CRC For Freshwater Ecology Project C240:

The effects of increasing salinity on ecosystem function, resilience and diversity.

FINAL REPORT
Activites 4/5 & 6

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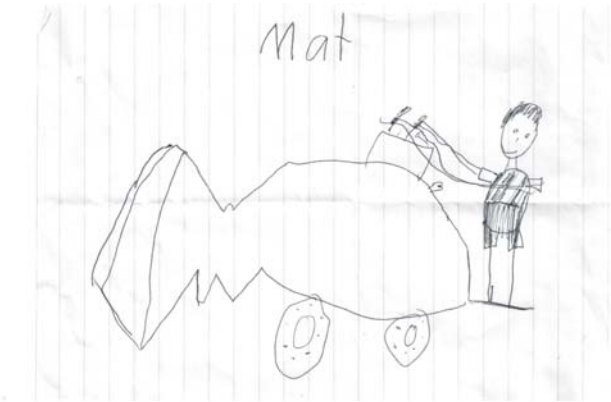

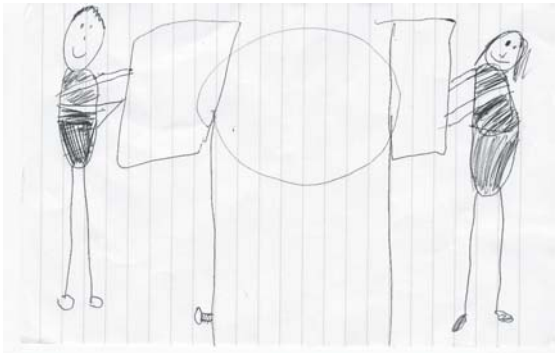

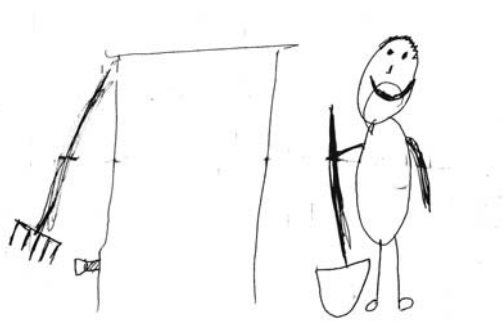

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Introduction

Vast areas of Australia's farmland and waterways are gradually being influenced by salinity. It is estimated that by the year 2050 an area the size of Victoria will be lost to traditional agriculture (PMSEIC 1998) and that 40,000 km of waterways and associated wetlands will be adversely affected by salt (Dillon and Lewis 2001). In 1990 the importance of rising salinity and associated deterioration in water quality within the Murray-Darling Basin was identified as a priority in the Murray-Darling Basin Natural Resources Management Strategy (Crabb 1995). More recently, the Prime Minister's Science, Engineering and Innovation Council (PMSEIC 1998) reviewed the scope of the salinity problem in Australia. In particular, the potential devastating impact of salinization on aquatic ecosystems was highlighted. The large spatial and temporal scales of the impact of salinity mean that if our current best land management practices were fully implemented, salinization would continue to increase in aquatic ecosystems throughout Australia.

Natural resource management organizations such as the Department of Land and Water Conservation (DLWC) in New South Wales (NSW) and the Murray-Darling Basin Commission (MDBC) have set interim end-of-catchment (or valley) targets for salinity based on existing knowledge. Available data suggest that aquatic biota will be adversely affected as salinity exceeds 1,000 mg L^{-1} (1,500 EC) but there is limited information on how increasing salinity will affect the various life stages of the biota in aquatic systems. While some native aquatic biota appear to be tolerant of increase in salinity above 10,000 mg/L (Williams and Williams 1991; Kefford *et al*. in press), it may be early life forms that are potentially most at risk from gradual increases in salinity. Although the effect of increasing salinity on aquatic biota has been extensively reviewed we have very limited understanding of the ecological consequences of salinization in Australian freshwaters (Hart *et al*. 1991; Bailey and James 1999; Nielsen and Hillman 2000; Clunie *et al*. 2002).

Previous work by Nielsen *et al*. (2003) has shown that salinity in combination with hydrology influence plant community species richness, composition and abundance as well as the richness and abundance of the microinvertebrate community. For a given salinity level, different communities develop from the same seed and egg bank when flooded. In general, species richness and abundance decline significantly at salinity levels over 1000 mg/L. Also, individual species have been shown to vary in their salinity tolerance depending on flooding depth.

Our understanding of how freshwater sediment chemistry is affected by increasing salt is not well developed. Recent work by Baldwin *et al*. (2005) has shown that nutrient and microbial dynamics can be altered if freshwater sediments are inundated with salt-containing waters. Longer term impacts of salt on sediments and nutrients are unknown.

