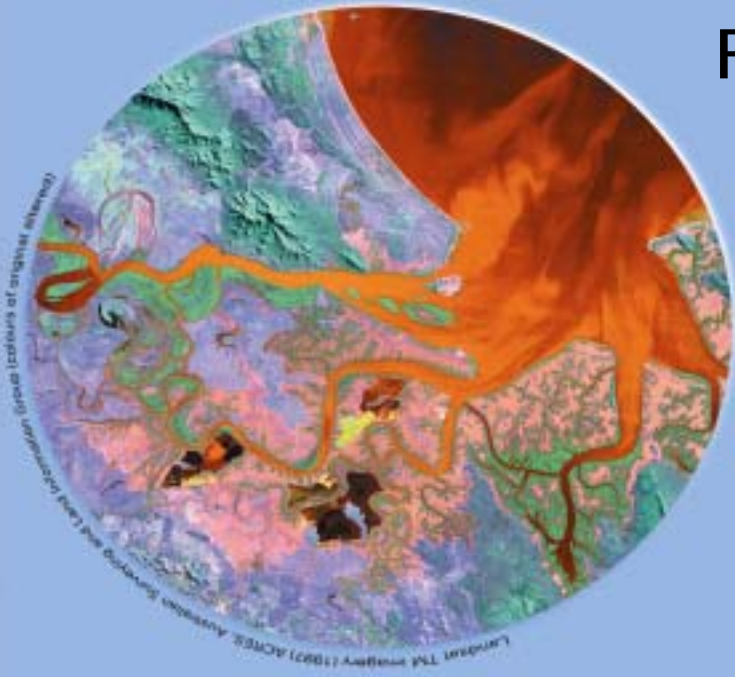




Cooperative Research Centre for Coastal Zone, Estuary and Waterway Management

Technical Report 35



Fitzroy River: Intertidal Mudflat Biogeochemistry

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December, 2005



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Non technical summary

The Fitzroy estuary supports a highly productive fishery, both professional and recreational. However, previous studies of the estuary have pointed out that the basis for this productivity is unclear. The growth of phytoplankton (the base of most food webs) is generally very low in the water for most of the year due to the high sediment loads. A potential base for this productivity which has not previously been investigated are the extensive mudflats and the algae which grow on them. These algae, often referred to as microphytobenthos, make use of the fact that they have a substrate to grow on and are uncovered or partially uncovered by the tides usually twice a day. This avoids one of the biggest factors limiting algal growth which is water turbidity and the consequent light limitation.

The aim of this study was to investigate mud flat productivity and to try and understand the factors which might control it and its possible relationship with the high and low flow cycles of the estuary.

The cycle of the estuary can be roughly divided into three periods, summer wet (High flow January to March), winter (Low flow, cool April to July) and summer dry (Low flow, hot September to December) and each of these is characterised by differences in the physical, chemical and biological nature of the estuary.

During summer wet conditions the estuary is dominated by warm fresh water which brings increased nutrients from the catchment. The effect of tides is low in that the mudflats are not exposed very much due to generally higher water levels. During this period, there is little algal activity on the mudflats but some of the nutrients being transported through the estuary are taken up and stored in the sediments.

As the flow decreases (winter period), cooler saline water again migrates up the estuary until the system becomes more marine in nature. During this time the mudflats start to become exposed again and algal growth accelerates. The cooler saline water contains fewer nutrients and what is available is rapidly taken up by the algae. This also sets in place a concentration gradient that sees nutrients stored in the sediments released and these are also rapidly taken up by the algae. The algae also make use of the cooler mudflat temperatures and algal growth reaches a peak around July. This period is therefore characterised by visible algae on the mudflats.

Following this period of growth, temperatures begin to increase (summer dry) which appears to cause the mudflats to eventually become too hot for the algae to flourish and they begin to decline rapidly which causes some nutrients to be released back into the water column.

Thus, in contrast to the more intensively studied temperate estuaries, it appears that the cooler months (winter period) are the more productive period for estuaries such as the Fitzroy River. These productive months could be the time during which the food for higher levels in the food web is established and, more importantly, it could be the mudflats that provide much of that food. Estimates of MPB production suggest that the main channel alone could produce 135 tonnes of carbon and this value could increase significantly if the extensive tidal creeks are included. Clearly this could be a significant source of material to the higher trophic levels.

Introduction

The Fitzroy River (Figure 1) is a macro tidal estuary on the central coast of Queensland, Australia. The river drains a very large catchment (almost 150 000 km²) that is dominated by farming and mining. The barrage at Rockhampton (60 km upstream of river mouth) and the large tidal range (up to 4 m) lead to the river being predominantly marine for most of the year. Large episodic rainfall events (Figure 2) during the summer result in annual flood events that bring very large quantities of freshwater and sediment to the estuary and out into Keppel Bay.

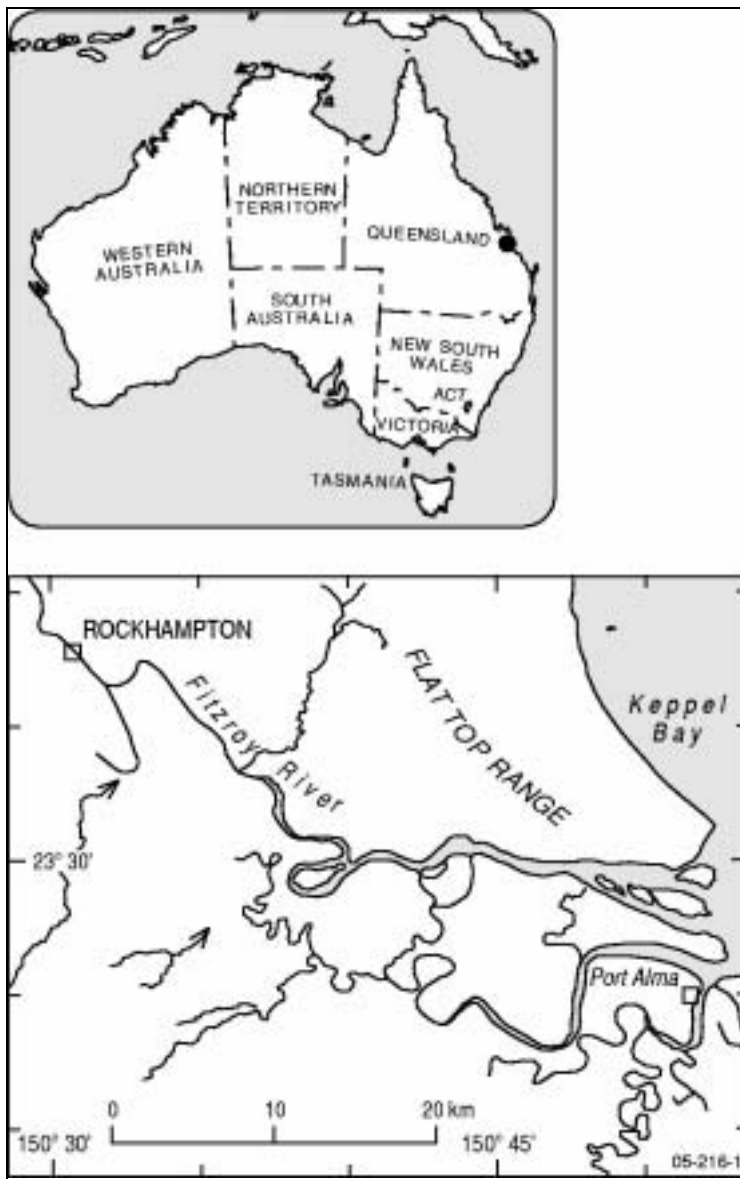


Figure 1: Fitzroy River Location

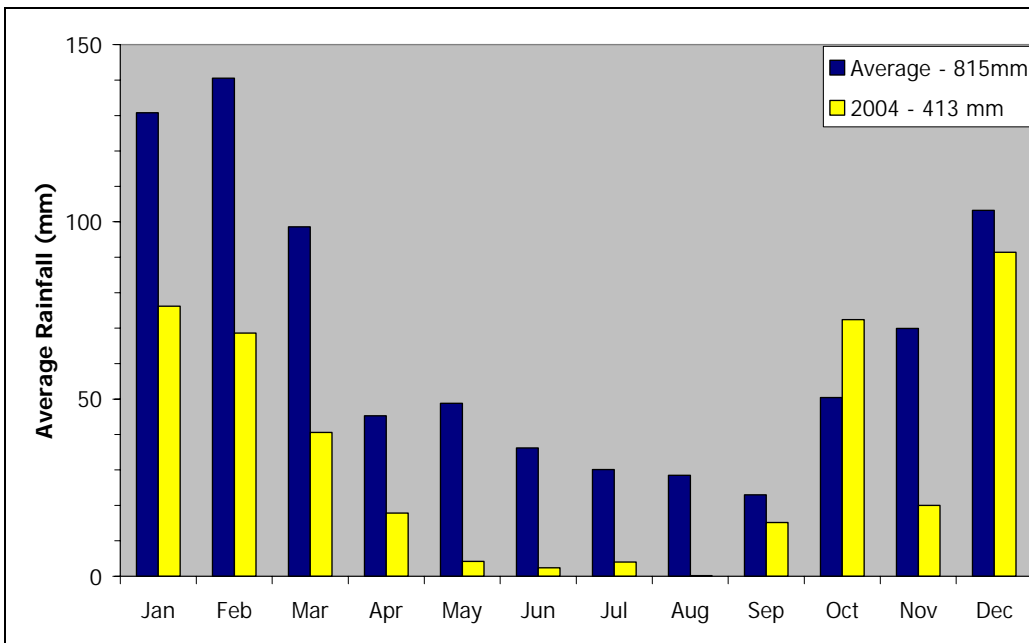


Figure 2: Long Term Monthly Average (65 years) and 2004 Monthly Rainfall data (data for Rockhampton airport from the Bureau of Meteorology)

As such, flows from the Fitzroy have the potential to interact with the Capricorn coast and the southern sections of the GBR-WHA. The area also supports extensive commercial and recreational fisheries.

Background

The Fitzroy estuary is classified as “anthropogenically modified” (OzEstuaries database) due to the significant changes since European settlement. The barrage, constructed in 1970, effectively halved the length of the estuary and determined that the estuary would be fully marine for much of the year and would experience prolonged flushing times, especially close to Rockhampton.

Changes in and around the Rockhampton area have also been mirrored in the catchment, notably extensive land clearing and a shift to grasslands. Associated with these changes is an increase in sediment loads and greater loss of nutrients with run-off, which ultimately enters the estuary.

Phase one of the Coastal CRC Fitzroy program (200 – 2003) provided the first attempt to bring detailed understanding of estuarine processes, the likely impacts of increased nutrients and sediment loads and the possible interactions with fisheries production (Noble *et al.*, 2005).

The phase one study concentrated primarily on water column biogeochemistry and attempted to develop nutrient budgets for the estuary, which indicated that while high

loads of nutrients enter the estuary, only around 4% is actually retained. The high flows responsible for transporting these nutrients also ensure that the majority are exported at the mouth (Douglas *et al*, 2005). This study also noted that water column primary productivity is both limited and patchy. For much of the year the system is light limited due to the high turbidity but at times of low flow the water column can become sufficiently clear for primary productivity to occur (Figure 3). The question remains however, given the restricted primary productivity, what drives the higher order ecosystem which yields commercial and recreational fisheries.

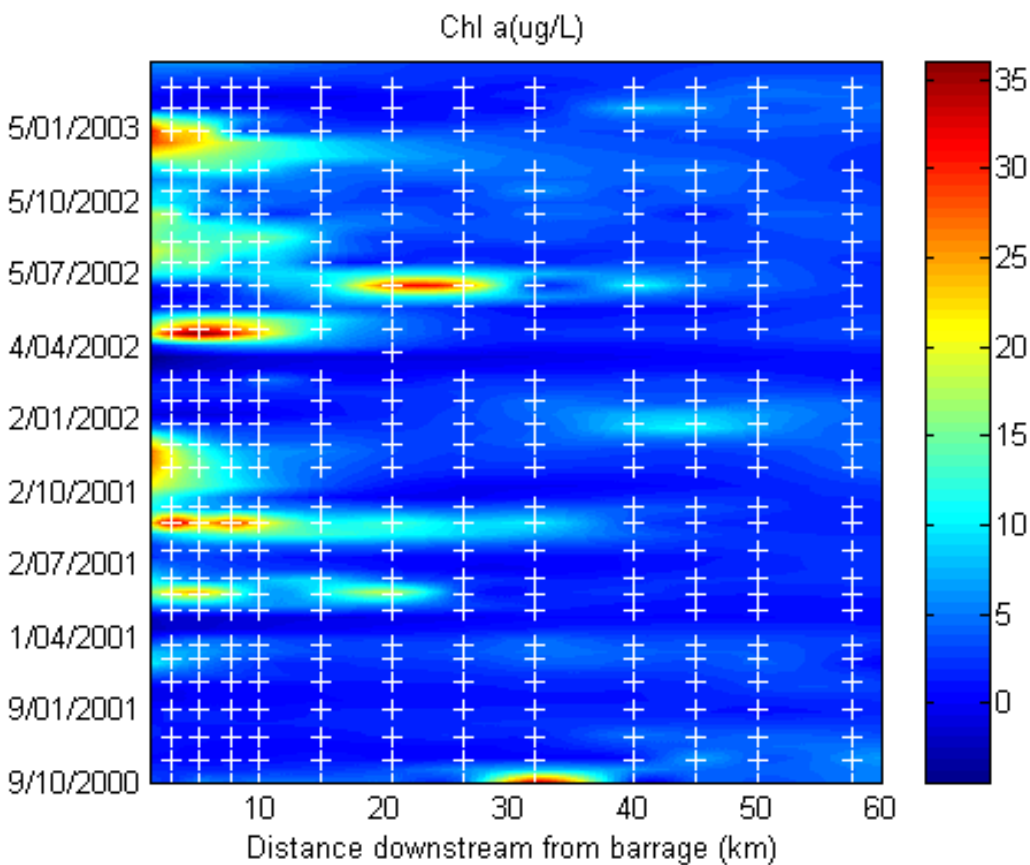


Figure 3: Fitzroy estuary Water Column Chlorophyll 2000 – 2003 (from Douglas *et al*, 2005)

Objectives

Phase one of the study noted that while the water column may be light limited, the same was not true for the areas of mudflats which visually supported significant levels of microphytobenthos (MPB) at various times of the year. MPB are effectively illuminated on every tidal cycle and can therefore be productive irrespective of the water column turbidity.

In addition, mud flats are known to be areas important for the uptake and remineralisation of nutrients that can be important for, and influenced by, MPB productivity. The extent to which mudflat productivity and nutrient cycling are important processes in the overall biogeochemistry of the Fitzroy estuary needs to be established in order for more accurate biogeochemical models to be developed, which in turn will facilitate enhanced management strategies.

Thus, the primary objectives for this part of the Fitzroy project were:

- Quantitation of mud flat nutrient cycling and identification of key processes
- An increased understanding and quantitative estimate of MPB productivity
- Integration of these processes into biogeochemical models

Methods

Sites

Rationale for site selection – representative sites

A preliminary field survey was performed by Craig Smith (Geoscience Australia) in September 2003 during which sediment samples were collected at a variety of sites along the estuary. Based on this data, a series of sites were chosen to be sampled over four seasons (detailed in milestone report AC22; Figure 4). Sites were chosen to span and be representative of the range of environments found in the estuary to assess benthic productivity. Also, based on the preliminary survey, it was decided that samples were to be collected at approximately the mid to high tide area, which appeared to be the most productive.

Sampling Program

The selected sites were sampled quarterly in March, July, September and December 2004. Sites were located according to recorded GPS positions and any useful visual marks. The samples collected at each site on each survey are detailed in Table 1.

Table 1: Sample Details for Field Surveys

Survey	Date	Biomarkers	Chl-a	Core Incubations
0	6-18/10/03	All sites	All sites	
1	14-22/3/04	All sites	All sites	3 sites
2	9-17/7/04	All sites	All sites	3 sites
3	4-12/9/04	All sites	All sites	3 sites
4	3-11/12/04	All sites	All sites	3 sites

In addition, at each station, water column temperature, dissolved oxygen and salinity were recorded as well as descriptive notes and digital photos to record the overall appearance of the site.

During the July survey, site 603 was sampled along with 4 replicates taken on an axis from the original site, parallel to the water, approximately 5 m apart. This was done in order to assess longitudinal variation along the mudflat. In December, site 613 was sampled in duplicate, as the last site on one day and the first site the following day in an attempt to assess any diurnal variation.

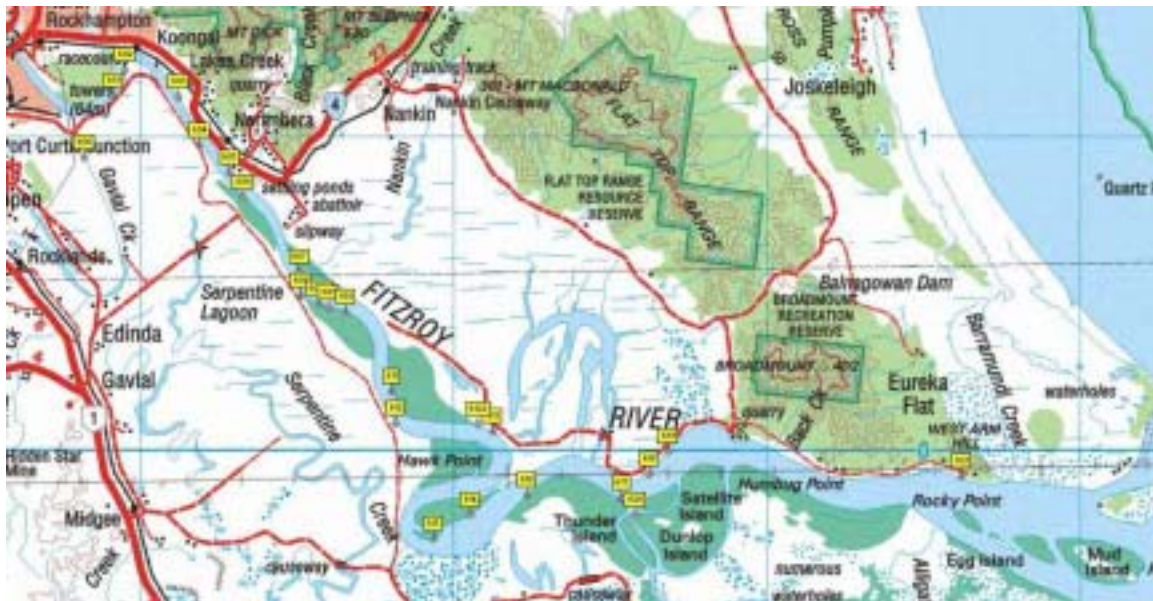


Figure 4: Mud Flat Sample Sites

Nutrient Flux Measurements

Sampling

Six sediment cores (14.5 cm internal diameter) were collected at each of three sites (613, 615 and 619 – Figure 4), three for dark incubations and three for light incubations. Core barrels used were 25 cm long, of which ~10 cm contained sediment and the remainder containing overlying site water. Upon return to the laboratory (usually within 2 hours), the cores were submerged in site water at in-situ temperature. All sediment cores were left overnight with paddle stirrers (60 rpm) loosely fitted in order to equilibrate. Light incubated cores were illuminated using 50 watt ($500\text{mE m}^{-2} \text{s}^{-1}$) halogen lamps while dark incubated cores were covered to eliminate light. (Dalsgaard *et al.*, 2000; Cook, 2002)

Core Incubations

Flux experiments commenced the morning following core collection. Lids containing the paddle stirrers were firmly screwed to the core barrels indicating time zero (T₀). Dissolved oxygen and pH of the overlying water were measured prior to sample draws using probes that are inserted through a port in the core lids. Samples were then drawn from the cores using a syringe at approximately 0, 2, 4 and 6 hours, with site water replacing the drawn volume. Samples were analysed for alkalinity, NH₄, NO_x, DON, SiO₄, PO₄ and N₂. pH and alkalinity were used to calculate total organic carbon (TCO₂), and nutrient fluxes were calculated from concentration changes over time.

