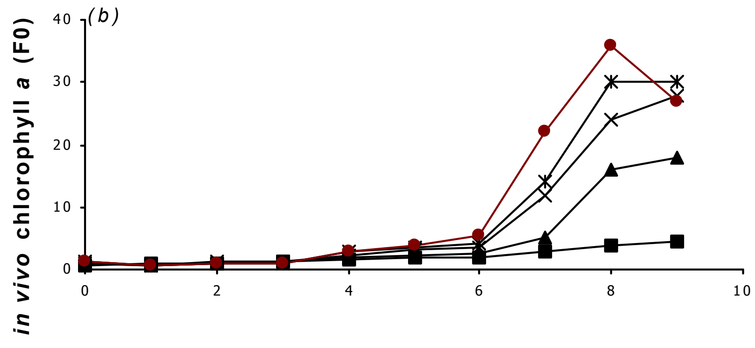
Sterilized Sediments

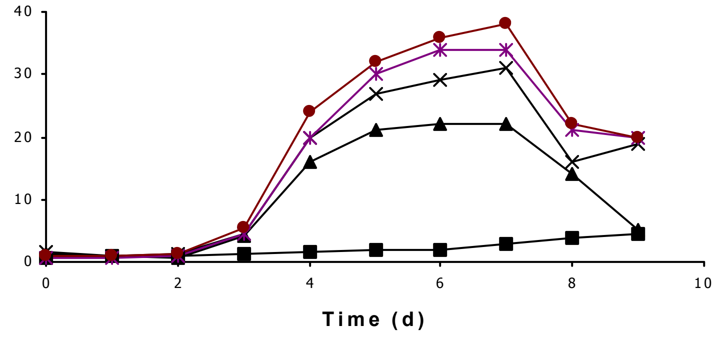


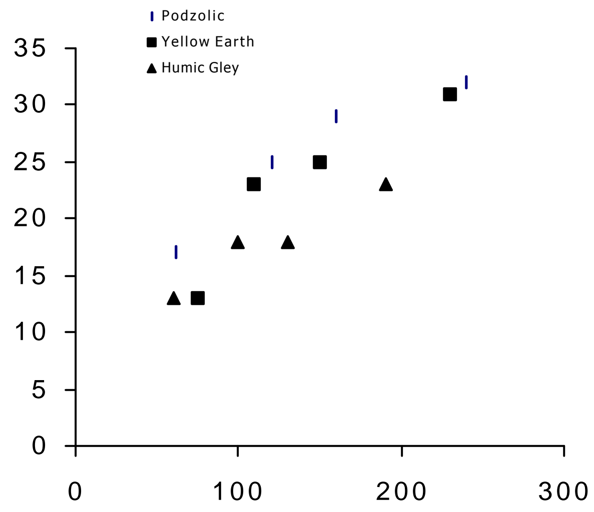
Unsterilized Sediments



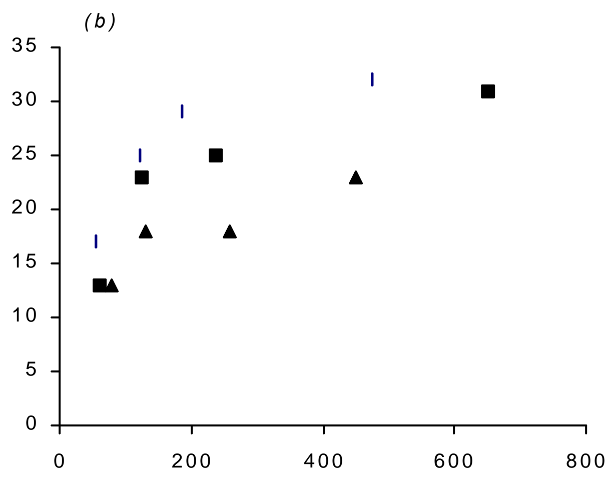

CON-P
0
50
100
200
40
30
20
10
0
0
2
4
6
8
10

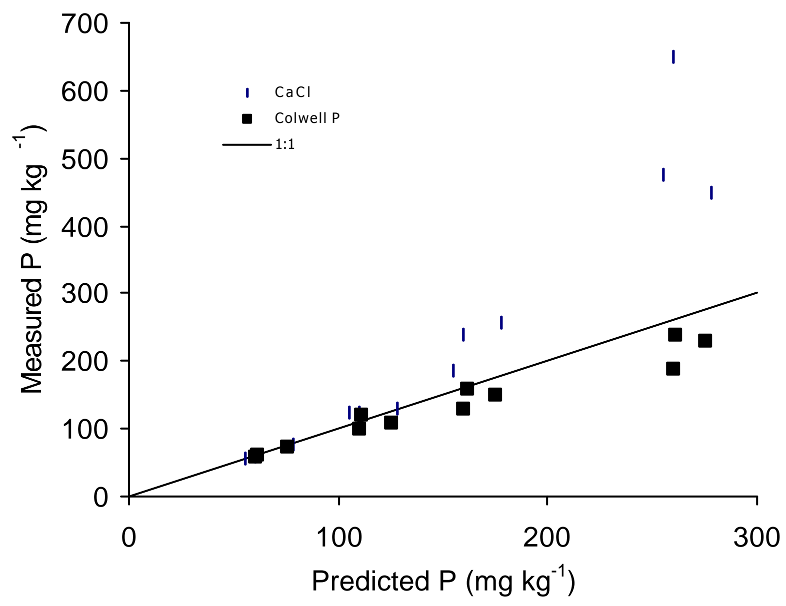

(b)
in vivo chlorophyll a (F0)
40
30
20
10
0
0
2
4
6
8
10


40
30
20
10
0
0
2
4
6
8
10
Time (d)



Nutrient Stimulated Bloom Potential (F0)





700
600
500
400
300
200
100
0
Measured P (mg kg -1)
CaCl
Colwell P
1:1
0
100
200
300
Predicted P (mg kg-1)