Over the next 100 years, salinity is predicted to increase up to or above 3000 mg L^{-1} in many freshwater ecosystems, aquatic systems will undergo significant changes to their biota. While all freshwater aquatic systems are under long term threat from salinization, wetlands are the systems that potentially are at greatest risk in the short term and therefore, will be the focus of this project.

Given this focus on wetlands, the research will include a combination of mesocosm experiments and field based studies. Before predicting the effects of salinization on aquatic ecosystems, we need to understand the process of aquatic ecosystem degradation. In particular, an understanding of the effects of increasing salinity on primary and secondary production, nutrient dynamics and food web structure and its relationship to biodiversity changes are needed. A series of mesocosm experiments will be undertaken that will allow determination of which processes are modified and the flow on effects to biodiversity. Results from the mesocosm experiments will be used to develop a conceptual model of salinity, diversity and resilience that can be tested in a natural system.

The objectives of the project are therefore: To quantify the effect of a gradient of salinity on key biodiversity (microbes, algae, zooplankton and aquatic plants) and ecological processes (microbial activity, algal production and respiration, zooplankton and aquatic plant recruitment) and, To explore the relationships between the function, diversity and resilience of aquatic systems along a gradient of salinity.

These objectives will test the following hypothesis

A. Community diversity and structure hypotheses

Increased salinity:

i) changes the species richness and abundance of biotic groups (aquatic plants, microinvertebrate, phytoplankton and microbes) under experimental mesocosm conditions.

ii) decreases the numbers and richness of grazers, causing the numbers and richness of algal populations to increase through reduced grazing and increased nutrient availability.

B. Functional hypotheses

Increased salinity:

iii) will promote a decrease in methanogenesis. If this occurs in association with sulfate-containing waters, a shift to sulfate reduction as the terminal anaerobic respiration process will result, leading to changes in phosphorus dynamics.

iv) decreases the sediment's capacity to retain ammonia, but increases mobilization of iron, leading to a decrease in available phosphorus in sediments and overlying water.

v) reduces the concentrations of dissolved nutrients so that the relative importance of benthic algae increases over phytoplankton.

vi) affects the capacity of algae to take in nutrients, with the impact being dependent on salt concentration, salt composition and relative nutrient concentration. This impacts productivity and community composition.

vii) differentially restricts the photobiology of algae causing changes in primary production, system productivity (and community composition).

viii) changes the capacity of biotic groups to reproduce.

ix) will change recruitment of microinvertebrates and aquatic plants.

Project methodologies

Mesocosm set up

Twenty four 800 L mesocosms were purchased and situated at Wonga Wetlands, Albury. Wonga Wetlands is situated on the outskirts of Albury and is a large wetland complex that has a well established education centre and associated facilities that make it ideally suited to being a research site.

Sediment was collected from a wetland called "Bluelight " on the Macquarie Marshes, NSW. Approximately 8 m^3 of sediment was removed from the surface 10cm and transported to laboratory. At the time of sampling, the wetland was dry. Sediment was placed in the mesocosms to a depth of 30 cm and the tanks filled with water from Albury town supply. Commercial sea salt (Aquasonic) was added to mesocosms to give replicate units in a randomized split plot design experiment with 4 replicates per treatment. Four salinity levels ware prepared to give mesocosms with <300 mg L^{-1}, 1000 mg L^{-1} 5000 mg L^{-1} and15000 mg L^{-1} salt, as a single dose treatment, and two further treatments adding salt (weekly additions) over 6 months to give final concentrations of 1000 mg L^{-1} and 5000 mg L^{-1} (Figure 1). Salinity in the tanks was maintained by addition of water of salt as required.

Pumps were fitted to the mesocosms to provide gentle mixing but did not disturb the sediment.

Selected parameters measured in the mesocosm studies (e.g. diversity of algae, zooplankton, plants, microbes and ecosystem process measures) will be tested along a field salinity gradient to aid our extrapolation from mesocosm results to the field. Results from these mesocosm experiments and parallel field studies will be used to develop and modify a conceptual model of how ecosystem function and diversity and resilience are modified along a gradient of increasing salinity