Denitrification and Nitrogen Fixation

Both the denitrification (determined by isotope pairing technique) and nitrogen fixation experiments were performed on samples taken from the original sediment cores.

The isotope pairing method involves the spiking of sediment cores with $^{15}\text{NO}_3^-$, then measuring the rate at which this is converted to N_2 gas. Samples were obtained from sub-cores (4.8 cm id x 25 cm) that were taken from the original sediment cores. The sub-cores contain approximately 8 cm of sediment and the remainder being water column.

Nitrogen fixation was determined using the acetylene reduction technique. Surface sediments (2 x 10 ml) from the original cores were placed into 125 ml glass bottles and capped with gas-tight septums. 40 ml of acetylene was then injected into the headspace and 3 ml samples of the headspace taken at 0, 3 and 6 hours. Any nitrogen fixing bacteria present will reduce the acetylene into ethylene in much the same way they would convert N_2 into ammonia. Samples were placed directly into 3 ml Vacutainers™ (Becton Dickinson) until analysis (of acetylene and ethylene) by Gas Chromatography.

Nutrient Analyses

TCO₂ calculated from pH and Alkalinity (Gran titration)

The alkalinity was determined by Gran titration, whilst the carbonate alkalinity (CA) was estimated by subtracting the alkalinity contribution of $\text{B}(\text{OH})_4^-$. Carbon dioxide (TCO₂) was estimated from pH and carbonate alkalinity according to Mehrbach *et al.* (1973) using:

$$\text{TCO}_2 = \text{CA} \frac{1 + K_2 / a_H + a_H / K_1}{1 + 2K_2 / a_H}$$

Where:

a_H is the activity of the hydrogen ion and K_1 and K_2 are the first and second ionisation constants of carbonic acid (H_2CO_3).

Samples for dissolved nitrate (NO_3^-) and nitrite (NO_2^-), referred to collectively as NO_x , and filterable reactive phosphate (PO_4^{3-}) analysis were frozen after sparging with N_2 and sent to Queensland Health Scientific Services for analysis.

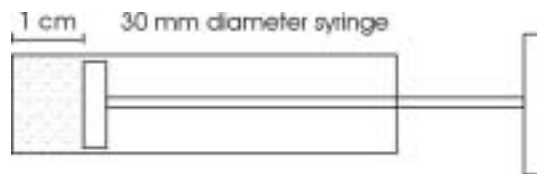
N₂ Measurement (Southern Cross University)

Southern Cross University Environmental Analysis Laboratory analysed the benthic chamber samples for N₂. The laboratory used the method and instrumentation of Kana *et al.* (1994) with the following modifications. Gases were detected with a Balzers QMS422 quadrupole mass spectrometer and a water bath (± 0.01 °C) was used to stabilize sample temperature in the water line upstream of the membrane. The effect of O₂ in the sample on the N₂ signal measured by the membrane inlet mass spectrometer was corrected by making a standard curve of O₂ concentration against N₂:Ar ratios using water standards made from the incubation water equilibrated with the atmosphere at constant temperature (Eyre *et al.* 2002).

Biomarkers

Sampling

Sediment samples were collected using disposable syringes (30 mm diameter) that had the Luer tip cut off thus making a simple barrel and plunger. The barrel was inserted into the sediment whilst holding the plunger still. The plunger was then used to extrude all but 1 cm of sediment out of the barrel. Five such “1 cm top sections” were combined to comprise a single sample from each site.



Sediments were extruded into clean jars, homogenised, sub-sampled into cryo-vials for pigment analysis and foil placed between the lid and contents. The samples were kept on ice until the jars could be frozen and the pigment samples placed in a liquid nitrogen dry shipper. Samples were later transported to CSIRO Marine research in Hobart for analysis.

Lipid Biomarker Analyses

Sediment samples for sterol and fatty acid analysis were extracted quantitatively by a modified one-phase CH₂Cl₂-MeOH Bligh and Dyer method (Bligh and Dyer, 1959). After phase separation, the lipids were recovered in the lower CH₂Cl₂ layer (solvents were removed in vacuo) and were made up to a known volume and stored sealed under nitrogen at -20 °C. The total sterol fraction was obtained following alkaline saponification of an aliquot of the total lipids. Fatty acids were recovered from the

aqueous fraction of the saponified mixture after the addition of 1 ml of HCl. Sterols were converted to their corresponding O-TMSi ethers by treatment with bis(trimethylsilyl)trifluoroacetamide (BSTFA, 100 μ L, 60 $^{\circ}$ C, 60 minutes). Fatty acids were converted to their methyl esters prior to analysis by treatment with acidified methanol.

Gas chromatography (GC) was performed using a Varian 3800, interfaced with Galaxy chromatography software. The gas chromatograph was equipped with a 50 m x 0.32 mm i.d. cross-linked 5% phenyl-methyl silicone (HP5) fused-silica capillary column; hydrogen was the carrier gas. Sterol and fatty acid fractions were analysed using a flame ionisation detector, with 5 β (H)-cholan-24-ol (Chiron AS, Norway) as the internal standard for sterols and the C₂₃ fatty acid methyl ester as the injection standard for fatty acid analysis. Peak identifications were based on retention times relative to authentic and laboratory standards and subsequent GCMS analysis.

Verification of the identity of individual sterols by GC-MS analyses was performed on a Thermoquest GCQ-Plus bench top mass spectrometer fitted with a direct capillary inlet and a split / splitless injector. Data were acquired in scan acquisition or selective ion monitoring and processed using Xcalibur software supplied with the instrument. The nonpolar column (HP5) and operating conditions were similar to that described above for GC-FID analyses, but helium was used as the carrier gas.

Pigment Analyses

Mudflat sediment samples were analysed for pigments by Lesley Clementson (CMAR). To extract the pigments, the samples were weighed and quantitatively transferred to 50 ml centrifuge tubes. 100% acetone (8-10 ml) was added to each tube and the tubes were vortexed for about 30 seconds and then sonicated in an ice-water bath for 15 minutes in the dark. The samples were then kept in the dark at 4 $^{\circ}$ C for approximately 15 hours. After this time the tubes were centrifuged and the supernatant from each tube decanted into a separate 25 ml volumetric flask which were stored in the dark at 4 $^{\circ}$ C. A second extraction was performed on each MPB sample with a resting time of only 3 hours. The samples were again centrifuged and the supernatant of the second extraction was added to the first. A pre-determined volume of water was added to each flask such that the final extract mixture was 90:10 acetone:water (vol:vol). Each flask was made to the 25 ml mark with 100% acetone and then filtered through a 0.2 μ m membrane filter (Whatman, anatope) prior to analysis by HPLC using a Waters - Alliance high performance liquid chromatography system, comprising a 2695XE separations module with column heater and refrigerated autosampler and a 2996 photo-diode array detector.

Immediately prior to injection the sample extract was mixed with a buffer solution (90:10 28 mM tetrabutyl ammonium acetate, pH 6.5 : methanol) within the sample loop. After injection pigments were separated using a Zorbax Eclipse XDB-C8 stainless steel 150 mm x 4.6 mm ID column with 3.5 µm particle size (Agilent Technologies) and the following gradient elution procedure:

Time	% Solvent A	% Solvent B
	95	5
11	45	55
15	45	55
22	5	95
29	5	95
31	95	5

Where solvent A is 70:30 28 mM tetrabutyl ammonium acetate, pH 6.5 : methanol and solvent B is 100% methanol.

The flow rate was 1.1 mL min⁻¹ and the column temperature was 55 °C. The separated pigments were detected at 436 nm and identified against standard spectra using Waters Empower software. Concentrations of chlorophyll a, chlorophyll b and β,β-carotene in sample chromatograms were determined from standards (Sigma) while all other pigment concentrations were determined from standards (DHI, Denmark).

Pigment concentrations were determined from standards of purified pigments isolated from algal cultures.

Stable Isotope Analysis

Sediment samples were dried at 55°C overnight, before being ground, homogenised and weighed into tin cups (Elemental Microanalysis Ltd., Okehampton, UK) for analysis. For the analysis of carbon, a few drops of sulphurous acid were added to remove any carbonates present. This was done within cups to prevent loss of acid soluble organic carbon (Verardo *et al.* 1990). Samples were re-dried and the cups closed prior to analysis. Samples were analysed for δ¹³C and δ¹⁵N using a Carlo Erba NA1500 CNS analyser interfaced via a Conflo II to a Finnigan Mat Delta S isotope ratio mass spectrometer operating in the continuous flow mode. Combustion and oxidation were achieved at 1090 °C and reduction at 650 °C. Samples were analysed at least in duplicate. Results are presented in standard δ notation:

$$\delta^{13}\text{C}(\text{‰}) = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 100\%$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. The standard for carbon is Vienna Pee Dee Belemnite (VPDB) while that for nitrogen is Air. The reproducibility of the stable isotope measurements was ~ 0.2 ‰ for carbon and 0.5 ‰ for nitrogen.

Results

Field Observations

During 2004 there was only a moderate flow event for the Fitzroy (Figure 5) but nonetheless, this was sufficient to decrease salinity along most of the estuary (Figure 6) such that the first survey was undertaken in relatively low salinity conditions, but this quickly reverted to saline conditions for the remaining surveys (Figure 6). It is worth noting that by December sites in the lower estuary are experiencing very high salinities (close to 40) which may be due to minimal water exchange and increased evaporation. During each survey the water column appeared to be well mixed. Water temperature exhibited a marked change between around 20 °C in the winter months to 28 °C in summer (Figure 7). Dissolved oxygen was lowest in March (Figure 8) but still had an average value of 89 %. There was a significant dip in DO at site 600 during the September and December surveys though the reasons for this are unclear.

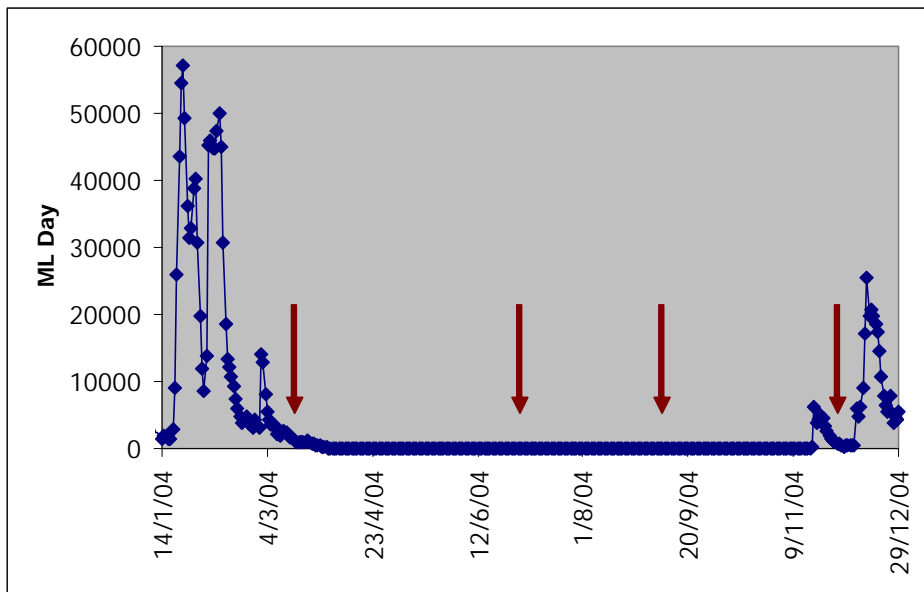


Figure 5: Flow measured at “The Gap” During the Survey Period. (Arrows indicate Field Surveys).

Fitzroy intertidal mudflat biogeochemistry

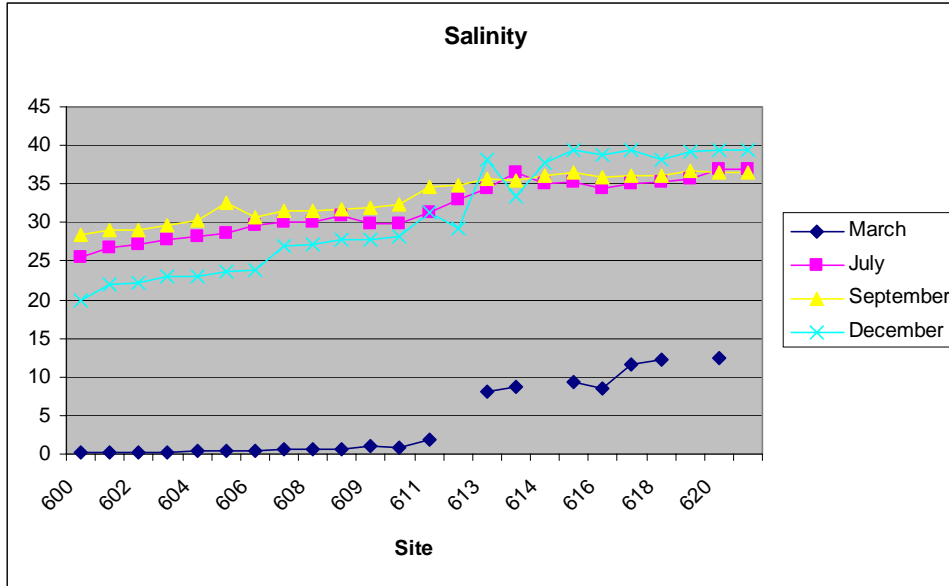


Figure 6: Water Column Salinity at each Site During Field Surveys

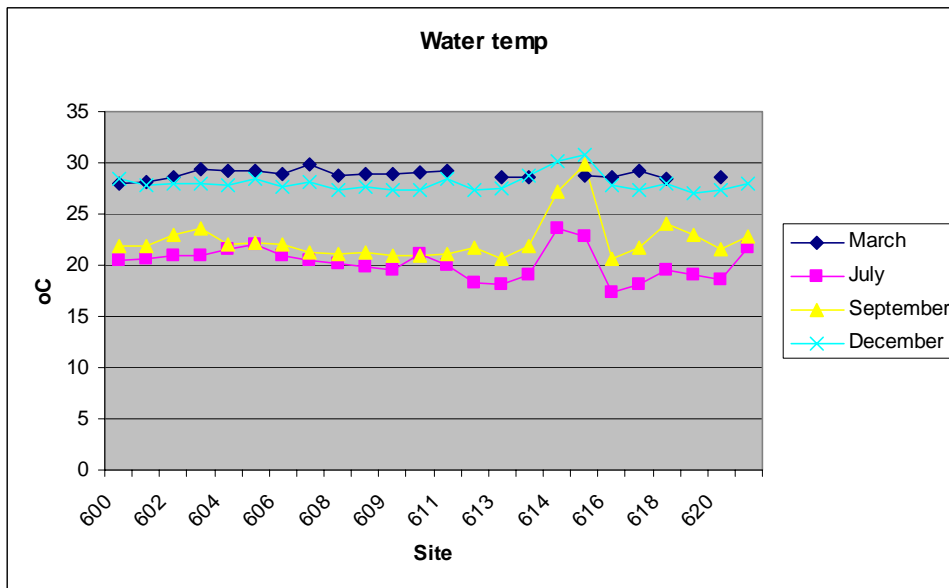


Figure 7: Water Column Temperature Measured at each Site During Field Surveys

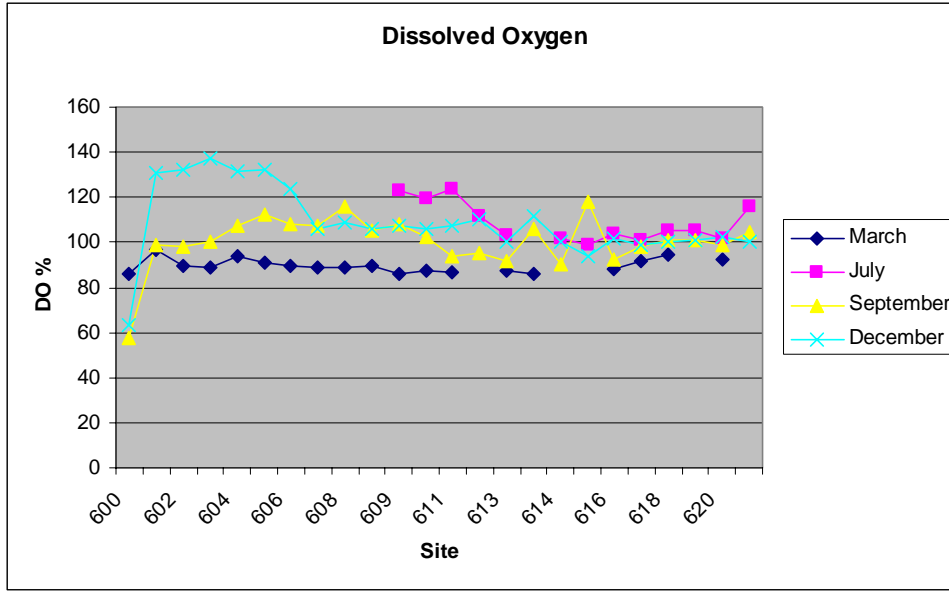


Figure 8: Water Column Dissolved Oxygen Recorded During Field Surveys

During 2004 mudflat productivity was visually highly variable, both spatially and temporally. In March and December there was little evidence for algal activity on the mudflats, whereas in winter there were clear indications of micro and macro algal production (Figures 9 and 10)



Figure 9: Abundant Filamentous Algae at Site 606 in July 2004



Figure 10: Abundant Diatoms (Dark Brown Areas) at Site 620 in July 2004

Bulk Carbon and Nitrogen

Organic Carbon and total nitrogen content of the mudflat sediments is shown in Figures 11 and 12. Both are highly variable both spatially and temporally with few identifiable trends, except that the sites within the arm of the cut-through (614 and 615) appear to be much more consistent over time compared to other sites within the estuary. When these values are expressed as C:N (Figure 13) the results are significantly more consistent temporally. Most sites have an average C:N of between 10 and 14 which is indicative of the organic matter being predominantly of terrestrial origin. Again, there are some exceptions, namely sites 614 and 615 and some sites in the upper part of the estuary (600 – 604), which tend to have average C:N values closer to 8 which is a much more “marine” signal. Site 613 gave a spuriously high C:N value in September which appears to be due to a low measured N, in keeping with values at this site for the other surveys but an elevated C value. This could be due to the sample containing material such as woody matter or charcoal.

Fitzroy intertidal mudflat biogeochemistry

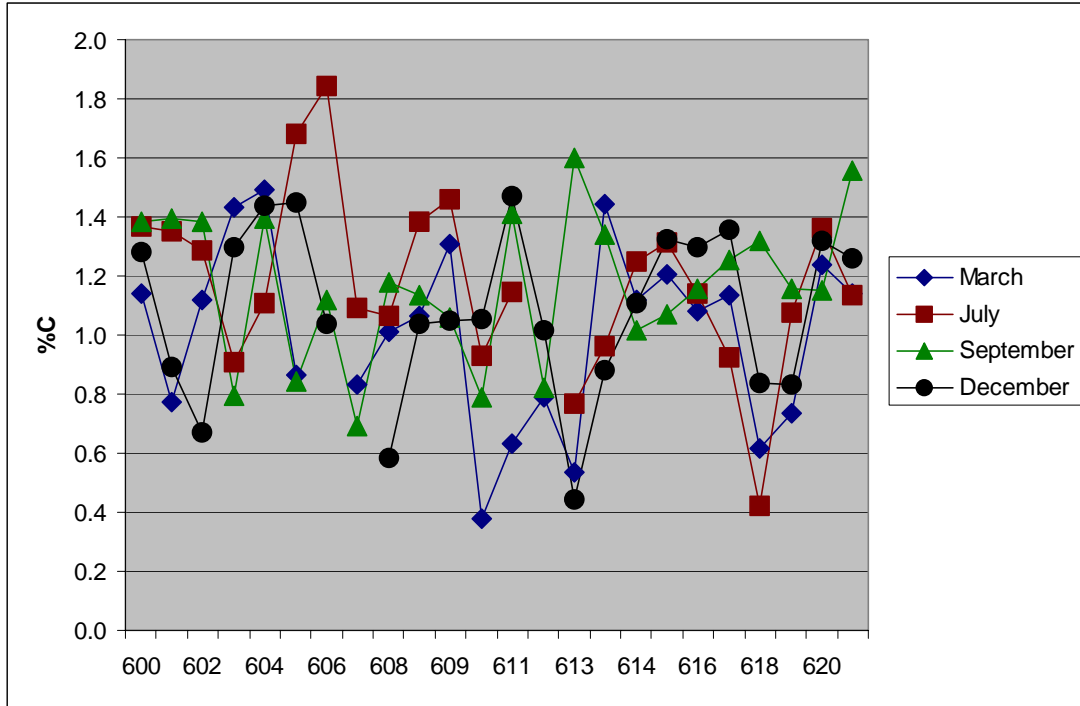


Figure 11: Mud Flat Organic Carbon Content (w/w)

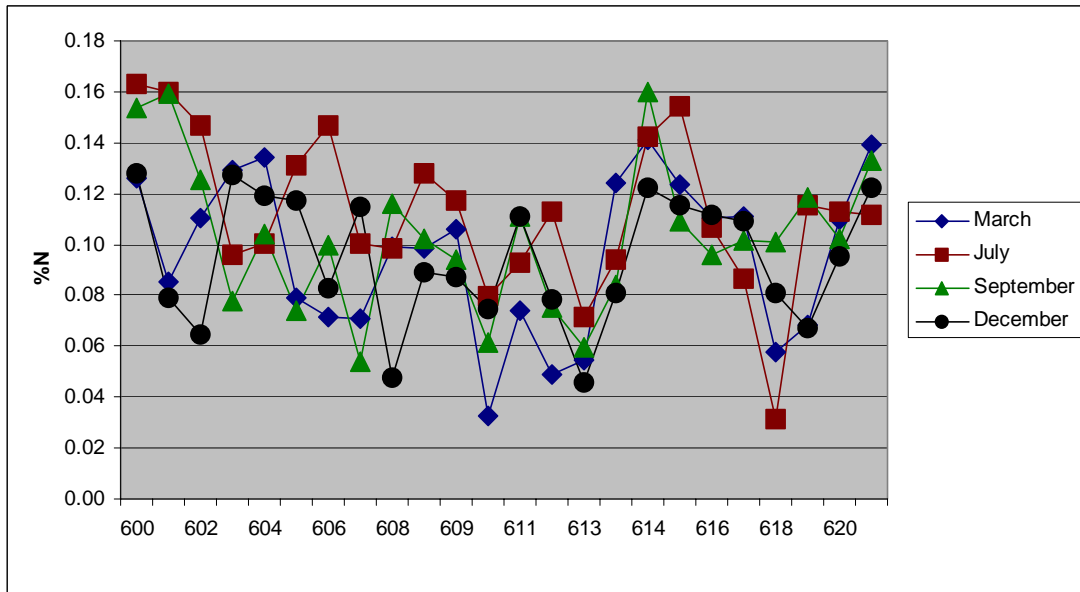


Figure 12: Mud Flat Nitrogen Content (w/w)

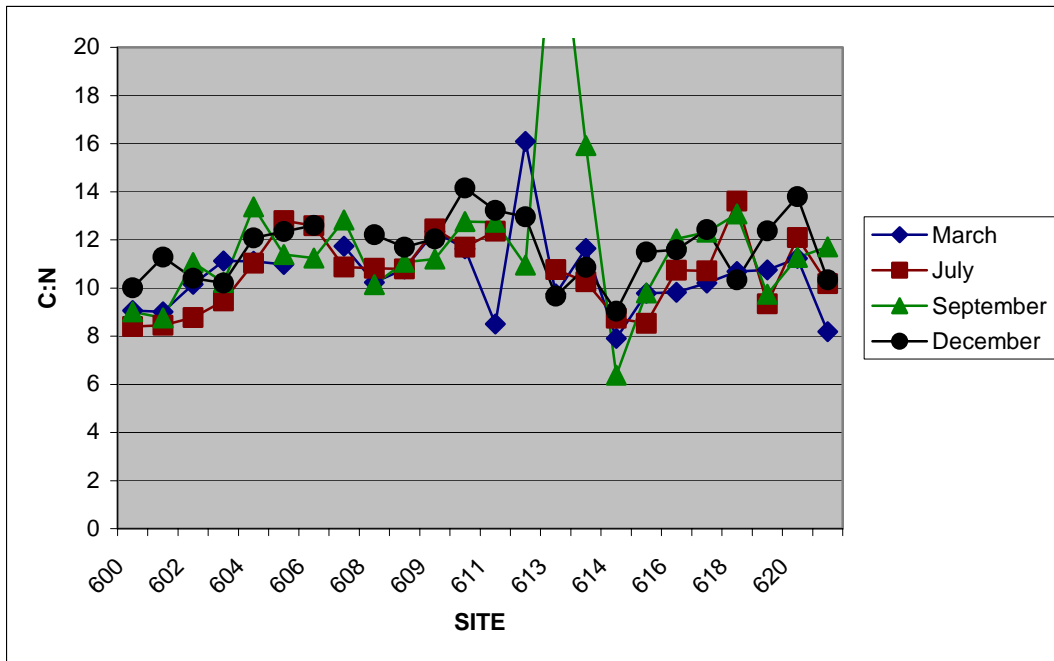


Figure 13: Mudflat sediment C:N (w/w)

Stable isotope values tend to show a more consistent trend across the sites (Figures 14 and 15). Summer $\delta^{13}\text{C}$ values tend to average between -21 ‰ and -22 ‰ along the estuary, though the lower half tends to be more variable (Figure 14). In winter, the pattern tends to become more variable with some sites exhibiting a shift to less negative values (more algal). $\delta^{15}\text{N}$ values tend to be more consistent across the surveys (Figure 15) except in the upper reaches of the estuary, where there is a distinct shift to less positive values (more terrestrial) at times of elevated flow (March and December). In general, the sites exhibit a distinct trend from more positive values (ca. +8 ‰) in the upper reaches to less positive (ca. +5 ‰ - +6 ‰) in the lower half of the estuary. This trend is the inverse of many estuaries which exhibit a trend from less positive (terrestrial) to more positive (marine) values.

Fitzroy intertidal mudflat biogeochemistry

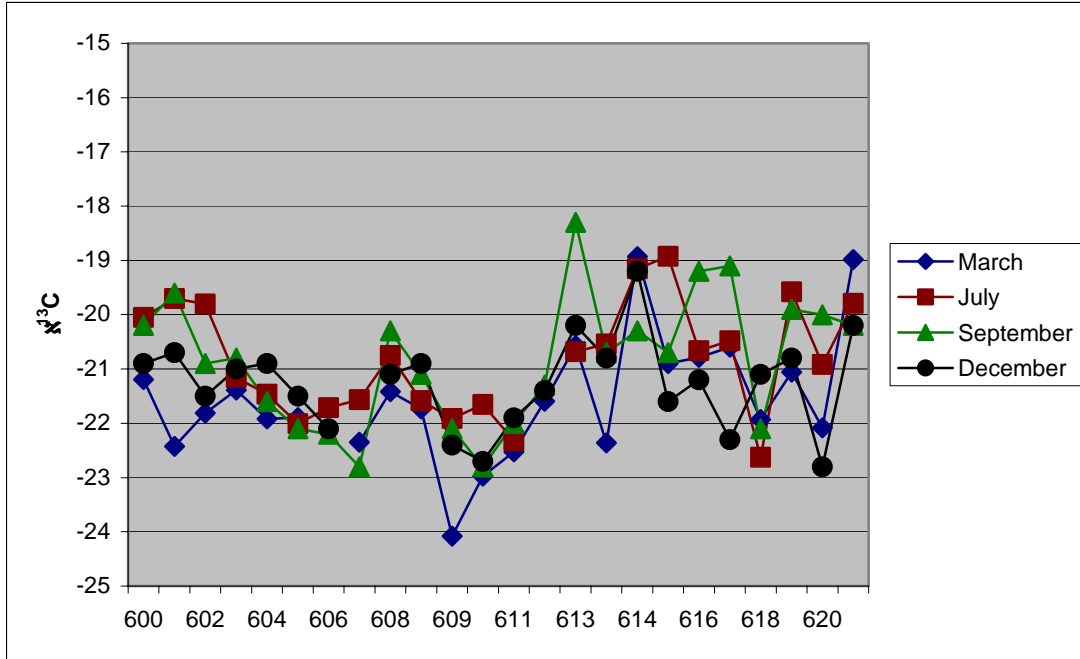


Figure 14: $\delta^{13}\text{C}$ Values of Mudflat Sediment Organic Carbon (vs VPDB)

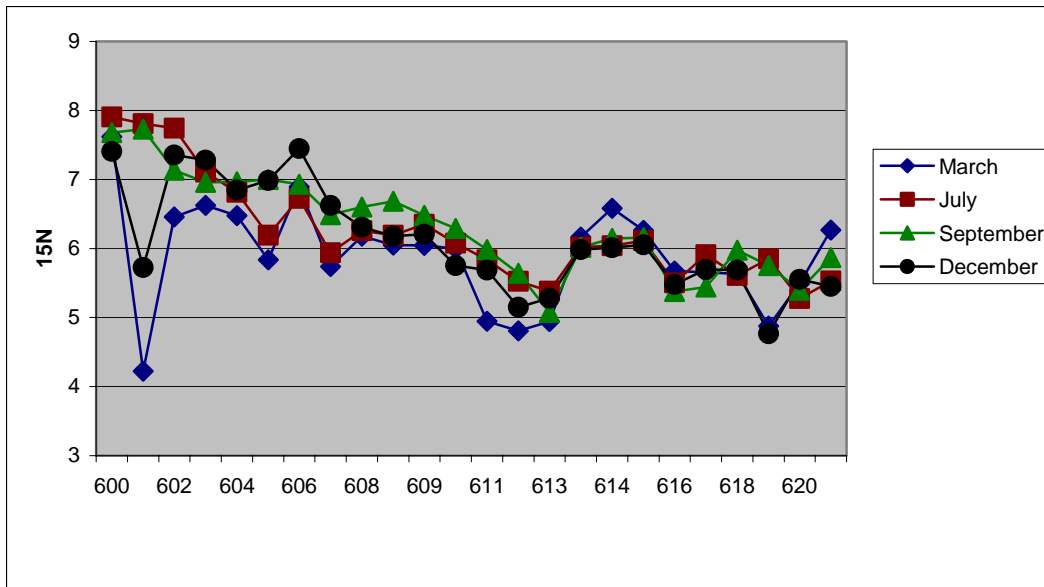


Figure 15: $\delta^{15}\text{N}$ Values of Mudflat Sediment Nitrogen (vs Air)

Lipid Biomarkers and Pigments

Principal tracers

The analysis of sterol, pigment and fatty acid biomarkers from intertidal sediments, was undertaken to provide context for the nutrient fluxes measured at sites 613-2, 615 and 619. Biomarkers can be used as proxies for algae (primary productivity), detritus (allochthonous organic carbon and nitrogen inputs) and heterotrophic bacteria (heterotrophic turn-over of resident organic matter). A total of 88 biomarkers were analysed – sterols, fatty acids and pigments. Of these 39 were abundant enough to provide information about species composition. Of these, we can distil down to a subset of eight to broadly trace the major sources of organic matter that dominate the system. These are; (i) primary productivity as a whole – [chlorophyll-a and phytol], (ii) terrestrial organic matter – 24-ethylcholesterol and hexacosanol, (iii) markers of the dominant algal group – [eicosapentanoic acid (EPA) and brassicasterol], (iv) heterotrophic bacteria [iso-15:0 fatty acid] and (v) *in*-fauna – [cholesterol] (Table 2).

Spatial Variation

Spatial variation was assessed both within site, and from the low to the high water mark. Within site variation was assessed by analysis of five samples taken randomly at site 603 during the July 2004 survey. The terrestrial plant markers 24-ethylcholesterol and hexacosanol had the lowest coefficients of variation which probably reflects the fact that the distribution of such allochthonous organic matter is determined more by physical distribution factors (tides and deposition) than is autochthonous matter (Table 2). Conversely, chlorophyll-a and eicosapentanoic acid (EPA) had the highest variance since a multitude of additional growth factors affect the abundance of resident autochthonous algae even at small scales. As might be expected, the variance of cholesterol (*in*-fauna) and the heterotrophic bacterial marker *i*-15:0 fell somewhere between as both markers represent organisms that turn over carbon from both algal and terrestrial organic matter sources, albeit at rates biased toward the more labile carbon available from the algae.

An additional factor in the high variance of chlorophyll as opposed to phytol is that the concentrations for the pigments were lower than expected. We believe this was a function of poor extraction efficiency of pigments from the fine sediments; this is discussed further in the section “Estimating MPB Biomass”. The other marker that had a high variance was EPA. Although the method used to elucidate fatty acids would not be expected to yield CV's as low as those for sterols, the high CV for EPA probably accurately represents the greater spatial variation of algal growth on the

mudflats as opposed to that of heterotrophic bacteria which utilize both algae and other forms of organic matter over extended periods. In summary, the CV values in Table 2 provide a pseudo-variance for the primary biomarkers against which site and seasonal differences can be assessed. The %CV for most of the biomarkers was < 15% and for EPA and chlorophyll was \approx 25%.

Table 2: Principal biomarkers used, the variance measured at site 603 (coefficient of variation – CV), and the primary origin of the marker.

Biomarker	CV (n=5)	Origin
Hexacosanol	2.7%	terrestrial plant matter
24-ethylcholesterol	8.5%	predominantly terrestrial
Brassicasterol	13.5%	algae
Phytol	13.7%	side-chain of chlorophyll
Eicosapentanoic acid (EPA - 20:5w3)	27.5%	algae - diatoms
Chlorophyll - a	22.9%	primary productivity
i15:0 fatty acid	11.2%	heterotrophic bacteria
Cholesterol	16.1%	Ubiquitous component of animal cell wall [in sediment largely in-fauna]

Variation from low to high water mark

The preliminary survey undertaken in September 2003 was used to choose where in the intertidal zone samples should be collected during the subsequent quarterly surveys. During this survey, five samples were collected across the intertidal zone [from low to high water mark] at sites 609 and 615. At each site, based on the distribution of phytol, the mid-tide sites were the ones where the microphytobenthos grew best (Figure 16). This result was more pronounced at site 609 in the main channel where the banks were steeper than at 615 in the cut-through where the more level mudflats remain moist for longer. There was a similar, but less pronounced distribution of the terrestrial plant marker hexacosanol suggesting that at least some of this effect may simply be depositional in nature (Figure 17).

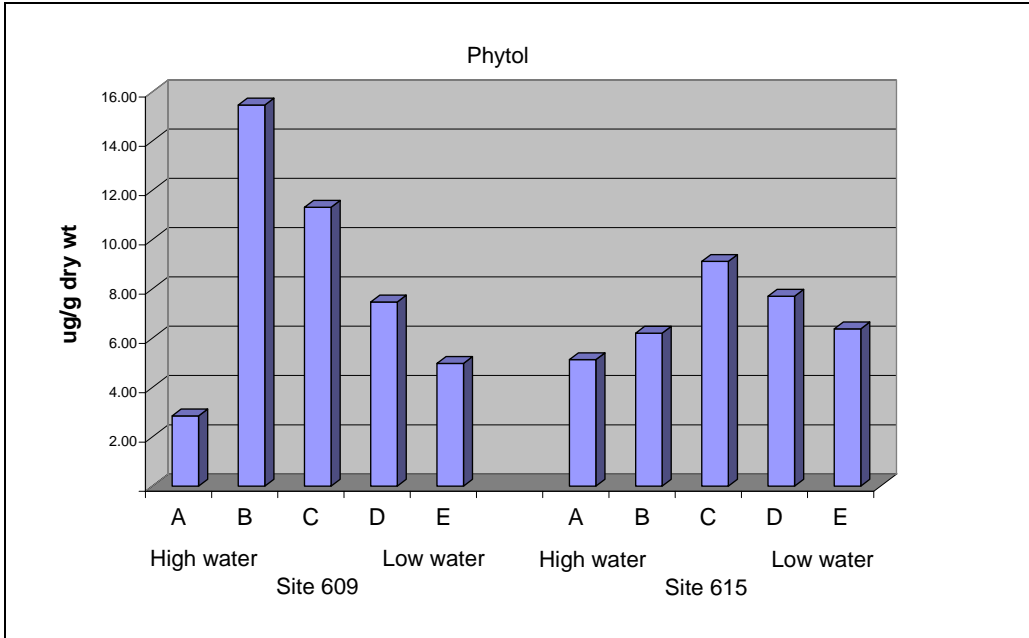


Figure 16: Concentration of phytol at two sites (609 and 615) from high to low water mark. These data were used to help determine the most suitable tidal point at which to take samples during subsequent surveys.

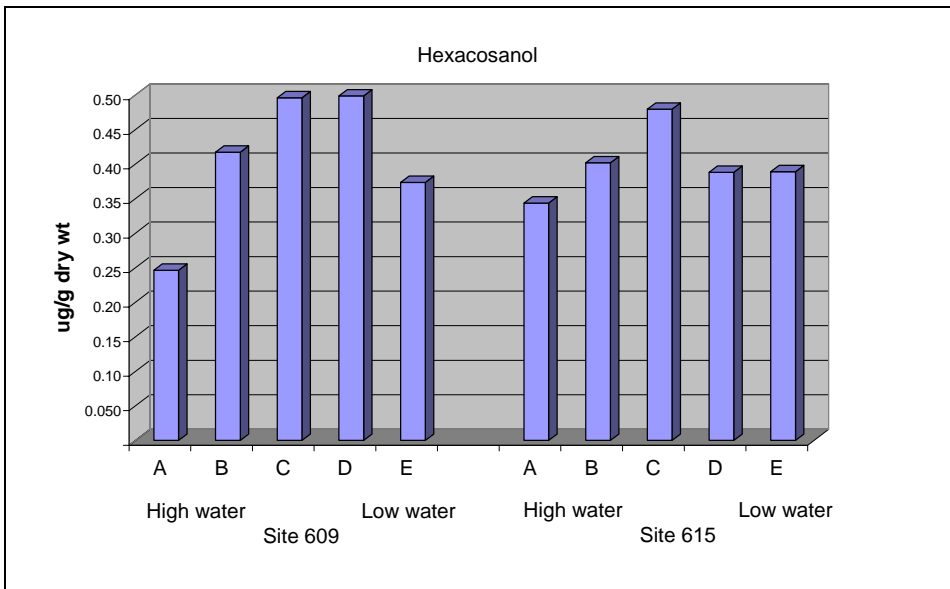


Figure 17: Concentration of hexacosanol at two sites (609 and 615) from high to low water mark. These data were used to help determine the most suitable tidal point at which to take samples during subsequent surveys.