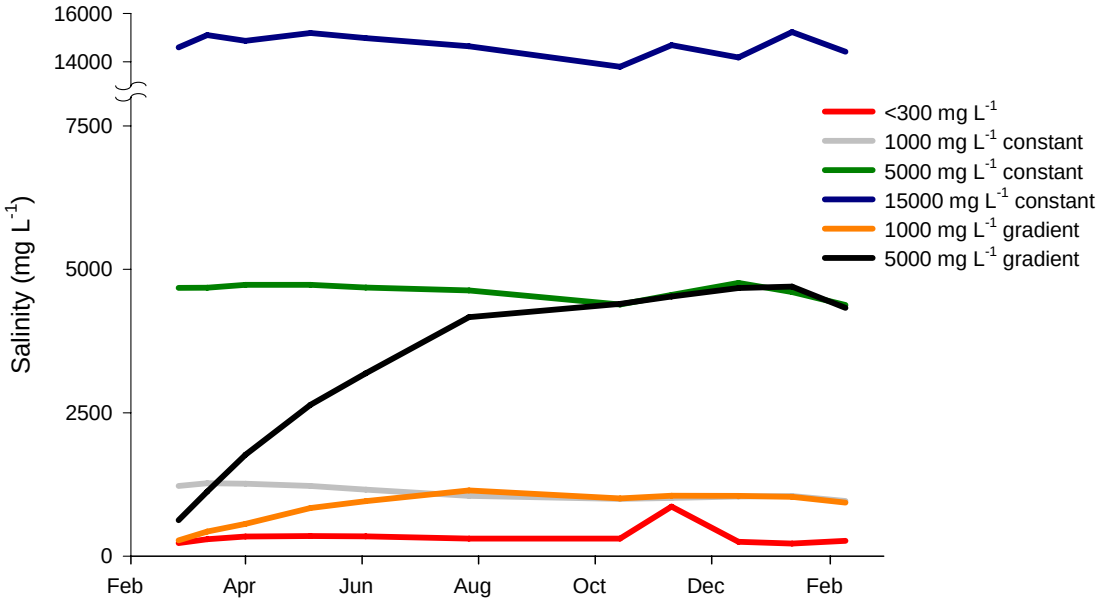

Figure 1: Salinity regimes imposed on the mesocosms

Biogeochemistry

Analyses of total nitrogen (TN), total phosphorus (TP), ammonia, NO_x, soluble reactive phosphate (srP) followed standard methods. Dissolved oxygen (DO), pH and temperature were made using an Horiba (U10) water quality meter. Temperature and DO also will be measured on occasions with multi-probes (in the course of making production and respiration measurements).

Terminal restriction fragment length polymorphism (T-RFLP) is a DNA-based technique and was used to examine microbial community structure in sediments. The technique essentially examines the diversity of a target gene. In our case, overall microbial community structure patterns were based on diversity of 16s rRNA genes and sulfate-reducing bacteria communities were based on dsrAB genes (which are specific to this group of microbes).

A suite of enzyme activities were measured in the water column of the tanks and were used to measure changes in microbial activity. Multivariate analysis of the enzyme activity used PERMANOVA (Anderson 2001; McArdle and Anderson 2001; Anderson 2005) to determine if there where changes in enzyme activity over time

Algal Productivity

Underwater light profiles were measured in each mesocosm on each occasion with cosine-corrected, underwater quantum sensors and the vertical attenuation coefficient (k_d) estimated by linear regression of natural log transformed irradiance values against depth. The euphotic zone was estimated as the depth to which 1% of the surface irradiance

penetrated. The mean irradiance within the water column ($\overline{E}$) was calculated from the expression (Riley, 1957):

$$\overline{E} = E_0(1 - e^{-k_d z_m}) / k_d z_m \qquad 1$$

E_0 is the irradiance just below the water surface and was calculated from the incident irradiance assuming a 10% surface loss. The mixing depth z_m was equal to the depth of the mesocosms (0.7m).

The biomass of planktonic algae was estimated from the concentration of chlorophyll-a. An integrated sub-surface water sample was collected in a 250mL opaque plastic bottle from each of the twenty four mesocosms. Chlorophyll concentration was measured in triplicate by extracting GF/C filtered samples into 10 ml of boiling 90% ethanol, storing at 4^{o}C for 12 h, clearing by centrifugation, and reading spectrophotometrically at 665 and 750 nm. Calculation followed the method of the International Standards Organization (ISO 10260:1992(E)), but without acid correction for phaeophytin.

Total metabolism was measured at intervals of 3-5 weeks in three of the four mesocosms that comprised each of the six salinity treatments. The eighteen mesocosms were selected randomly on the first sampling occasion then utilized throughout the rest of the season. Total mesocosm metabolism was estimated from the diel changes in dissolved oxygen concentration measured using Clarke-type oxygen sensors (YSI). Oxygen probes were submerged to a depth of 30cm and held in a frame with a submersible pump that flushed water across the membrane surface. Measurements of oxygen concentration and temperature were recorded at 5 or 10 min intervals for a period of 48 h starting from the middle of the first day. The measurements were used to calculate metabolic rates by the single station method (Odum 1956; Meyer and Edwards 1990; Young and Huryn 1996). Total system metabolism was only measured during the first season.