Seasonal Variation in Productivity

Large differences in primary productivity between the four quarterly surveys were apparent based on the mean concentration of phytol (Figure 18). There was 3 times more phytol in the mudflat sediment in July and 2½ times more in September than in either of the hotter months of December or March. The results for the more specific algal markers brassicasterol, EPA and other algal biomarkers showed the same trend (Figure 18). These results indicate that peak growth of microphytobenthos occurred after the flood in late February 2004 and continued through the cooler months.

The mean concentration of iso-15:0 fatty acid showed a similar but more muted pattern to phytol as the number of heterotrophic bacteria increased to take advantage of additional labile organic matter from the microphytobenthos. In contrast, the terrestrial plant marker hexacosanol showed a relatively even distribution year round which, other than December, varied by < 5% (Figure 18).

Fitzroy intertidal mudflat biogeochemistry

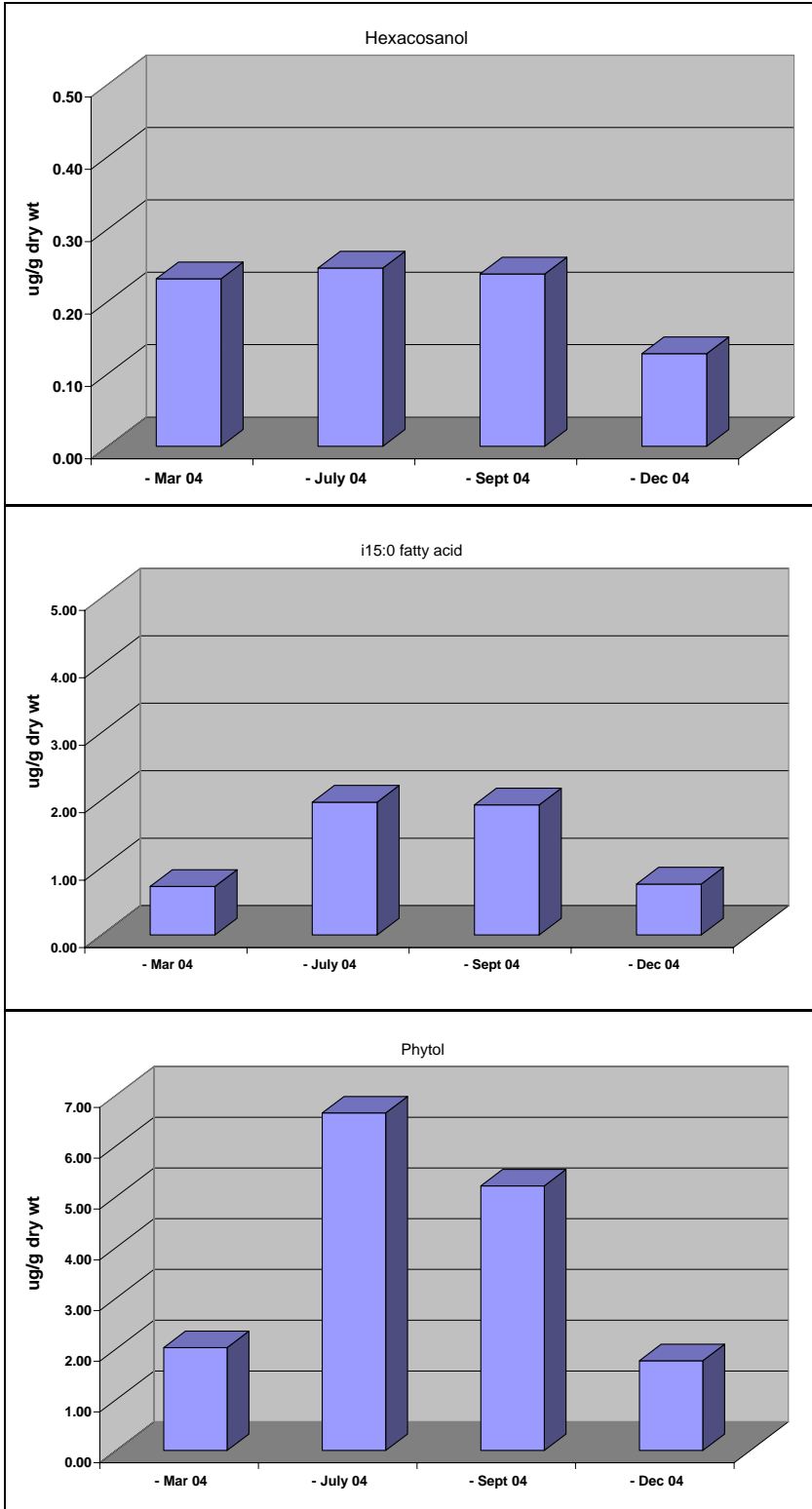


Figure 18: The mean concentration of phytol, hexacosanol and iso-15:0 fatty acid [mean from all sites] for each of the four quarterly surveys. Primary production was highest in winter after the autumn flood whereas terrestrial organic matter was fairly consistent year round

Distribution of Organic Matter along the River

In contrast to the seasonal uniformity, the concentration of hexacosanol varied by up to eight times at sites along the river. Phytol and the other algal markers varied by more than 10 times. As was seen in Table 2, the %CV for the primary biomarkers was generally less than 15 % indicating that measured differences between sites were not environmental and/or analytical variation. Furthermore, differences between sites co-varied significantly across all the surveys. Data for co-variance analysis were standardized to account for large differences between summer and winter – [Std. Score = (raw score - mean)/Std. deviation]. Hexacosanol ($p < 0.01$, Figure 19) co-varied more closely than brassicasterol ($p < 0.01$, Figure 20) and phytol ($p < 0.05$, Figure 21) and both of these more closely than EPA ($p < 0.1$). This evidence suggests that the measured differences between sites along the river are largely determined by physical factors like geomorphology and tidal force that in turn determine current velocities. This then results in greater or lesser deposition of allochthonous organic matter and influences scouring of microphytobenthos from the mudflats. In the case of the microphytobenthos, the algal biomarkers don't co-vary quite as well as the terrestrial markers due to the additional growth factors of light, nutrients and substrate that probably vary, not only between sites, but over time.

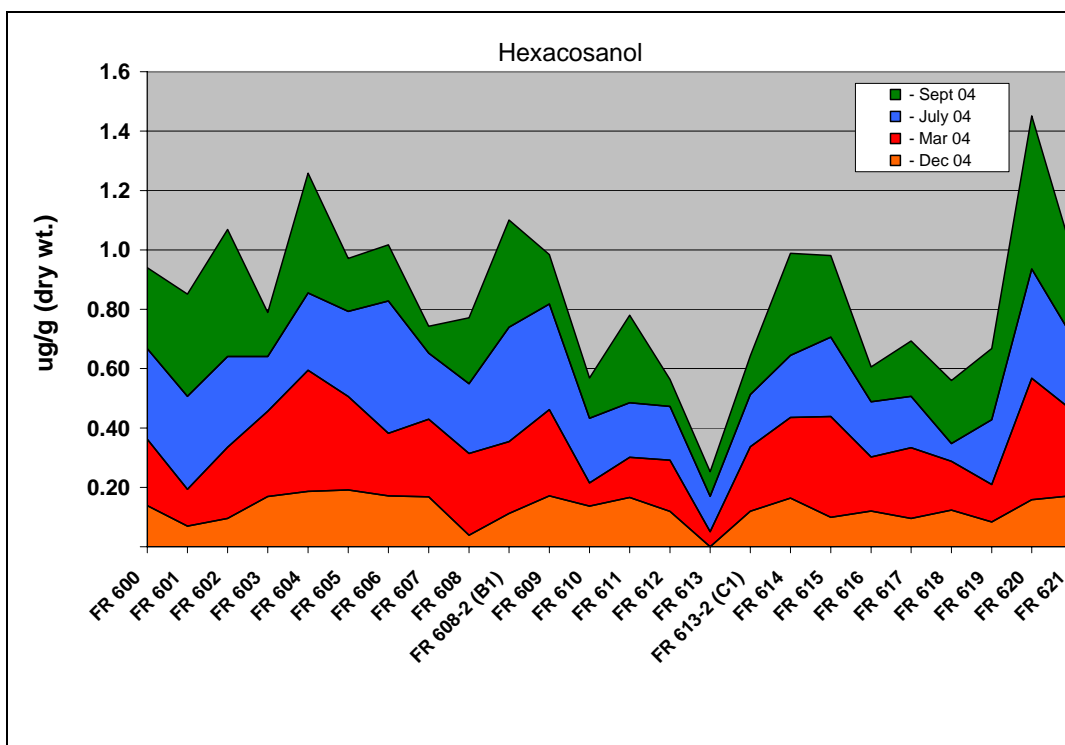


Figure 19: The concentration of hexacosanol at all sites over the four surveys (ug/g dry wt.). Note the significant co-variance by site over the four surveys ($p < 0.01$).

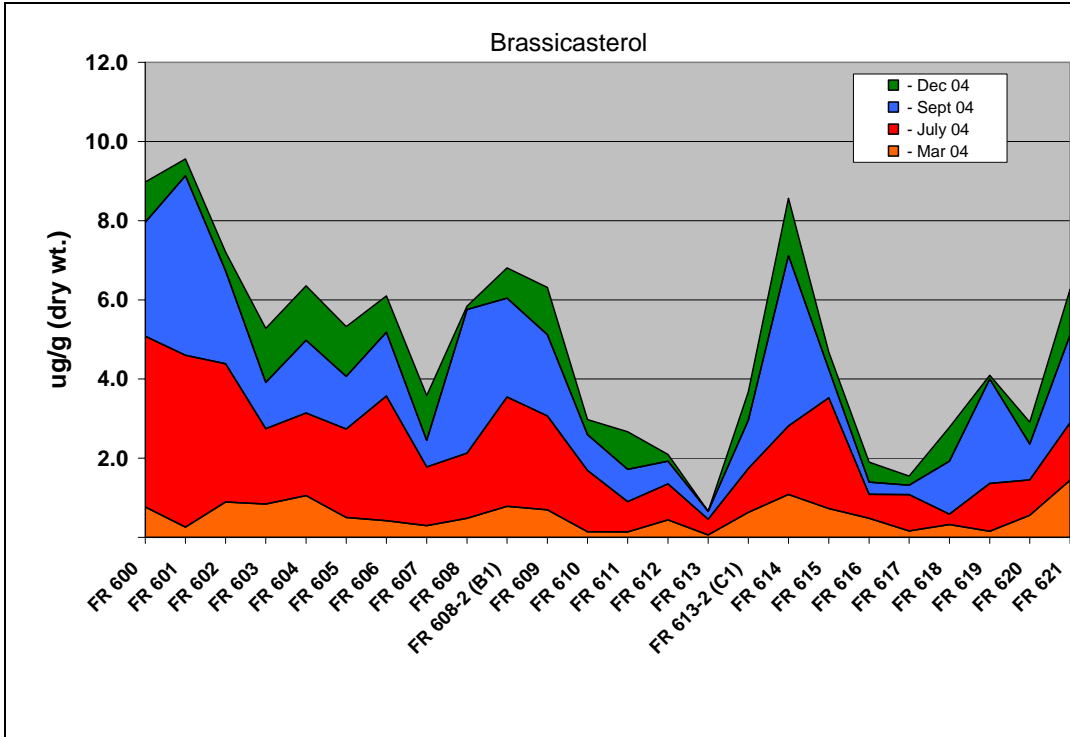


Figure 20: The concentration of brassicasterol at all sites over the four surveys (ug/g dry wt.). Co-variance by site over the four surveys was significant ($p < 0.01$).

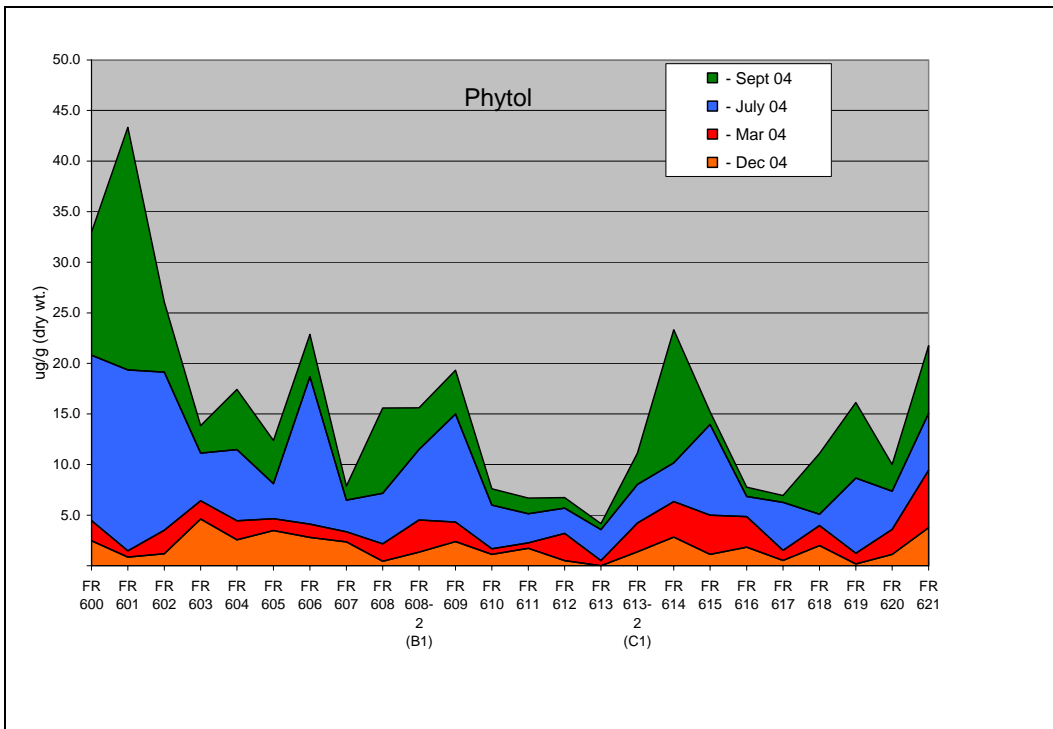


Figure 21: The concentration of phytol at all sites over the four surveys (ug/g dry wt.). Co-variance by site over the four surveys was less than for hexacosanol but still significant ($p < 0.05$).

Species composition

The MPB community composition was dominated by diatoms at all stations and in all surveys. Diatom markers such as EPA, fucoxanthin and brassicasterol were the dominant individual markers within their compound classes (Figures 22 and 23).

Prasinophytes (indicated by prasinoxanthin) were abundant in all surveys except March and dinoflagellates (indicated by peridinin) and cryptophytes (indicated by alloxanthin) also present at many sites, but were in much lesser amounts compared to the diatom markers. Green algae or euglenophytes (indicated by chlorophyll b) were also present in increasing amounts after the February flood and mats of green filamentous algae were particularly noticeable at several sites in the mid-reaches of the river (around site 609).

Notable anomalies in the results included the lack of Chlorophytes (indicated by lutein) at any station during any of the surveys other than in December at stations 604 and 610. Also, there was an unusual 19:w5 fatty acid identified in the fatty acid chromatograms, which was only abundant in July and September. Consistent with measured increases in biomass after March, pigments indicative of chlorophyll-a degradation (Phaeophytin a) were also present in increasing amounts after the March survey throughout the stations.

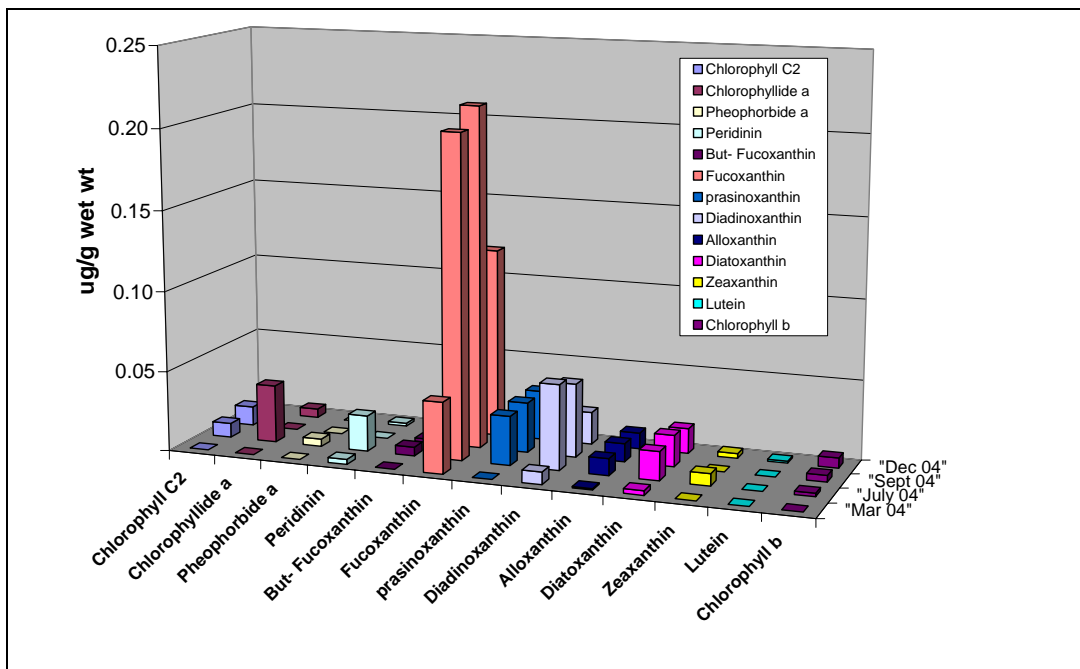


Figure 22: The summed concentration of pigments (ug/g wet wt.) at all sites during the four surveys. The diatom pigments fucoxanthin (Fuco) and diadinoxanthin (Diadino) were the dominant individual components followed by prasinoxanthin (prasinophytes).

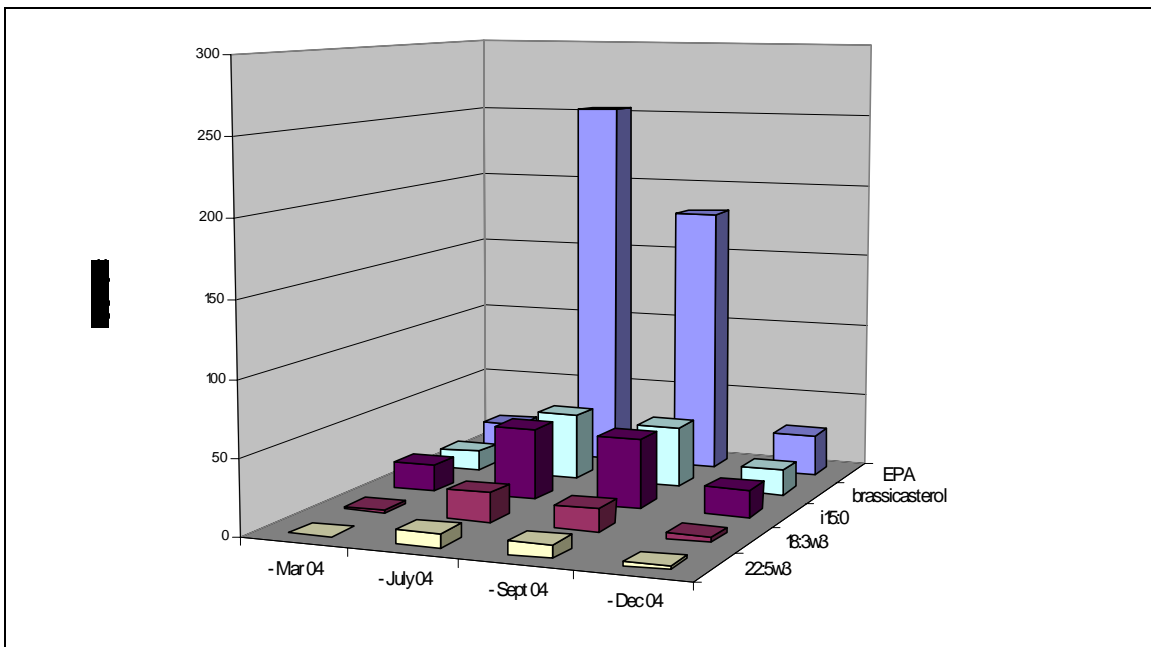


Figure 23: The summed concentrations (ug/g dry wt.) of diatom markers brassicasterol and EPA versus the 18:3w3 and 20:4w6 fatty acids over the four surveys. 18:3w3 and 20:4w6 fatty acids are associated with green and red algae respectively.

After normalizing the biomarker data to percent composition, a matrix correlation was performed to help indicate whether markers from different compound classes represented similar or different sources. The most important result, from the view of tracking primary production, showed that phytol (the side-chain of chlorophyll) was well correlated with the algal markers and poorly correlated with the terrestrial organic markers. The best correlations were in July and September when primary production was at its measured peak. The R-squared values for EPA and brassicasterol correlated to phytol were both > 0.9. In March and December these values fell to > 0.8 but in comparison, R-squared values for hexacosanol, 24-ethylcholesterol and stigmasterol were all < 0.7. These results show that phytol can confidently be used as a surrogate for primary productivity in conceptual and biogeochemical models for the Fitzroy.

Within the more “source specific” biomarkers, there were some compounds that did not correlate as well as might be expected. EPA and brassicasterol correlated reasonably well in the July survey ($R^2 = 0.82$) but this had decreased markedly by December ($R^2 = 0.63$). This result suggests that related groups with different but overlapping constituent biomarkers were present in different quantities in July as compared to December. Cyanophytes (indicated by zeaxanthin) did not figure

prominently in the pigment assays, but this does not mean that cyanobacteria do not make a significant contribution to the algal flora of the mudflats. As noted above and discussed further below, pigment concentrations were not as high as expected based on other data and we suspect poor extraction efficiency. Furthermore, there are other cyanophyte markers that could not be assayed in these surveys and importantly, as was seen in the section above, phytol did not co-vary as significantly as many of the individual algal markers because phytol is a part-constituent of many varieties of algae including cyanobacteria.

Nutrients

Water Column Conditions

A majority of the annual rainfall in the Fitzroy River catchment falls in large episodic events during the summer months (Figure 2). These large rainfall events lead to increased runoff that can cause river discharge to spill over the Rockhampton barrage. Figure 5 shows the river discharge during 2004 at The Gap, approximately 80 km upstream of Rockhampton. It shows that the discharge occurred in January to March and again in November and December. Although 2004 was a relatively dry year (413 mm rainfall in 2004 compared to average of 805 mm/yr), river discharge was enough to cause a small annual flood with freshwater flowing over the Rockhampton barrage. For a majority of the year, the catchment rainfall is insufficient to cause enough runoff to breach the barrage.

Figure 24 shows the salinity, TCO₂ and dissolved nutrient concentrations at each site during each sampling period in the Fitzroy River. The impact of the annual flood is evident in the salinity plot that shows salinities of around 10 during March (approximately 1 month after the flood). When the floodwaters stop flowing, the strong tides then dominate the system returning it to marine conditions prior to July (salinities 35.1 to 35.9) and even hyper-saline by December (salinities up to 40.3).

Water column temperatures in the main channel showed distinct seasonal differences with cooler temperatures in the winter (20.8 to 23.6 °C) and warmer temperatures in the summer months (27.5 to 29.7 °C). The cut-through had more stable temperatures all year round due to its semi-isolated position and shallower water column (25.4 to 29.8 °C).

TCO₂ was lower following the flood (1765 – 1852 µM in the channel and 1126 µM in the cut-through). Concentrations returned to 2639 – 2809 µM by July and remained at similar concentrations for the remainder of the year (2420 – 2664 µM).

The highest NO_x , PO_4 and SiO_4 concentrations were found in the channel following the flood (27.13 μM , 1.36 μM and 131.74 μM respectively) before decreasing significantly for the remainder of the year. This suggests that the fresher floodwaters are the source of higher nutrient concentrations. The cut-through site received the fresher floodwaters (as indicated by the drop in salinity) however, NO_x , PO_4 and, to some degree, SiO_4 appear to have been rapidly taken up, possibly by microphytobenthos (MPB).

NH_4 concentrations were low in the channel (0.05 – 1.02 μM) all year around. Highest concentrations were found in the cut-through during July (16.0 μM), September (4.5 μM) and December (7.8 μM).

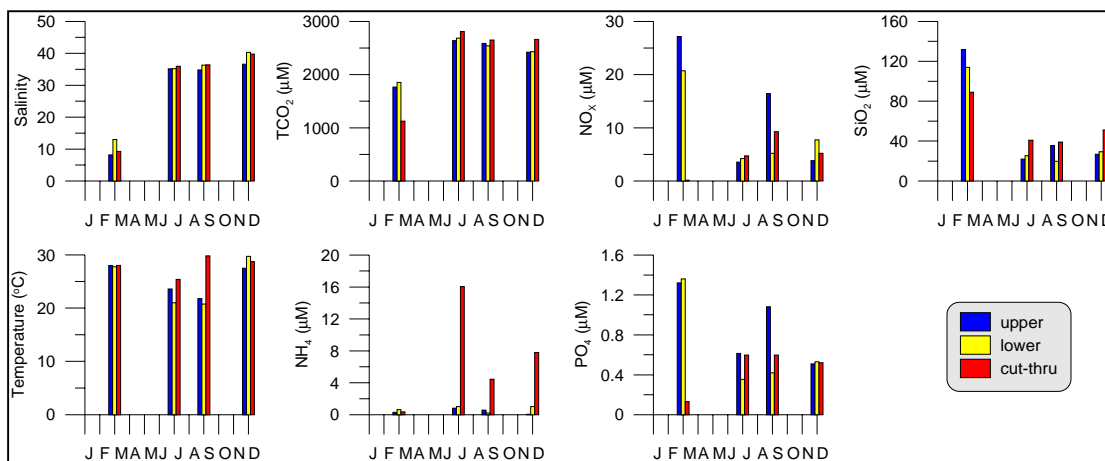


Figure 24: Water column conditions at the three benthic flux sites

Benthic Fluxes

The benthic fluxes shown in this results section are averages that were calculated from fluxes (light and dark) obtained from replicate core incubations at each site during each survey. Raw flux values are available on the data CD.

TCO₂/O₂

Dark TCO₂ fluxes (respiration) in the upper channel ranged from 31.7 to 64.1 mmol m⁻² d⁻¹ from March until September before dropping to 6.2 mmol m⁻² d⁻¹ in December (Figure 25). In the lower channel the dark respiration rate fell from 86.6 mmol m⁻² d⁻¹ in March to 27.6 in December. The cut-through site showed a similar trend to the lower channel, with 92.7 mmol m⁻² d⁻¹ in March and 34.5 in December.

The light TCO₂ fluxes showed that several sites had evidence of photosynthetic activity, in particular the upper channel in July (-7.7 mmol m⁻² d⁻¹) and the lower channel in September (-1.2 mmol m⁻² d⁻¹) that were net photosynthetic. An

interesting observation is the upper channel in March, the cut-through in September, and all sites in December showed light TCO₂ fluxes greater the dark TCO₂ fluxes.

Dark O₂ fluxes (consumption) at all sites decreased from March to December. The upper channel fell from -30.7 to 4.1 mmol m⁻² d⁻¹ and the lower channel fell from -47.3 to 4.0 mmol m⁻² d⁻¹. Highest consumption rates were found in the cut-through site (-63.1 mmol m⁻² d⁻¹ in March).

At the channel sites, the light O₂ fluxes showed similar trends to the dark fluxes with the lower channel in September having net O₂ production (9.6 mmol m⁻² d⁻¹). In the cut-through, the light O₂ flux did not follow the dark O₂ flux, lowest consumption rates were found in March (-5.6 mmol m⁻² d⁻¹).

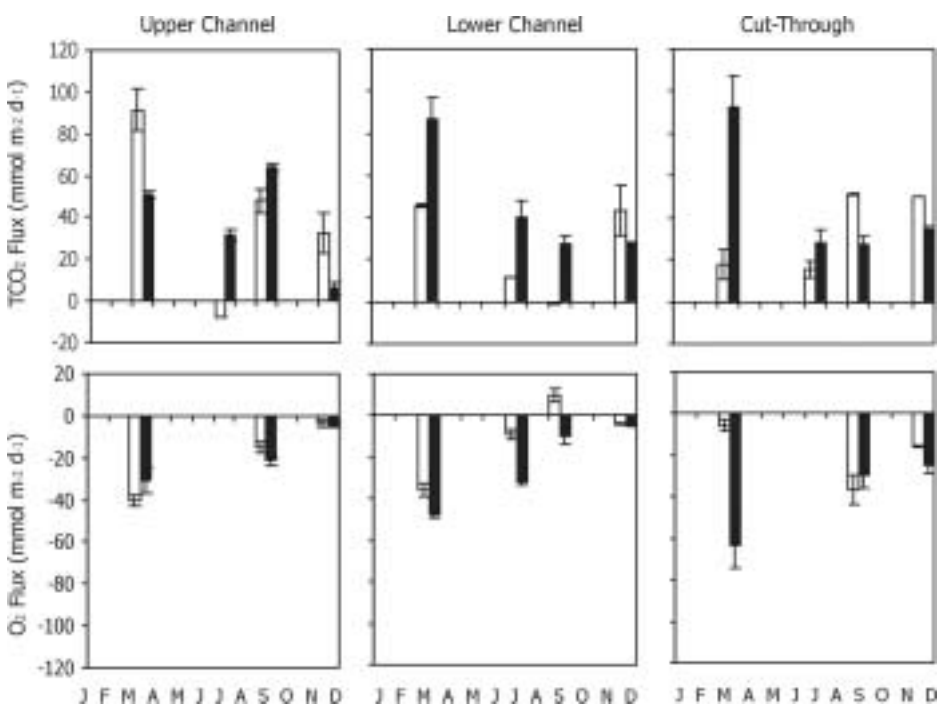


Figure 25: Average TCO₂ and O₂ fluxes. Open bars represent light fluxes and solid bars represent dark fluxes.

At the channel sites, the light O₂ fluxes showed similar trends to the dark fluxes with the lower channel in September having net O₂ production (9.6 mmol m⁻² d⁻¹). In the cut-through, the light O₂ flux did not follow the dark O₂ flux, lowest consumption rates were found in March (-5.6 mmol m⁻² d⁻¹).

NH₄/ NO_x

NH₄ fluxes in the channel sites were generally low and showed no trends within sites or between light and dark rates (Figure 26). Highest fluxes were found in the upper

channel in March where there was an uptake in the light ($-0.7 \text{ mmol m}^{-2} \text{ d}^{-1}$) and release in the dark ($1.2 \text{ mmol m}^{-2} \text{ d}^{-1}$). At all other times, except the lower channel in the dark ($0.6 \text{ mmol m}^{-2} \text{ d}^{-1}$), NH_4 fluxes were below $0.3 \text{ mmol m}^{-2} \text{ d}^{-1}$ in the channel.

The highest NH_4 fluxes were found at the cut-through site; however, like the channel no trends were evident. Light fluxes ranged from $-1.8 \text{ mmol m}^{-2} \text{ d}^{-1}$ in July to $2.3 \text{ mmol m}^{-2} \text{ d}^{-1}$ in September, while dark fluxes ranged from $-0.3 \text{ mmol m}^{-2} \text{ d}^{-1}$ in March to $1.8 \text{ mmol m}^{-2} \text{ d}^{-1}$ in July.

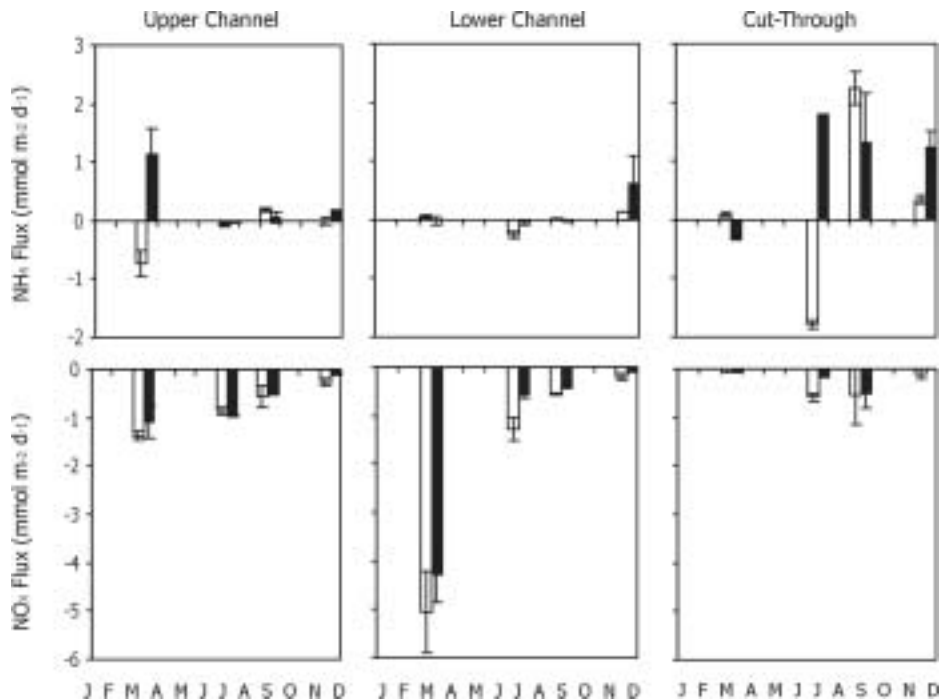


Figure 26: Average NH_4 and NO_x fluxes. Open bars represent light fluxes and solid bars represent dark fluxes.

NO_x fluxes at all sites were negative, indicating uptake of this nutrient in both the light and dark. Both the channel sites showed a similar trend with the largest uptake in March (up to $1.38 \text{ mmol m}^{-2} \text{ d}^{-1}$ at the upper channel site and up to $5.06 \text{ mmol m}^{-2} \text{ d}^{-1}$ at the lower channel site) and a decrease throughout the year. By December, the uptake rates at both the channel sites were very low (down to 0.08 at the lower channel site). Uptake of NO_x in the cut-through was lower than in the channel with highest rates being in July and September (up to $-0.6 \text{ mmol m}^{-2} \text{ d}^{-1}$).

N_2/DON

N_2 fluxes (Figure 27) alternated between uptake and release in the light and dark at all sites. All fluxes were between -8.4 and $5.5 \text{ mmol m}^{-2} \text{ d}^{-1}$ except for the light flux of the lower channel in December, which had a very large N_2 release

($35.2 \text{ mmol m}^{-2} \text{ d}^{-1}$). No trends were evident and there was no correlation between light and dark fluxes.

Dissolved Organic Nitrogen (DON) was only measured for the July and September surveys. DON fluxes at all sites were all considerable and increased from July to September in both light and dark incubations. Light fluxes were larger than dark fluxes in the channel sites whereas dark fluxes exceeded light fluxes in the cut-through site.

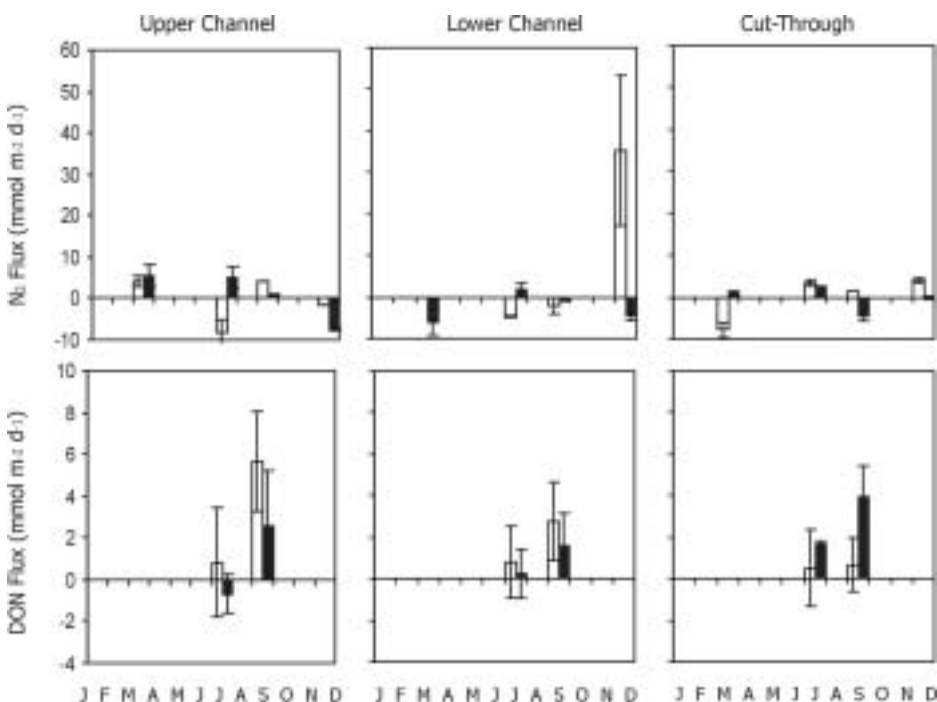


Figure 27: Average N_2 and DON fluxes. Open bars represent light fluxes and solid bars represent dark fluxes.

PO_4/SiO_4

PO_4 fluxes (Figure 28) in the channel sites were all negative, indicating uptake in both the light and dark. Highest uptake was generally found in March and lowest in December. Uptake rates ranged from 0.39 to $0.03 \text{ mmol m}^{-2} \text{ d}^{-1}$. Uptake rates were also usually higher in the light compared to the dark. In the cut-through, uptake rates were lower (less than $0.1 \text{ mmol m}^{-2} \text{ d}^{-1}$) and some PO_4 release (positive fluxes) was measured (0.12 and $0.03 \text{ mmol m}^{-2} \text{ d}^{-1}$ in the light in July and the dark in December respectively).

SiO₄ fluxes were generally negative (uptake) up until September before becoming positive (release) in December at all sites. The highest uptake rates were recorded in March at each site (up to 9.1 mmol m⁻² d⁻¹), with light fluxes generally larger than dark fluxes in the channel sites and dark fluxes greater than light fluxes in the cut-through site.