The metabolism of planktonic organisms was determined by enclosing a water sample in a 1000 ml clear Perspex incubation chamber in each of the mesocosms. The chambers were fitted with an externally mounted submersible pump that recirculated water from one end of the chamber to the other through plastic tubing fitted with an in-line YSI oxygen electrode. Chambers were submerged 30cm under the water, filled and closed, and oxygen concentration and temperature logged at 5 or 10 minute intervals for periods of 36h. The chlorophyll concentration of each enclosed sample was measured following incubation. The night time reduction in oxygen concentration was used to estimate community respiration while day time changes in oxygen concentration were used to estimate net community production during the light period. The 24 hour respiration (CR) was calculated from the average hourly night time rate assuming that day time respiration rates were equivalent to night time rates. Gross community primary production (GPP) was calculated from the day time net production by correcting for the estimated respiration losses over the light period. The 24 hour net production (NPP) was estimated by reducing the measured daytime net production by the measured night time respiration.

Algal Communities & Biomass

Two water samples were taken from each tank on each sampling occasion (18 February – 28 July 2004) for phytoplankton estimation.

To assist in later counting, one sample was examined live for the presence of any small flagellates by concentrating it via filtration (0.45 um filter) and examining the resuspended residue under the microscope for the presence of motile cells.

The other sample was preserved at the time of sampling using Lugol's Iodine, and stored in a refrigerator until counted using a Lund cell and a Zeiss Axioskop microscope. Counts were made of the algal taxa present judged to be contributing one percent or greater to total algal biovolume. The various taxa were then aggregated into the groupings Centric diatoms, Pennate diatoms, Euglenophyceae, Green flagellates, Green non-flagellates, Cyanobacteria and Other.

Algal biovolumes were estimated for selected samples (usually one per salinity treatment) by relating each algal taxon to a known geometric shape, and aggregating the data into the above groupings.

Multivariate analysis of the community data was performed using Primer (V5). Non-metric multidimensional scaling (nMDS) derived from a Bray-Curtis distance metric was used to display community data and Analysis of Similarity (ANOSIM) was used to determine if communities developing under the different salinity regimes were significantly different. All data were square-root transformed prior to analysis.

Aquatic plant communities

The response of plants was tested for both salinity and water regime. Germination was assessed from pots hanging in two water regimes (damp and flooded) as well as from the sediment in the bottom of the tanks.

Pots (17cm diameter, 17cm deep, surface area 0.024 m2) were filled to within 5cm of the top with sandy loam and then filled to the top with wetland sediment. A metal mesh frame was fixed above each tank from which pots (containing wetland sediment) were hung using chains and hooks. The pots were placed in each of the tanks and hung at 'damp' level so that the soil was waterlogged but not submerged for the first 24 hours. The flooded pots were then submerged to 40 cm below the surface and the damp pots remained waterlogged.

Plant scores were then made monthly from the pots by lifting the pots out of the water and counting the number and types of plants germinating. For the plant communities developing from the sediment at the bottom of the tanks a percentage cover score was estimated and number of taxa recorded using a viewing tube. Identification and nomenclature of angiosperms and ferns follows Harden (1990-1993), liverworts follows Sainty & Jacobs (1981).

Analysis of variance using Systat (version10) was used to determine if significant differences occurred between treatments. Where significant difference between treatments was indicated, Bonferroni pairwise comparisons were used to explore which treatments were different. Data were subjected to a logarithmic transformation ($\log\{x+1\}$) prior to analysis, which removed heterogeneity of variances, as a pre-requisite for parametric analysis (Underwood, 1997).

Zooplankton communities

Zooplankton were collected using a 12 volt submersible inline pump with a pumping capacity of capacity 25 L min^{-1}. On each sampling occasion a 20 L volume of water was collected and filtered through a 50 μm mesh net before being preserved in 70% ethanol. Samples were collected from each tank by moving the pump up and down throughout the water column. Samples were collected monthly from February 2004 t to February 2005

Samples were counted in a Sedgewick-Rafter counting chamber and identified using a darkfield microscope. Animals were identified to the level of Family or Genus following keys in Shiel (1995), with the exception of ostracods that were identified to Class.

All counts were converted to abundance of animals (number L^{-1}) prior to analysis. Repeated measures analysis of variance using Systat (version10) was used to determine if significant differences occurred between treatments. Where significant difference between treatments was indicated, Bonferroni pairwise comparisons were used to explore which treatments were different. Data were subjected to a logarithmic transformation (log{x+1}) prior to analysis, which removed heterogeneity of variances, as a pre-requisite for parametric analysis (Underwood, 1997).

Results

Biogeochemistry

Nutrient transformations.