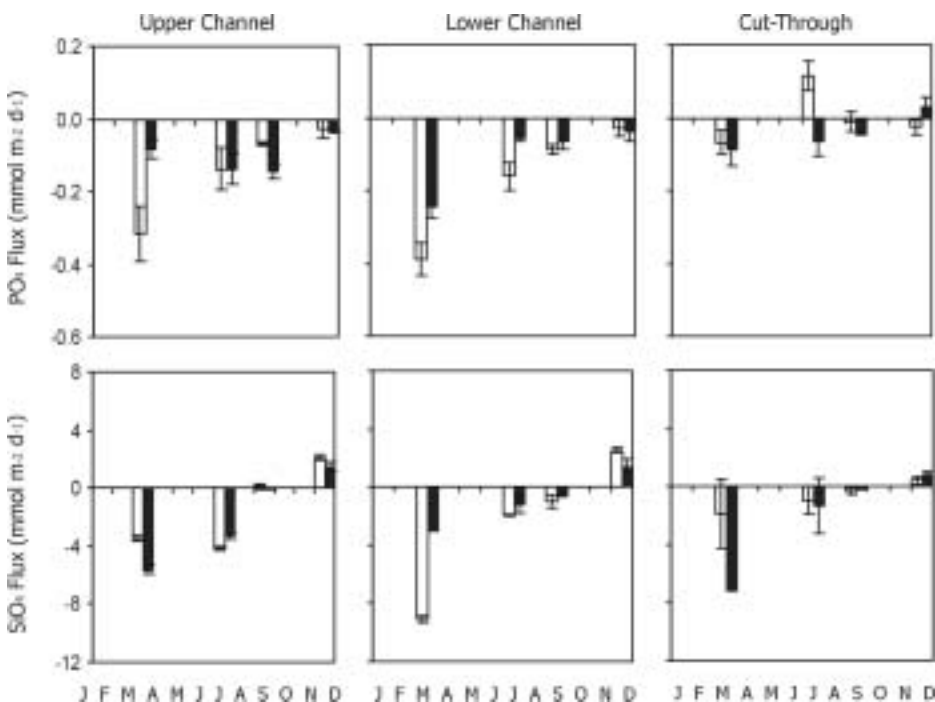


Figure 28: Average PO₄ and SiO₄ fluxes. Open bars represent light fluxes and solid bars represent dark fluxes.

Denitrification and Nitrogen Fixation

Denitrification rates (Figure 29) were low at all sites in both the light and dark (less than 0.3 mmol m⁻² d⁻¹). The dark rates appear to show an upward trend throughout the year in the upper channel and cut-through, whereas they show a downward trend in the lower channel.

Nitrogen fixation rates were an order of magnitude higher than the measured denitrification rates (between 0.8 and 3.1 mmol m⁻² d⁻¹). Light and dark rates were similar in the upper channel site, rates were highest in March and December (2.1 to 2.6 mmol m⁻² d⁻¹) and lowest in cooler month of July and September (0.9 to 1.3 mmol m⁻² d⁻¹). The lower channel site had almost the opposite trend with highest nitrogen fixation rates in July and September (up to 2.4 mmol m⁻² d⁻¹) and lowest rates in March and December (1.0 to 1.5 mmol m⁻² d⁻¹). The cut-through site showed a

greater variation between light and dark nitrogen fixation rates with the light rates highest in March and July (2.0 and 2.5 mmol m⁻² d⁻¹) and the dark rates highest in September and December (2.6 and 3.1 mmol m⁻² d⁻¹)

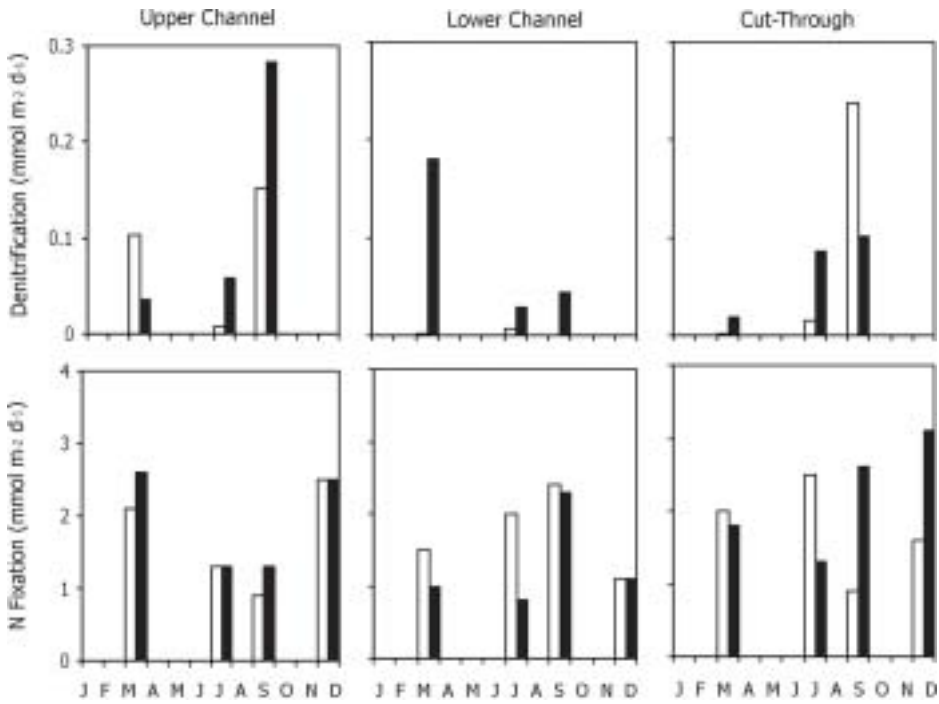


Figure 29: Average Denitrification and N-Fixation rates. Open bars represent light fluxes and solid bars represent dark fluxes.

Discussion

Sources of Organic Matter

In order to account for and trace the primary productivity that drives the lower Fitzroy River system, the first step was to trace the abundance and distributions of the major potential allochthonous and autochthonous sources of organic matter and their associated nutrients.

The results of the four surveys firstly shows that, despite a C:N ratio that indicates the greatest proportion of the total organic carbon is of terrestrial origin, that this organic matter is not tightly coupled to the pulse of algal growth evident in the cooler winter months. To begin with, the terrestrial plant markers are poorly correlated with the algal markers (Table 3). The rain events in late January and early February 2004 (Figure 5) had no apparent affect on the concentrations of hexacosanol (Figure 18) along the river banks. Taraxerol and 24-ethylcholesterol (appendix 1), which are also predominantly terrestrial plant markers, showed the same even trend of concentrations throughout the year. These results suggest that while there is undoubtedly a large mass of detrital material washed into the Fitzroy during these rain events, the vast bulk of it simply flows right through it with little deposition.

Indeed, nutrient budgets calculated during the FH1 study showed the same characteristic, with dissolved and particulate organic matter outputs from the system as high as or higher than inputs during wet events. These data are also corroborated by the hydrodynamic models (Herzfeld *et al.*, 2005) which demonstrate short residence times after a flood. Another factor to this pool of carbon is that, in comparison to utilization of senescent algal biomass, terrestrial plant matter is more refractory. For example, terrestrial plant matter has little or no polyunsaturated fatty acid constituents. The effect of increases in July and September in algal-derived polyunsaturates in the sediments is the corresponding increase in heterotrophic bacteria (Figure 18). This is also borne out by the higher sedimentary O² respiration rates occurring when algal biomass is at its highest rather than consistent through the year as would be the case if terrestrial plant matter was a significant energy source for heterotrophs. The concentration of cholesterol also follows the same pattern suggesting that in-fauna also utilize the increase in labile carbon although probably at multiple levels within the food chain.

In terms of other significant sources of allochthonous organic matter (carbon and / or nitrogen) there are two other possibilities. The first is tidal intrusion from the marine end of the system. However, this can be largely discounted from the results of hydrodynamic modelling. Logically water flows down the river and the tidal influence

has been shown to slop the same water back and forth on its migration to the sea with no prospect for any significant net upstream movement of nutrients. The second possibility is runoff from urban Rockhampton itself including the discharge of sewage effluent. The Rockhampton sewage treatment plant has been estimated to release 76 tonnes of nitrogen per year Douglas *et al.* (2005) to the river just below the barrage. However, there was no evidence of the human faecal sterol coprostanol in any of the mudflat sediments in any of the surveys. That is not to say coprostanol would not have been found in the river water nearby the sewage outfall, rather it is indicative that the river is efficient at assimilating that amount of sewage derived carbon and nitrogen without leaving a residual signature of the original material. The only signature that was present was a slight elevation of the ^{15}N isotopic signature in sediments from sites 600 and 601. The only detectable effect on the river was that the algal biomass (based on both phytol and specific algal biomarkers) at site 601 (closest to the sewage outfall) was more than double the average of all other sites during July and September. However, biomass levels at site 601 in March and December were no different to any other part of the river despite the continued input of effluents. From site 602 and on downstream there was no obvious indication of unusually high or slowly declining quantity of biomass. The reason for this is examined in more detail below. Suffice to say, the effect of effluents and runoff from Rockhampton on MPB production is comparatively small, localized and limited to the cooler months.

Table 3: Correlation coefficients for phytol versus various biomarkers. Coefficients calculated using concentration data (ug/g) across all 24 sites for each quarterly survey.

	Phytol correlated to ↓	Mar-04	Jul-04	Sep-04	Dec-04
algal markers	EPA (20:5w3) fatty acid	0.54	0.96	0.94	0.77
	Brassicasterol	0.81	0.92	0.90	0.89
	22-dehydrocholesterol	0.60	0.88	0.79	0.80
	18:3w6 fatty acid	0.70	0.85	0.93	0.74
	Total Chlorophyll a	0.79	0.82	0.70	0.83
	Total Pigments	0.79	0.81	0.71	0.82
	Diadinoxanthin	0.64	0.81	0.68	0.63
	Carotene pigments	0.42	0.88	0.67	0.35
	Fucoxanthin	0.74	0.73	0.65	0.69
Alloxanthin	0.24	0.74	0.59	0.60	
heterotrophic bacteria	i15:0 fatty acid	0.53	0.88	0.92	0.50
	a15:0 fatty acid	0.48	0.81	0.88	0.63
methanogens	10me 16:0 fatty acid	0.28	0.57	0.43	0.55
In-fauna	Cholesterol	0.88	0.63	0.82	0.95
Terrestrial organic matter	Hexacosanol	0.46	0.68	0.42	0.78
	Stigmasterol	0.27	0.75	0.74	0.56
	24-ethylcholesterol	0.31	0.56	0.80	0.71
	taraxerol	0.29	-0.23	0.35	0.19

Mudflat Primary Productivity

Measurement of pigments and phytol across the surveys can be used to indicate changes in species composition and relative biomass of microphytobenthos during this time. Theoretically, since phytol is the side chain of chlorophyll (liberated during lipid analysis), the two measurements, although made in different ways, should closely follow each other if the primary source is *in-situ* production. Phytol is also liberated via biodegradation of chlorophyll thus any allochthonous inputs can potentially contribute so-called free phytol. However, the correlation matrix in Table 3 indicates that the two do indeed correlate reasonably well which suggests the two are predominantly related to active MPB production.

Seasonal variation in biomass is quite marked, with large increases in MPB during the months of July and September compared with March and December (Figure 18). This increase coincides with cooler water (and presumably mud flat) temperatures, increased salinity and lower nutrients. Changes in species composition are more subtle, since at most times the MPB community appears to be dominated by diatoms, with changes in the minor species.

Effect of Salinity and Flow

It is likely that the decreased salinity and the associated high flow event have several possible effects. The high flows are likely to lead to increased re-suspension and possible export of MPB (as PON) from the estuary. Hydrodynamic modelling (Herzfeld *et al.*, 2005) shows that residence time within the estuary during these events is in the order of days. In addition, during high flow events, the mudflats will not become exposed and the system will continue to be light limited. Decreased salinity is likely to cause a shift in the species composition of MPB as many diatoms are not able to withstand large salinity changes. However, the pigment and phytol data indicates that there are still some live phytoplankton during this time and that the MPB are still dominated by diatoms.

High flows are also responsible for the transport of dissolved nutrients through the estuary into Keppel bay. Only a small proportion of these are retained within the estuary (FH1 report) possibly due to some uptake by phytoplankton and diffusion into the sediments. However, it does seem that these nutrients may be responsible for an enhanced proportion of pelagic diatoms in Keppel Bay (Ford *et al.*, 2005). An interesting question, and one which cannot be answered from this study, is to what extent this population is “seeded” by resuspended benthic diatoms from the estuary and the part that these might play in re-populating the benthic communities as the salinity front moves back up the estuary.

Effect of Temperature

Water temperature shows a marked difference of approximately 10 °C between winter and summer months. Temperatures on the mud flat surface may well vary much more than this due to changes in the incident solar radiation. In temperate estuaries, an increase in temperature is accompanied by an increase in growth rate/productivity, with many algae having an optimum growth temperature of around 25 °C. However, above this temperature, Blanchard *et al* (1996) showed that in light saturated conditions, there was a marked decrease in the productivity of MPB (Figure 30).

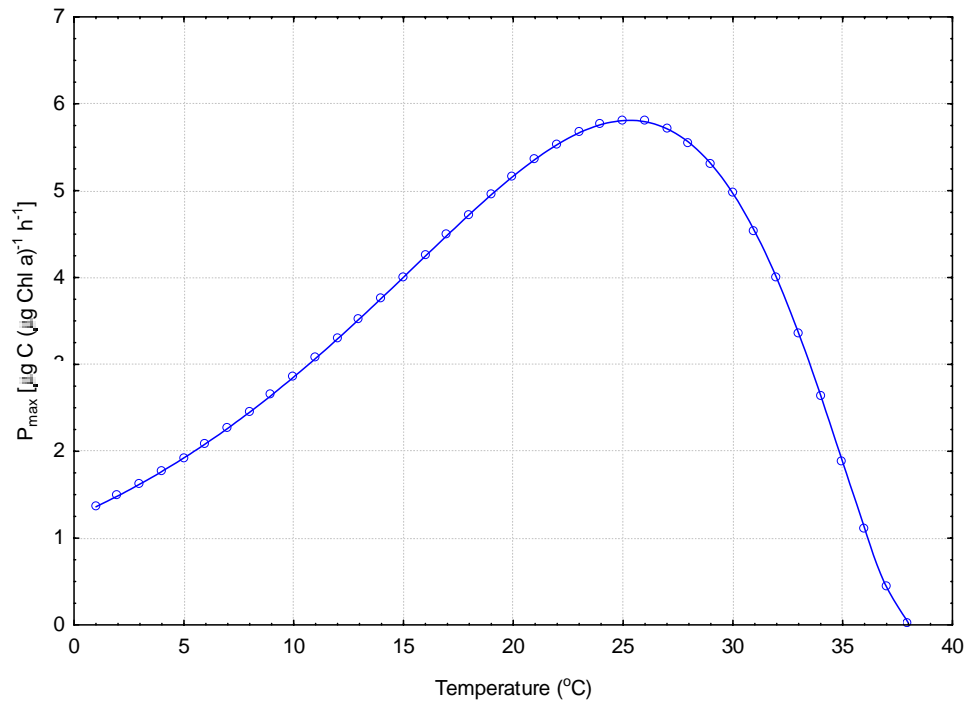


Figure 30: Relationship between Temperature and MPB Productivity (Blanchard et al., 1996)

Thus, it appears most likely that the cooler months provide the best opportunity for increased MPB production and that the higher temperatures on the exposed mud flats will be too high for optimal production. It appears therefore that temperature could be an important control on MPB production in this estuary and in contrast to temperate systems, it is winter which is the more productive period with the limiting factor being high temperatures. A direct comparison of phytol, as a surrogate for production, and temperature over the survey period (Figure 31) clearly demonstrates this close relationship.

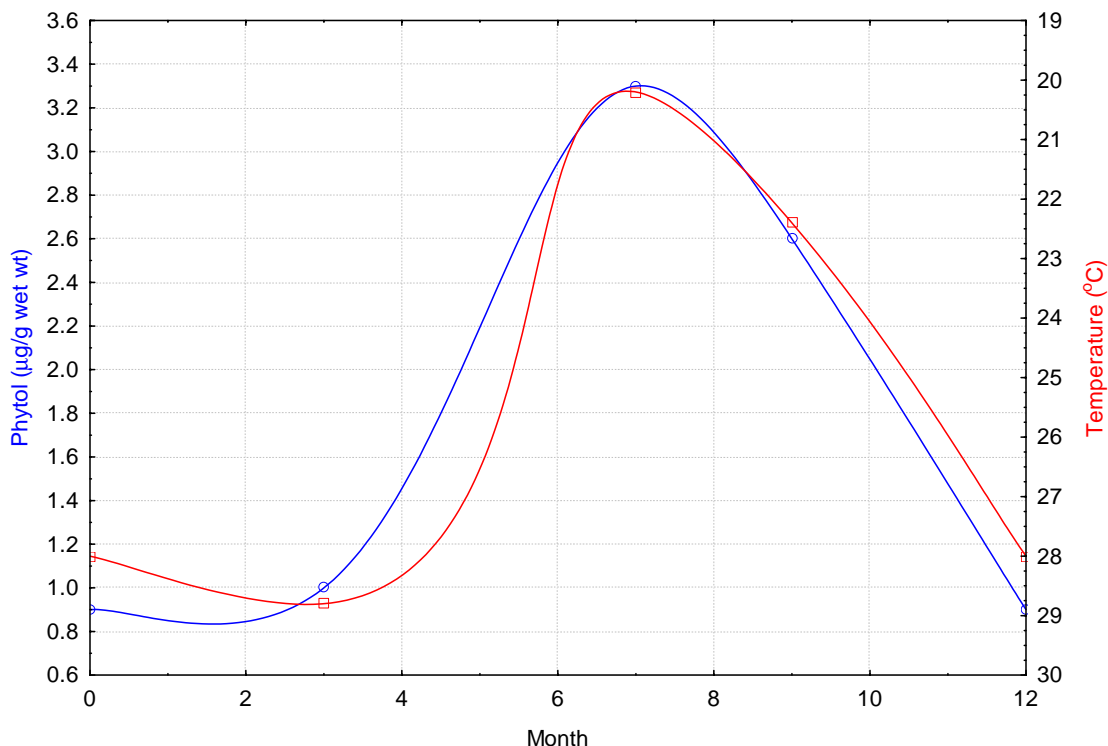


Figure 31: Comparison of phytol and mean water temperature over the survey period. Note: temperature scale is inverted to more clearly show the strong relationship

Support for this hypothesis is apparent if we consider sites 600 and 601. These sites are located close to the township and more importantly, close to the sewage outfall. In times of low flow (May – Dec) Douglas *et al.* (2005) estimated 95 Tonnes of nitrogen are imported into the estuary, and approximately 80% of this originates from the STP outfall. There is evidence from the stable isotope data that this nitrogen is being utilised by algae in the upper reaches of the estuary and in July and September sites 600 and 601 are the most productive. However, in December, still with low flow but elevated temperatures, productivity falls dramatically (Figure 32)