The mesocosms studies supported earlier observations on the effects of increasing salinity on nutrient dynamics (Baldwin *et al*. 2005). Initial releases of ammonia release ranged from 0 to150 μg L^{-1} in the 5000 constant treatments and ranged from 1250 – 2500 μg L^{-1} in the 15000C treatments (Figure 2) The impact of changing salinity on the release of ammonium from sediments has been documented in seawater systems and is thought to occur as a consequence of cations competing with ammonium ion, and subsequently blocking cation exchange sites on the surface of sediment particles (Seitzger *et al*. 1991).

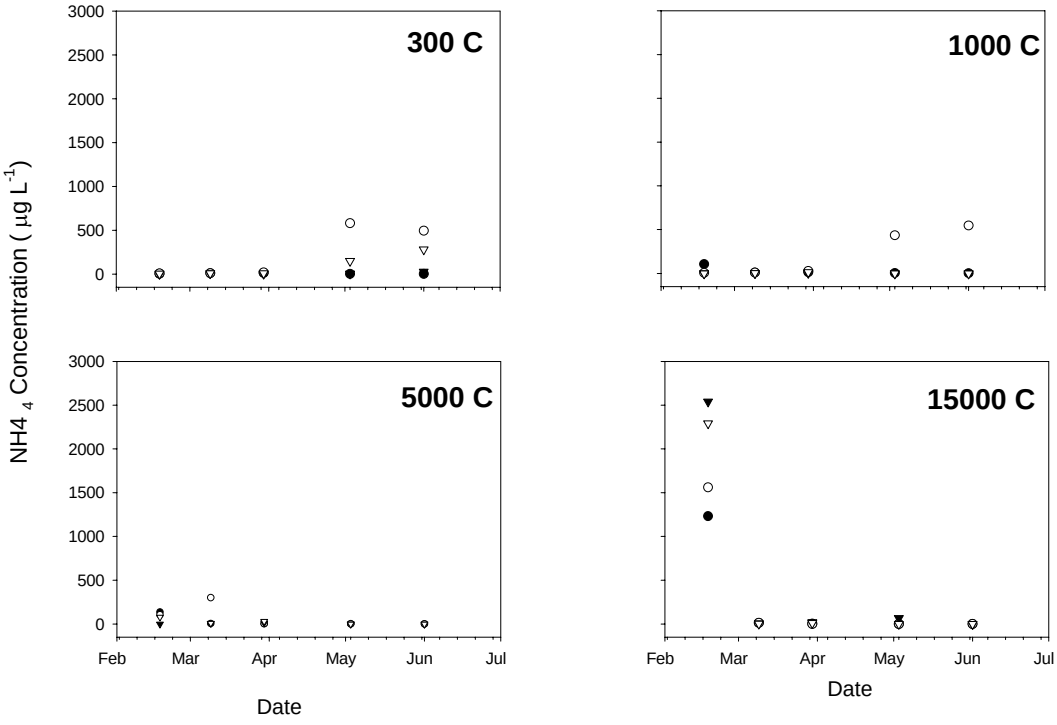

Figure 2. Ammonium response to different salt additions. Each point represents an individual mesocosm (C = constant salinity).

High nitrate concentrations were present at the start of the experiment in two of the 1000 mg L^{-1} constant treatments and very high levels in two of the 5000 mg L^{-1} constant treatment. The nitrate concentrations subsequently remained relatively low for the duration of the experiment (data not shown).

Filterable reactive phosphate remained relatively high throughout the experiment in the <300, 1000 and 5000 mg L^{-1} constant treatments, but very low in the 15000 mg L^{-1} constant treatment, on occasions below the level of detection (Figure 3). In our experiment, P clearly was not limiting at the lowest salinity, but was at limiting concentrations at the highest salinity. The anaerobic cycling of phosphate in aquatic sediments is closely linked to both the Fe and S cycles (Baldwin *et al.* 2002).

P is often associated with Fe and to a lesser extent Mn particles and can be release from sediments to the pore water through microbial activity. However, high salinities can lead to decreased P availability as increased salinity increased leads to solubilization of Fe and any phosphate that is released would form insoluble complexes with the free ferrous or ferric ions and precipitate from solution (Roden and Edmonds 1997). High productivity can also lead to consumption of P and a combination of both processes may have lead to very low P levels at highest salinity.