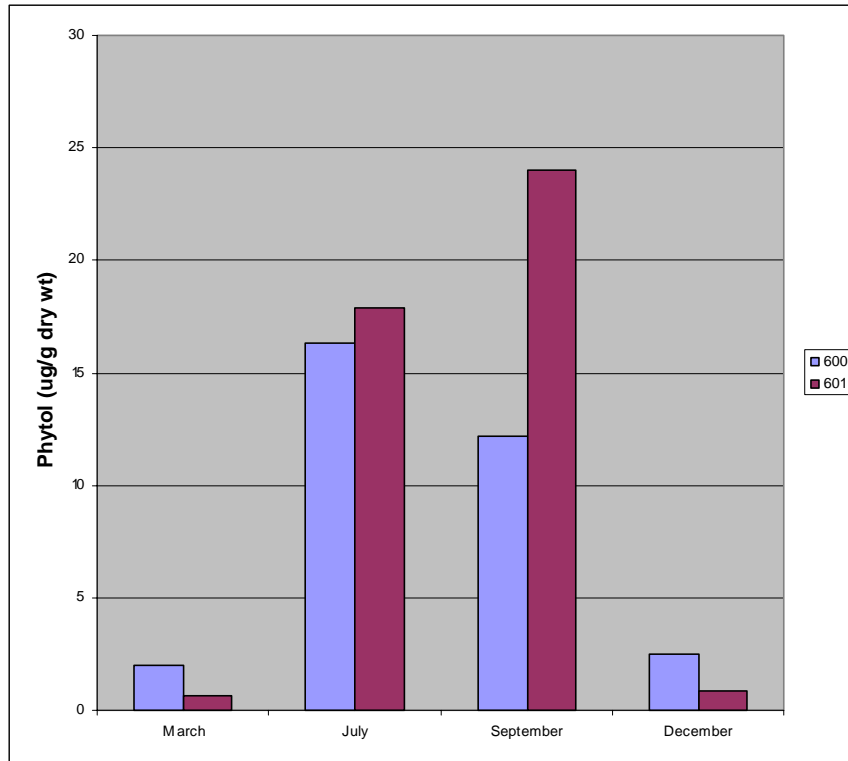


Figure 32: Productivity at sites 600 and 601 indicated by phytol concentrations

Effect of Nutrients

While large quantities of nitrogen and phosphorous are imported into the estuary during high flow events (Douglas *et al*/2005) it appears that the vast majority passes through the system without being utilised although there does appear to be some diffusion into the sediments (see nutrient flux section). The fact that there is only a relatively low level of MPB biomass in the post flood period (March survey) would seem to indicate that there is some limiting factor preventing uptake and subsequent growth by the MPB. The most likely factors would seem to be:

- i. A low starting biomass therefore only small uptake
- ii. A lack of exposed mud flat for much of the flow event causing light limitation
- iii. Elevated temperatures

It is only once these limiting factors have been removed that increased productivity can occur, and this appears to correspond to the winter months. Therefore, it appears that the nutrients imported into (and exported from) the estuary play little immediate role in stimulating MPB production. It follows from this, that the observed

increase in productivity must be supported by nutrients from alternative sources and/or reservoirs. These could be one or more of:

- i. Re-mineralisation of terrestrial organic matter
- ii. Utilisation of sediment nutrient pool which accumulated during the flow event
- iii. Inputs from anthropogenic sources (STP, meat works, etc.)
- iv. Nitrogen fixation by cyanobacteria

Terrestrial organic matter is generally regarded as containing a high proportion of refractory material and therefore not readily available for remineralisation. However, there is a general background of terrestrially derived material which may reflect its refractory nature but also facilitate some pre-adaptation to being able to utilise this material. Douglas *et al* (2005) estimated that there was some loss of DOC from the water column to the sediments during high flow conditions. It's unclear what the composition of this material is, though typically humic material does not contain much nitrogen. Similarly, Douglas *et al* (2005) estimated a loss of dissolved organic nitrogen and particulate nitrogen to the sediments and in this study we have measured a flux of nitrate into the sediments after the high flow event. Once the system is in low flow and water column nutrients are much reduced, there is likely to be a diffusion gradient out of the sediments, which could be utilised by MPB.

As stated in the previous section, there is some evidence for enhanced growth at stations in the upper part of the estuary and that this may be due at least in part to STP derived nitrogen. However, stable isotopes would suggest that this effect diminishes quickly with distance down stream as there is no evidence of higher $\delta^{15}\text{N}$ below site 601. It should also be stated that at this stage there is no specific evidence linking the elevated $\delta^{15}\text{N}$ values with an output from the STP but these effects have been observed elsewhere (Costanzo *et al.*, 2001). Isotope values can be relatively sensitive to such inputs and there is no evidence for faecal markers in the sediments.

Cyanobacteria can often make up a significant proportion of the MPB biomass in situations where light and/or nutrients are limiting. This is due to a more efficient photosystem and the ability to fix atmospheric nitrogen. In this study we were able to measure nitrogen fixation, which was occurring at all times of the year while pigment analysis suggests that cyanobacteria were only ever a minor component of the MPB population. In this study, the cyanobacterial contribution was assessed based on the presence of zeaxanthin, which is a less specific marker than pigments such as phycocyanin but we did not have the ability to measure these in this study. A

confounding factor in assessing the presence and/or relative importance of cyanobacteria is the efficiency with which they can be extracted. For example, in March the estuary was predominantly fresh and freshwater species of algae are known to be potentially difficult to extract for pigments due to thicker cell walls, thus if cyanobacteria were an important species at this time they could conceivably be underestimated. Some cyanobacteria (though not all) produce a C₃₂ hopanol (Summons *et al.*, 1999) which we detected in lipid extracts, and this can be utilised to examine the relative occurrence of cyanobacteria over the survey period. However, this cannot be used to infer their importance relative to other groups as there is little information on amounts of such markers relative to biomass and this would also most likely be highly variable parameter. Interestingly, this particular C₃₂ hopanol can be detected in sediments from each survey at similar levels, which does not correspond to the pigment (zeaxanthin) analysis, which only detected the presence of cyanobacteria in July and September. Neither of these parameters correlates with the measured N₂-fixation, which was greatest in March. These discrepancies highlight the difficulty in utilising markers (pigments or lipids) to infer the presence or absence of particular species or processes. Factors such as the amount of biomass per unit of marker, individual species composition and variability and the degradation rates of individual compounds can all contribute to confuse the interpretation.

Estimating MPB Biomass

Mudflat MPB biomass can potentially be assessed in two ways:

- i. Using chlorophyll a concentrations and an estimated C:Chl value (e.g. 30 is often used for diatoms (Claustre *et al.*, 1994))
- ii. Phytol, the side chain of chlorophyll, which is liberated during lipid analysis and occurs at a theoretical chl:phytol value of 3 (and therefore a C:phytol value of 90)

Data from the seasonal surveys indicates that the pigment and phytol data correlate reasonably closely (Table 3) indicating the two are predominantly from the same source. However, a comparison of raw data (see data CD) indicates that phytol appears to be present at levels far in “excess” of what might be expected for the measured chlorophyll values (Table 4). What is immediately apparent from this data is that there is a decreasing trend from March to December. Clearly this could be due to Chlorophyll being underestimated or Phytol being overestimated.

In the previous section it was noted that there can be several reasons for a reduced efficiency of pigment extraction. Freshwater species can be more difficult to extract due to thicker cell walls and presumably the same may be true for some marine benthic species, where a thicker cell wall offers more protection from the sediment matrix. The fact that the discrepancy is largest in March when the estuary is predominantly fresh would suggest that species composition has at least some part to play.

Table 4: Mean phytol:chl values across all sample sites for each survey (theoretical phytol:chl = 0.3)

Survey	Mean phytol:chl
March	102
July	64
September	53
December	25

Overestimation of phytol is possible if there are significant quantities of allochthonous material being brought into the estuary, including degraded chlorophyll. Phytol is a natural product of chlorophyll degradation and generally thought to be degraded relatively quickly ($t_{1/2}$ = ca 7 days; Sun *et al.*, 1998) but can potentially be found in a “free” state. To address this, we analysed one sample (site 602; July; phytol:Chl = 143) for phytol **prior** to the saponification step in our extraction protocol which liberates phytol from the chlorophyll molecule. When this was derivatised and analysed by gas chromatography (Figure 33) only a very small amount of phytol (ca. 5% of the total) could be detected indicating that this is not a potential mechanism for overestimating phytol in these samples. Another alternative mechanism for overestimation would be if there were significant quantities of chlorophyll degradation products not detected by the pigment methods used here but that still contain an intact phytol side chain. Degradation of chlorophyll by bacteria and other processes can produce colourless residues (Gillan and Johns, 1980; Sun *et al.*, 1994) which may not be detected in the methodology used here. Although the majority of chlorophyll degradation products appear to lose their phytol side chain (Louda *et al.*, 1998). With the above in mind we can utilise the pigment and chlorophyll data to give an estimate of the possible range of MPB production. Assuming a C:Chl ratio of 30 (MPB appear to be predominantly diatoms) and an estimated mud flat area of 240,000 m² (this assumes a 2m wide band of maximum production along both sides) for the main channel (not including the tidal creeks) we can calculate a standing biomass of MPB for each survey (Table 5).

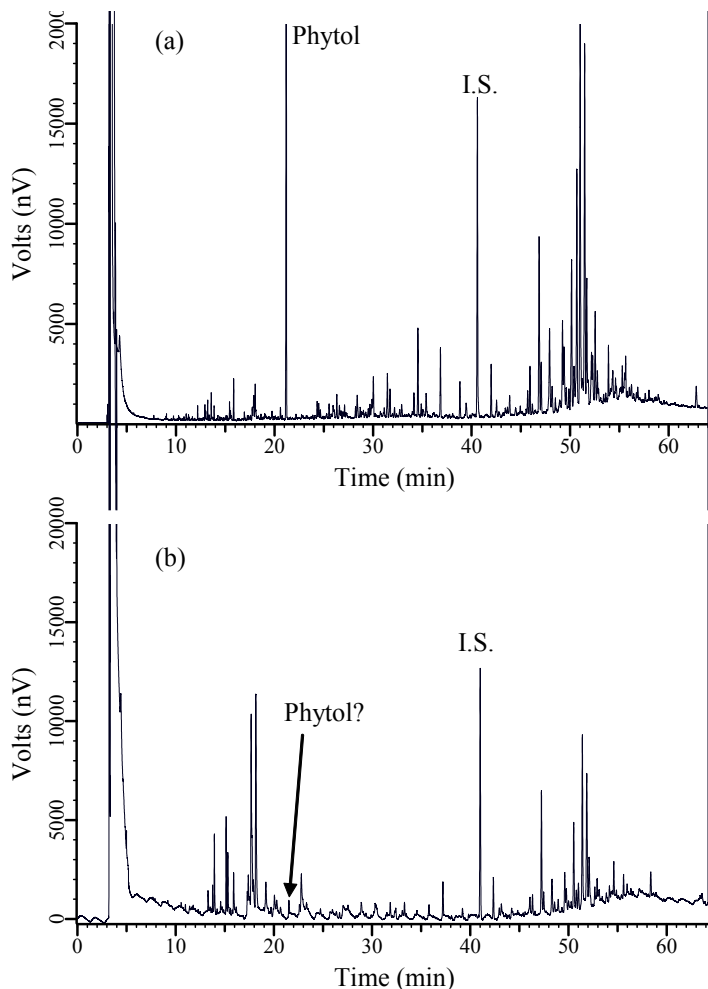


Figure 33: Comparison of (a) total and (b) “free” phytol in the solvent extract of sediment from site 620 sampled in July 2004

Table 5: Estimates of mean standing MPB biomass based on Chlorophyll and Phytol

Month	Mean Standing Biomass mgC/m ² (total C Kg)	
	Based on Chl	Based on Phytol
March	6 (1.5)	1862 (447)
July	32 (7.7)	6106 (1466)
September	30 (7.2)	4786 (1149)
December	22 (5.4)	1695 (407)

Davidson *et al.* (2002) present data on the relationship between carbon and cell biovolume and Hillebrand *et al.* (1999) suggest appropriate biovolume calculations for a variety of species, including diatoms. Thus using the equation presented in Davidson *et al.*

$$\text{Cell carbon (pg)} = 0.109[\text{live cell volume } (\mu\text{m}^3)]$$

And an estimated average diatom cell volume from Hillebrand *et al.* (1999) of 30000 μm^3 it is possible to estimate cell densities:

Table 6: Estimation of MPB cell densities based on Chlorophyll and Phytol

Month	Estimated number of cells m^{-2}	
	Based on Chl	Based on Phytol
March	1851421	569546302
July	9776154	1.867E+09
September	9160108	1.464E+09
December	6844586	518265602

From this data it is apparent that standing biomass triples between March and July/September before declining again to December. It is not possible to estimate growth rates from this type of data as there is no grazing information; however it is desirable to attempt to estimate gross production on the mudflats. Montani *et al.* (2003) used a combination of field and laboratory studies to estimate an MPB growth term of 1.68 d^{-1} for *Navicula*, a common benthic species, in an estuary in Japan. In this instance, if we assume lower growth rates than this and estimate them according to the observed biomass and assign them for quarterly periods, we might have a growth distribution as highlighted in table 7.

Table 7: Estimates for possible growth rates based on literature values and observed biomass.

Period	Estimated $\mu \text{ (d}^{-1}\text{)}$
March - May	0.25
June - August	0.5
September - November	0.5
December - February	0.2

If we then apply these growth rates to the calculated biomass in Table 5, we can estimate a possible MPB production (Table 8).

Table 8: Estimates of MPB gross production based on assumed growth rates and biomass calculations

Period	Estimated MPB Production ($\text{gCm}^{-2}\text{d}^{-1}$) (Total C (Kg))	
	Based on Chl	Based on Phytol
March - May	0.0015 (33)	0.47 (10,000)
June - August	0.016 (345)	3.1 (65,900)
September - November	0.015 (323)	2.4 (51,700)
December - February	0.004 (97)	0.34 (7,300)
Annual (gCm^{-2})	3.3 (800)	1.56 (135,000)

Obviously these estimates are critically dependent on the assumptions of growth rates and the biomass estimates but the values based on phytol compare favourably

with Montani *et al.* (2003) who found a range of seasonal production values from $0.63 \text{ gCm}^{-2}\text{d}^{-1}$ in the northern winter to $1.75 \text{ gCm}^{-2}\text{d}^{-1}$ in the northern summer.

Direct comparison between the MPB and water column productivity is difficult due to the variable and transient nature of periods of elevated water column productivity (Figure 3). However, the following points can be made:

- MPB production is much more seasonally and spatially consistent than water column productivity
- The reasonably conservative estimates of MPB production presented here are still highly significant.
- The data presented here represents only the main channel. Inclusion of the extensive tidal creeks would see these figures increase, potentially by orders of magnitude.

Mudflat carbon Export

With the observed MPB production it is important to formulate some hypotheses as to the most likely method of export of this carbon from the mudflat, assuming it is utilised by the wider food chain. Although no specific in-faunal studies were undertaken as part of this project, it is possible to gain at least a basic insight as to the possible importance of grazing. Cholesterol is a ubiquitous marker in sediments having a predominantly animal source. It is also present in lower relative and absolute amounts in some algal species, but this rarely affects the overall “animal fingerprint”. Therefore, in fine sediments such as these, concentrations of cholesterol are often associated with the presence of in-fauna. In this study, cholesterol levels closely followed the MPB production, as did the bacterial markers indicating both these groups were responding to the changes in biomass. In-fauna are likely to be grazing directly on the MPB or the bacteria, while the bacteria are most likely consuming either senescent cells or extracellular polymeric substances (eps) produced by the MPB. Benthic diatoms are known to produce eps (eg. Smith and Underwood, 2000; Wolfstein and Stal, 2002) and it has been postulated that this material can play a previously underestimated role in the heterotrophic pathway of tidal flats (Goto *et al.*, 1999; Hoskins *et al.*, 2003) and may play a role in stabilising the sediments (Andersen *et al.*, 2005).

It has been suggested that grazing of algal cells can lead to a predominance of the chlorophyll degradation products known as phaeophorbides, while senescence and the subsequent bacterial degradation produces phaeophytin (eg Barranguet *et al.*, 1997). In each of our surveys Phaeophytin was detected but phaeophorbide was

generally absent. Indirectly, this suggests that senescence and bacterial pathways are potentially more important, but it is by no means conclusive and further studies would be required to fully investigate this. Certainly in the winter months small crabs were observed “grazing” on the mud flats but it is not clear what these were consuming. Similarly small fish can be seen at the waters edge and these may also be grazing on the MPB, either directly off the mudflat or on re-suspended diatoms at the tidal front (Lucas, 2003).

Nutrient Cycling

Respiration

Rates

During the process of organic matter degradation, O_2 is consumed, TCO_2 produced and nutrients (NH_4 , NO_x , PO_4 , SiO_4) released. If oxygen is unavailable (or fully consumed) then another oxidant (generally sulphate) will be used in this process. The released nutrients are available for biological uptake and can (in some cases) be consumed as quickly as they are produced. The release of TCO_2 from the sediment is a direct measure of the amount of organic matter being degraded. The organic matter degradation process can be complicated by the presence of microphytobenthos (MPB) and other aquatic plants, which produce O_2 , and take up TCO_2 and nutrients. Figure 34 shows that all sites within the Fitzroy River are net respiratory (i.e. net positive TCO_2 fluxes – calculated from the average of light and dark fluxes) and that the highest rates for each site were in March following the annual flood.

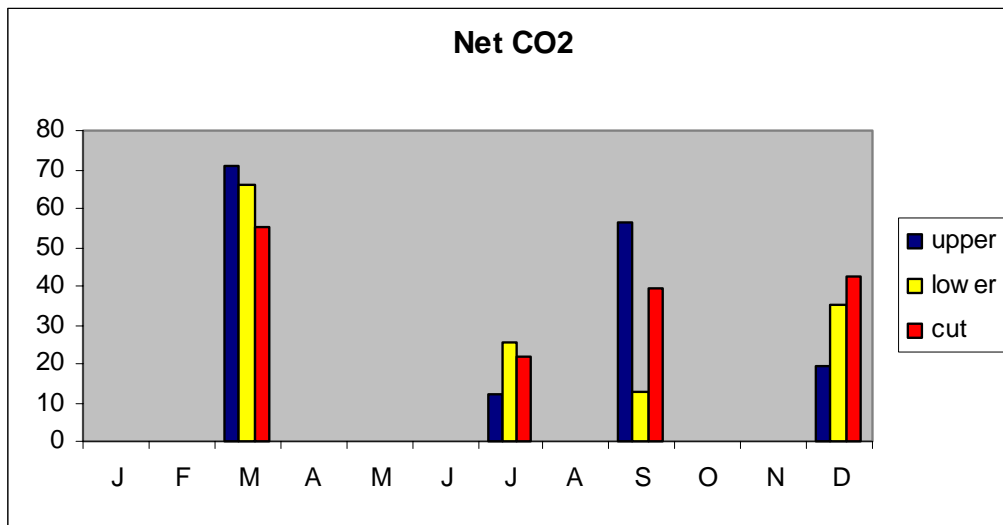


Figure 34: Net TCO_2 fluxes (calculated from the average of the light and dark TCO_2 fluxes)

The dominance of respiration at all sites would usually indicate that the intertidal sediments of the Fitzroy River are net heterotrophic, meaning that bacterial composition of organic matter in the sediments exceeds the creation of organic matter by benthic plants. However, when looking at the light and dark TCO₂ fluxes (Figure 25), we see light fluxes exceed dark fluxes (particularly in December) indicating that autotrophic production of TCO₂ dominating heterotrophic production.

Type

Figure 35 shows that, generally, more TCO₂ is produced than O₂ consumed. This result indicates that another oxidant (besides oxygen) is contributing to the organic matter degradation. Sulphate reduction is an anaerobic process whereby sulphate oxidises organic matter producing hydrogen sulphide. This is the most likely process to occur in coastal sediments once oxygen is depleted.