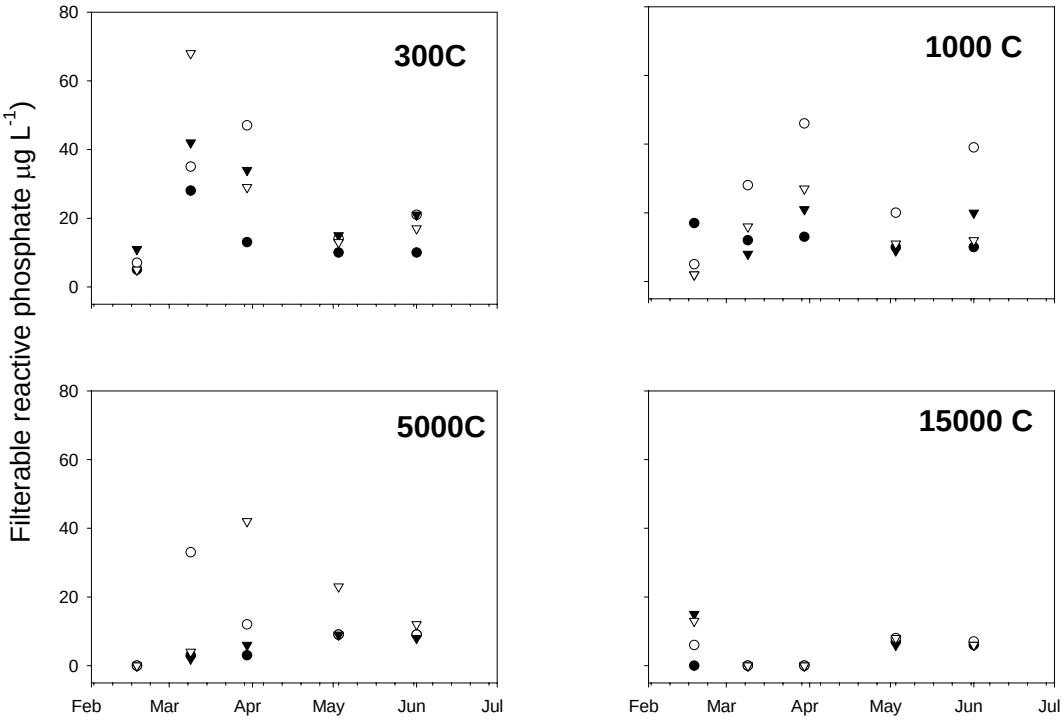

Figure 3. Phosphate (as filterable reactive P) response to different salt additions. Each point represents an individual mesocosm (C = constant salinity).

Sediment microbial communities

Only after 5 months was it apparent that salinity had an effect on the overall `sediment microbial communities structure (Figure 4) and that this tend could only be seen when comparing the 15000 constant treatment with other treatments. Pairwise analysis of similarity (ANOSIM) comparisons for the 15000 constant treatment and the <300 constant, 1000 constant, 5000 constant, 1000 gradient and 5000 gradient gave significance levels of 5.7%, 2.9%, 8.6%, 5.7% and 17% respectively. Examining the trends in community structure over time show that time of sampling had a greater effect on the community than the salinity (Figure 5). A major change in community structure occurred between the first and second sample periods and would be consistent with microbes responding to initial flushes of carbon and nutrients that occur on re-wetting of sediments.

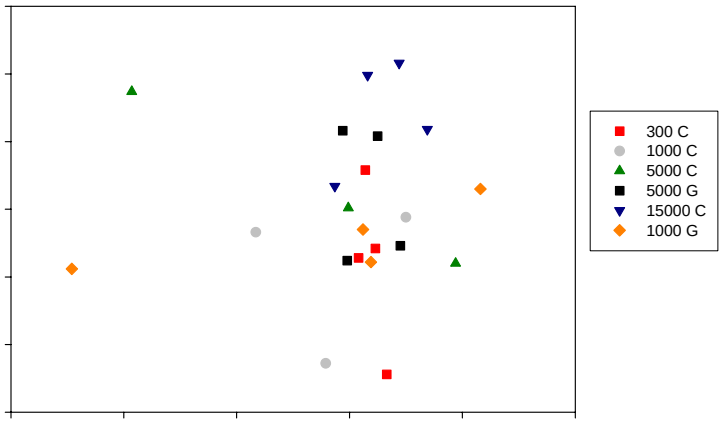

Figure 4. Ordination of the sediment microbial community structure in the mesocosms on 27 July 2005. Each point represents an individual mesocosm. Stress of plot = 0.16 (C= constant; G = gradient)