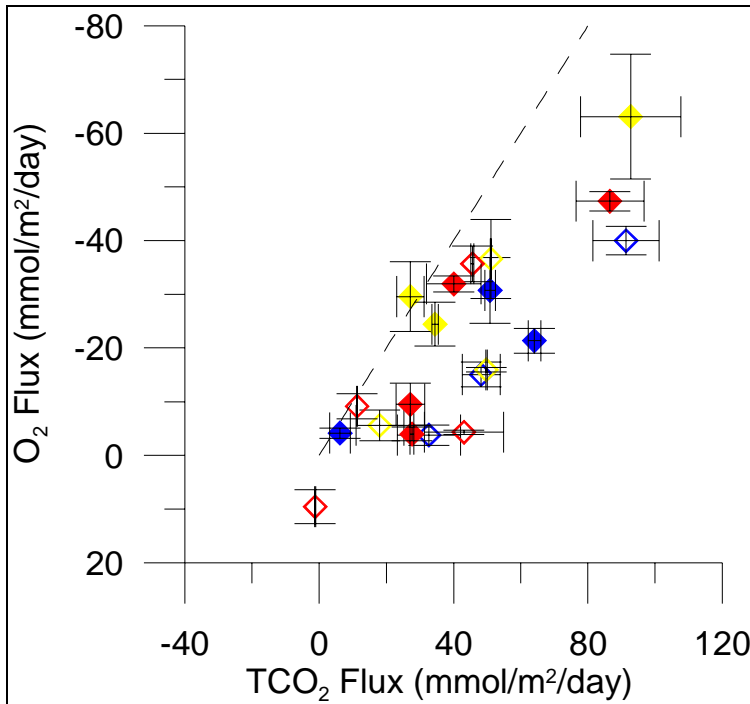


Figure 35: TCO₂ flux versus O₂ flux

Source

The annual floods that occur each summer have a severe impact on the organic material on the intertidal mudflats. It is highly likely that the floodwaters “clear” all surface organic material from the banks and deposit fresh, terrestrial-derived organic material. As the year progresses, this fresh terrestrial organic matter will be depleted

and replaced with new marine organic material transported upstream by the strong tides.

Table 9: C:N for Fitzroy River Benthic flux sites

Survey	Upper	Lower	Cut-through
March	11.6	10.8	9.8
July	10.3	9.3	8.5
September	15.9**	9.7	9.8
December	10.9	12.4	11.5

** Contaminated?? (Redfield 6.625)

The C:N ratio (Table 9) shows that, throughout the year, all sites had a greater ratio than expected from the degradation of a marine source of organic matter (Redfield ratio of 106C:16N or 6.625). The C:N were derived from sediments collected from the top 2 cm of the intertidal mudflats. Any marine organic matter (deposited on the incoming tides post-flood) would only occupy the very top (several mm) of the intertidal zone. It is therefore likely that the C:N we measure incorporate deeper terrestrial derived organic matter and newer marine derived organic matter.

Nutrient Fluxes

Ammonia (NH₄) and Oxidised Nitrogen (NO_x)

Figure 36 shows the net fluxes of NH₄ and NO_x at each of the sites within the Fitzroy River. Apart from the lower channel site in December and the cut-through site in September and December, all surveys showed a net uptake of dissolved inorganic nitrogen (DIN) species. In the upper channel site, NO_x uptake dominated any release of NH₄. The upper channel site showed a similar trend except for the December survey whereby a release of NH₄ was larger than the NO_x uptake. The cut-through site showed a net release of DIN that was dominated by NH₄.

The net uptake of DIN (particularly in the channel sites) indicates either an uptake by biota (MPB) or advective (bioirrigation) transport into the sediments. If advective transport of nutrients was taking place, then the loss of NH₄ and NO_x would occur at similar rates, this was not the case (data not shown). Therefore, it is assumed that uptake of these nutrients is the result of active MPB on the sediment surface.

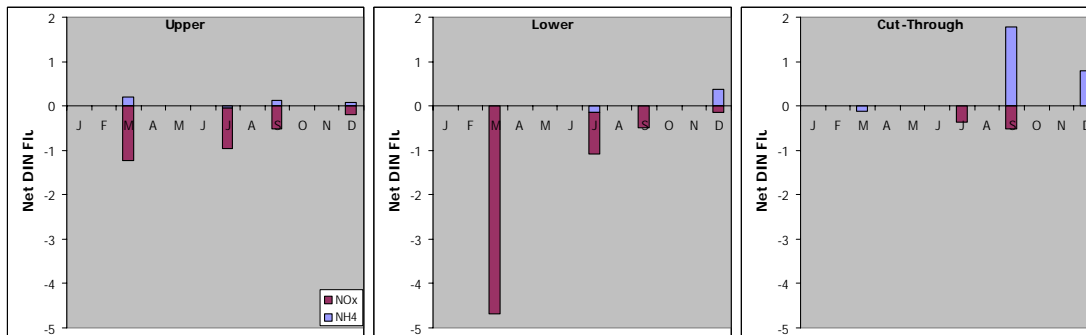


Figure 36: Net Dissolved Inorganic Nitrogen (NH_4 and NO_x) fluxes (calculated from the average of the light and dark fluxes)

Dinitrogen (N_2), Denitrification and Nitrogen Fixation

Denitrification, the reduction of nitrate to nitrogen gas (N_2), is an important process in coastal environments. It is a mechanism by which nitrogen, that would otherwise be available for phototrophic uptake, is removed from the system. Nitrogen fixation is essentially the reverse of denitrification, nitrogen gas is converted back into the biologically-available form (NH_4) by certain types of bacteria (including the cyanobacteria). This study undertook measurements of denitrification (via the isotope pairing technique), nitrogen fixation (via the acetylene reduction technique) and nitrogen gas (via the N_2 :Ar method: see Methods section). It was hoped that there would be some correlation between the pool of nitrogen gas and the amount being produced (denitrification) and consumed (nitrogen fixation). In theory, the N_2 flux should equal the denitrification rate minus the rate of nitrogen fixation. A positive result means a net denitrifying environment and a negative result means a net nitrogen-fixing environment.

The N_2 fluxes, obtained from the N_2 :Ar method, do not correlate with the denitrification rates measured via isotope pairing. The denitrification rates were all very small ($< 0.3 \text{ mmol m}^{-2} \text{ d}^{-1}$) and could not account for some of the positive N_2 fluxes measured. Similarly, the nitrogen fixation rates do not correlate with the N_2 fluxes measured. Nitrogen fixation rates are all between 0.8 and $3.1 \text{ mmol m}^{-2} \text{ d}^{-1}$ and, along with the small denitrification rates, should result in a net uptake of N_2 at all sites throughout the year. However, positive N_2 fluxes (release) were measured at each site during some of the surveys. Therefore, either some other process is generating the N_2 or the nitrogen fixation rates are significantly smaller than recorded. Anammox (anaerobic ammonia oxidation) is the only other known process that has N_2 as an end product and may be responsible for the excess N_2 (Devol,

2003). Another possibility is an over estimate of the 'true' rates of nitrogen fixation. The acetylene reduction technique only estimates potential rates of nitrogen fixation, and 'real' rates could be much less.

The very small denitrification rates could be attributed to the presence of MPB on the intertidal sediments. MPB have been shown to have a significant impact on denitrification (Risgaard-Petersen, 2003) by competing with nitrifying/denitrifying bacteria for nitrogen. Sundback *et al.*, (2004) found that MPB nitrogen assimilation often exceeded nitrogen removal by denitrification partly because MPB activity suppressed denitrification. It has also been shown that dissolved organic nitrogen (DON) can be greater than DIN in the presence of MPB, and that a significant fraction of the nitrogen assimilated by MPB is returned to the water column as DON (Sundback *et al.*, 1991; Eyre & Ferguson 2002). This is consistent with the DON results we obtained from the July and September surveys.

Phosphate (PO₄) and Silicate (SiO₄)

The net PO₄ and SiO₄ fluxes (Figure 37) show similar trends to each other. Both nutrients are predominantly taken up (negative fluxes) and the largest rates of uptake are immediately following the flood (March). The uptake of PO₄ and SiO₄ also corresponds with the uptake of NO_x (Figure 36) and as such is also attributed to MPB activity on the intertidal sediments. The uptake of nutrients is shown to be stimulated by the nutrient-enriched floodwaters, but then decreases throughout the year. The net SiO₄ fluxes even show that in December, this nutrient is released from the sediments, indicating that nutrients released from the degradation of organic matter is dominating nutrients taken up by MPB.

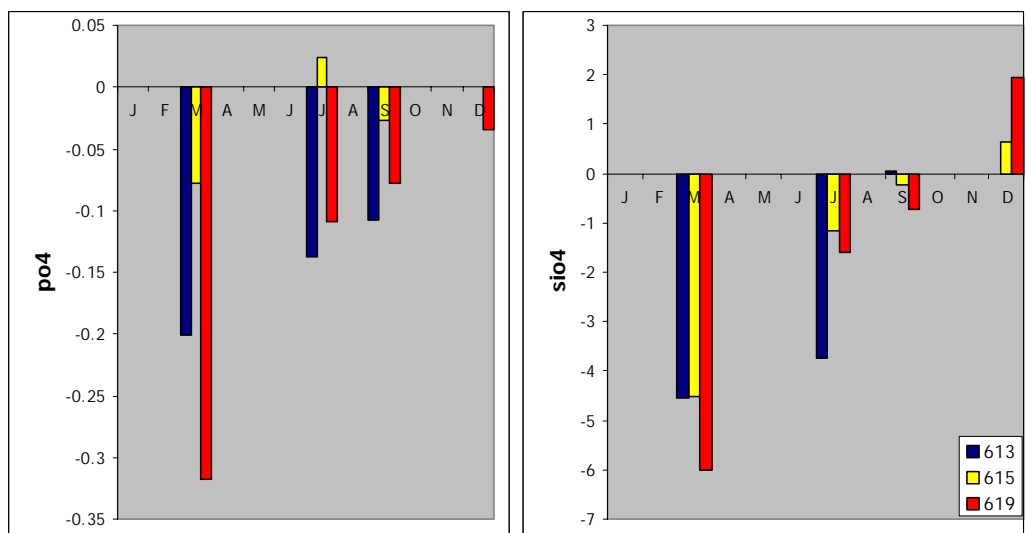


Figure 37: Net PO₄ and SiO₄ fluxes (calculated from the average of the light and dark fluxes)

Loads

The net PO_4 and SiO_4 fluxes (Figure 37) show similar trends to each other. Both nutrients are predominantly taken up (negative fluxes) and the largest rates of uptake are immediately following the flood (March). The uptake of PO_4 and SiO_4 also corresponds with the uptake of NO_x (Figure 36) and as such is also attributed to MPB activity on the intertidal sediments. The uptake of nutrients is shown to be stimulated by the nutrient-enriched floodwaters, but then decreases throughout the year. The net SiO_4 fluxes even show that in December, this nutrient is released from the sediments, indicating that nutrients released from the degradation of organic matter is dominating nutrients taken up by MPB.

Table 10: Calculated sediment nutrient loads

Site	N Load (T/yr)	P Load (T/yr)	Si Load (T/yr)
Upper Channel	-0.76	-0.32	-3.97
Lower Channel	-1.84	-0.37	-3.91
Cut-through	0.48	-0.06	-3.21

Conclusions

The main findings of this study are based around the initial objective of increasing understanding of the interrelationship between flow, nutrients and microphytobenthic production. During the study, it became apparent that there were three main periods in the estuary cycle, a summer wet period (high flow post flood), winter (cooler, low flow) and summer dry (warm, low flow). Thus, the conclusions from the work are best outlined according to these periods.

Summer Wet (Post-flood – March)

The Summer Wet period comprises the time immediately following the annual flood. This is the only time of the year when river flows dominate tidal flows and terrestrial inputs make it past the barrage at Rockhampton. During the Summer Wet:

- Annual floods bring nutrient-rich freshwater into the Fitzroy estuary;
- The Fitzroy River becomes predominantly fresh to the mouth of the estuary (depending on size of flood);
- The water column has high temperatures and high turbidity;
- Nutrients (particularly NO_x , PO_4 and SiO_4) are taken up at high rates, presumably by the biota;
- Standing MPB biomass is low, presumably due to poor growth conditions during the flood (low salinity, low light, high temperatures, reduced mudflat area)
- Significant growth will occur between now and July

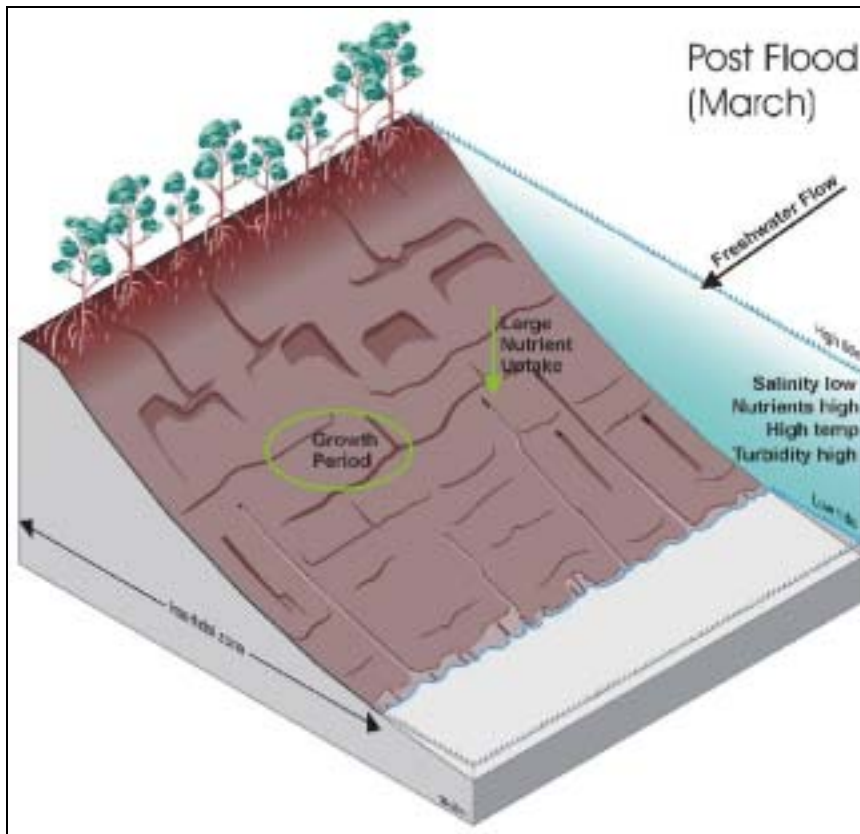


Figure 38: Conceptual model for the post flood period (March)

Winter (July – September)

The winter period encompasses the July and September surveys, and may extend a month or two either side of this. This period is characterised by:

- No riverine flows, therefore the Fitzroy estuary is fully marine and tidally influenced;
- Cooler water temperatures;
- Nutrient uptake reduced from the Summer Wet period, but still significant;
- Significantly elevated standing biomass of MPB – at its maximum in this period
- Physical conditions are at optimum for growth during this period

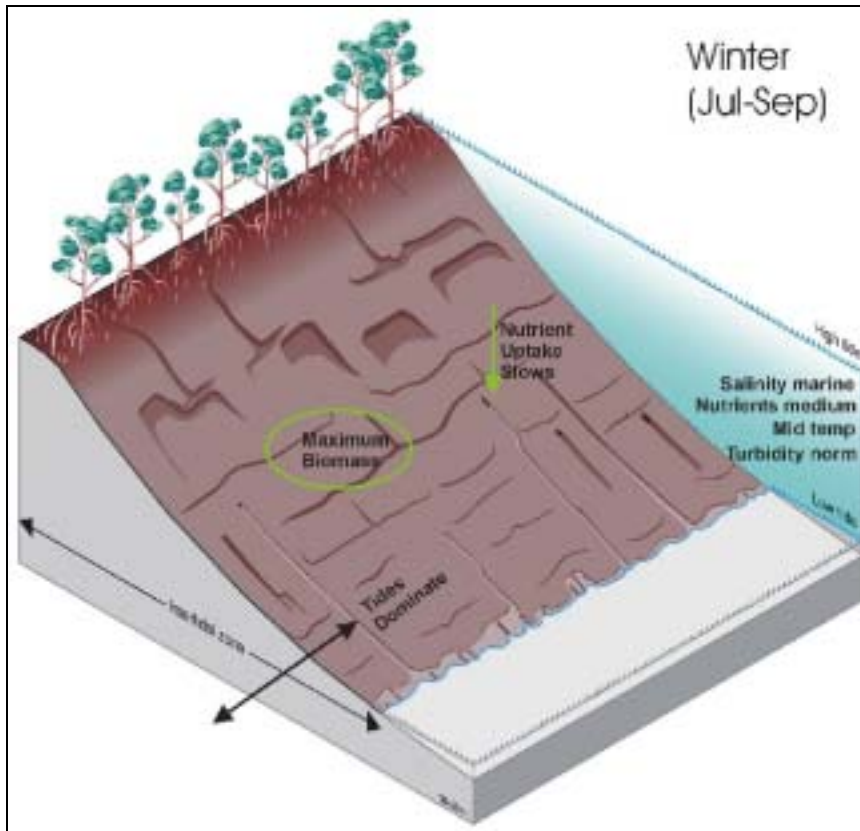


Figure 39: Conceptual model for the winter period (July – September)

Summer Dry (Pre-flood December)

The Summer Dry period comprises the summer months immediately prior to the annual flood. During this period:

- There are still no riverine flows and the Fitzroy estuary has become hypersaline in places due to large water residence times and evaporation;
- Water column temperatures are high;
- Nutrients are either being taken up at very low rates, or are being released (by the degradation of organic matter);
- Conditions become much less suitable for MPB growth due to elevated temperatures and MPB biomass has declined significantly

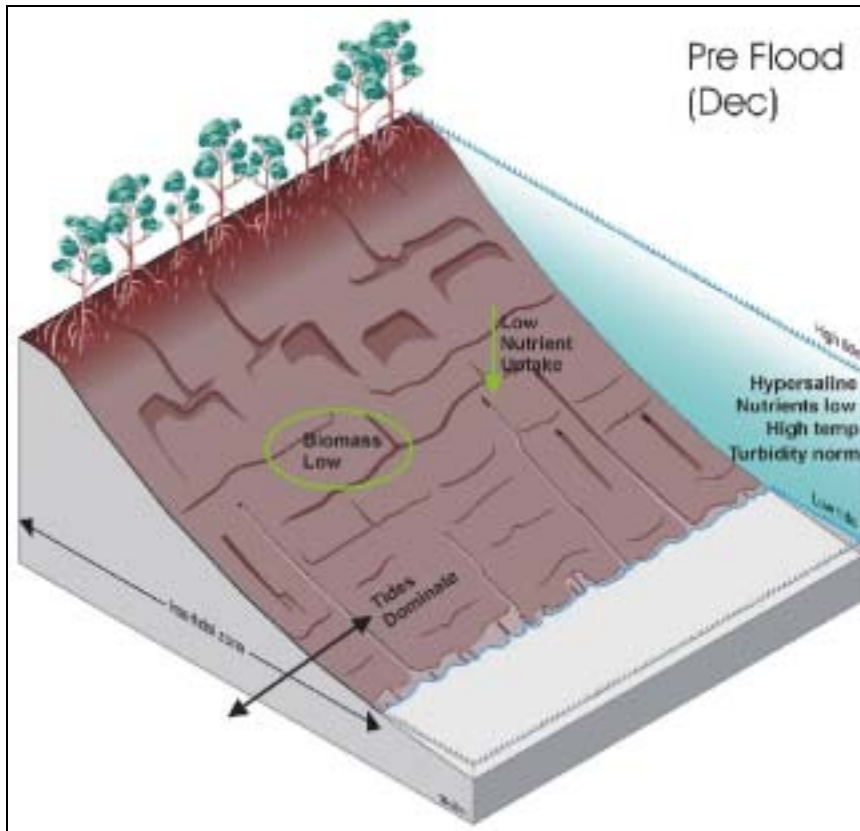


Figure 40: Conceptual model for the pre flood period (December)

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