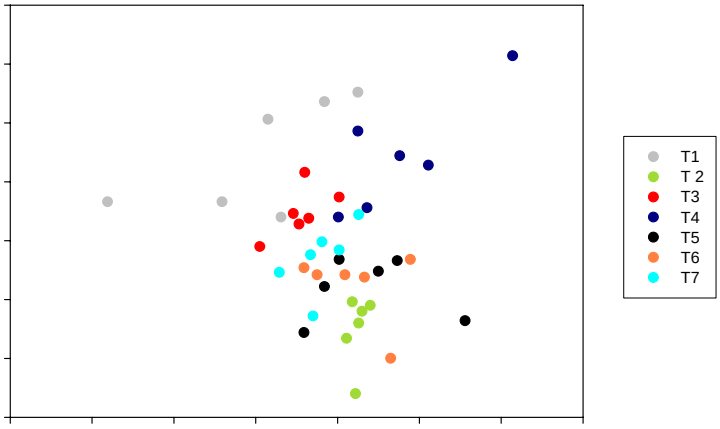

Figure 5. Ordination of microbial community structure changes over time. Colour groups represent each sample time and each point within a colour group represents one of the treatments within that time. Stress of plot = 0.2 (C= constant; G = gradient)

Although the overall microbial community showed resilience to salinity, the time-frame of this experiment was sufficient for different microbial groups to have changed. Notably, high activity by sulfate-reducing bacteria occurred at the highest salt level (Figure 6).

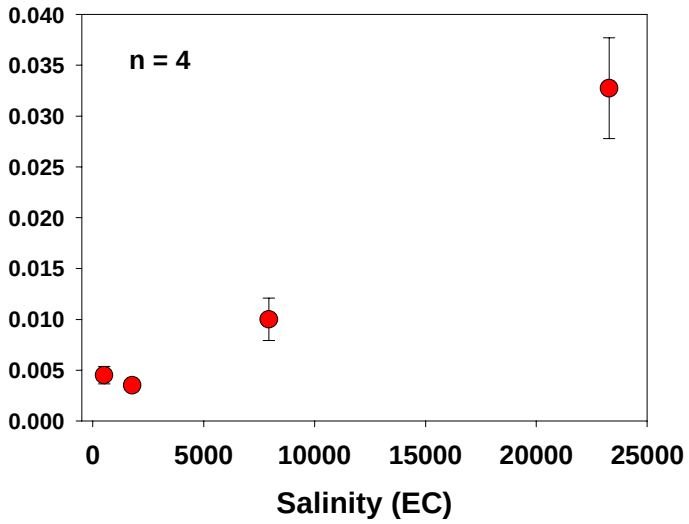

Figure 6. Formation of acid volatile sulfur (Iron sulfide) in response to increasing salinity.

Water column microbial activity.

Enzyme activities in the water column of the 15000 and 5000 constant treatments were not significantly different from each other, but were significantly different from all the other treatments (PERMANOVA, $P < 0.05$). The differences between the high salt treatments were apparent from the first sampling date (x days after inundation) and remained different for the duration of the experiment. Activities in the 5000 gradient were similar to those in the low salt treatments, until the salt level had been adjusted to 5000. Once the salt additions gave an electrical conductivity of 5000, the enzyme activities were similar to the high salinity treatments.

High variability was measured across the 1000 constant treatment, which was attributed to very high activities in mesocosms number 6. Activities in the latter mesocosm were vastly greater than in all other mesocosms with the equivalent salt treatment. Reasons for the differences remain unresolved at this time.

Enzyme activities were generally well correlated with algal production. Following log transformation for chlorophyll content and enzyme activities, coefficients (r) for esterase, phosphatase, amino peptidase, β-glucosidase, α-glucosidase, β-xylosidase were 0.469, 0.56, 0.661, 0.321, 0.525 and 0.522 respectively. Excluding the β-glucosidase, chlorophyll content was explaining approximately 50-60 % of the variation in the enzyme activity. A relationship with salt is apparent, however, the correlation with production is confounding simple analysis, since production also is correlated with salinity.

Algal Productivity

Biomass patterns

Average planktonic chlorophyll concentrations commenced at similar levels and initially increased in all treatments except the 5000 gradient treatment (Figure 7, note logarithmic axis). After 31/03/04 (42 days) consistent differences between treatments became evident.

In constant salinities of 1000 mg L^{-1} and less the planktonic biomass decreased over several weeks to levels less than 20 mg L^{-1} and remained at low levels for the remainder of the first season. In contrast the 5000 and 15000 mg L^{-1} constant treatments consistently contained biomass levels greater than 50 mg L^{-1}. In the second season the average chlorophyll concentrations showed similar patterns to the first season with a distinct separation between the lower and higher salinity treatments although in most cases the biomass had increased from the first season. The average chlorophyll concentration for the 1000 constant treatment is based on three replicates as one of the mesocosms (tank 6), was physically different from the others having extremely high turbidity through out the experimental period. The reason for the elevated turbidity is not known but this replicate was removed from the biomass analysis as the impact of the high turbidity was likely to over-ride salinity influences for the plankton.

Planktonic biomass pattens in the gradient treatments were similar to the low constant salinity treatments during the first season, reflecting that salinities were initially low and increased slowly. In the second season the 5000 mg L^{-1} gradient tanks, which had reached there maximum salinity level at the end of the prior season, contained chlorophyll concentrations similar to those of the high constant salinity treatments. In contrast the 1000 mg L^{-1} gradient treatments retained relatively low biomass levels. Chlorophyll concentrations increased to very high values in the 15000 mg L^{-1} constant treatment during the second season exceeding 1000 µg L^{-1} in two of the mesocosms.

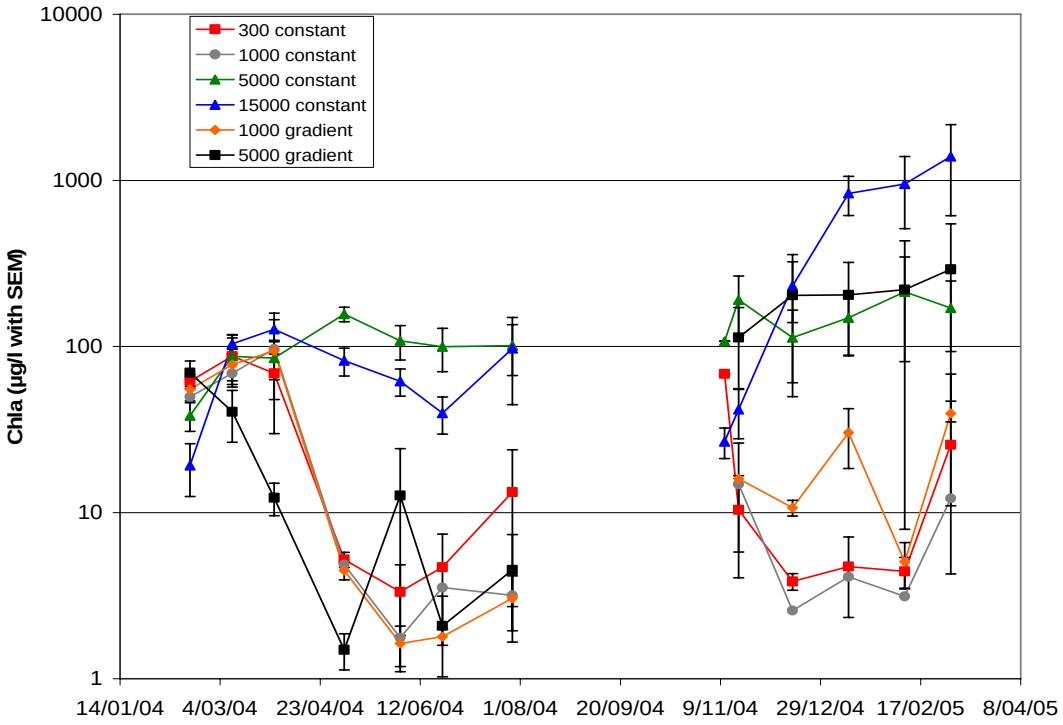

Figure 7: Average chlorophyll concentrations in the six salinity treatments ± standard errors of the mean (SEM).

Light conditions

In all treatments the calculated average euphotic depth exceeded the mesocosom depth and light penetrated to the bottom of the tanks. The quantity of light reaching the sediments tended to decline with increasing salinity, but despite this difference the depth of mean irradiance in the mesocosms was relatively constant across treatments at 0.25 to 0.3 metres. This depth is similar to the depth at which planktonic chambers were incubated allowing comparison of the planktonic and total measurements of GPP as well as CR.

Metabolism

Total system

The total system GPP tended to be high initially and then to decline overtime in each of the treatments except the 5000 mg L^{-1} gradient treatmetn which increased before declining. By 02/06/04 (105 days) GPP of most treatments was at a minimum between 2 & 3 gO_2 m^{-3} d^{-1} but the 15000 mg L^{-1} constant treatment was significantly higher at 4.5 gO_2 m^{-3} d^{-1} (Figure 8Figure 7). This pattern reflects the planktonic biomass changes except for the 5000 mg L^{-1} constant treatment which had a biomass similar to the 15000 mg L^{-1} constant treatment but a significantly lower rate of GPP. The 15000 mg L^{-1} constant treatment remained significantly higher than other treatments for the following two months.

Total system respiration rates initially increased (more negative) and then declined but lower salinity treatments showed larger maximum community respiration rates than higher salinity treatments (Figure 9). The <300 constant treatment reached a CR rate three times greater than that of the 15000 mg L^{-1} constant treatment. Respiration rates remained larger in the <300 mg L^{-1} constant and 1000 mg L^{-1} gradient treatments until 23/06/04 when respiration rates became similar across all treatments. This pattern does not match that of the phytoplankton biomass.

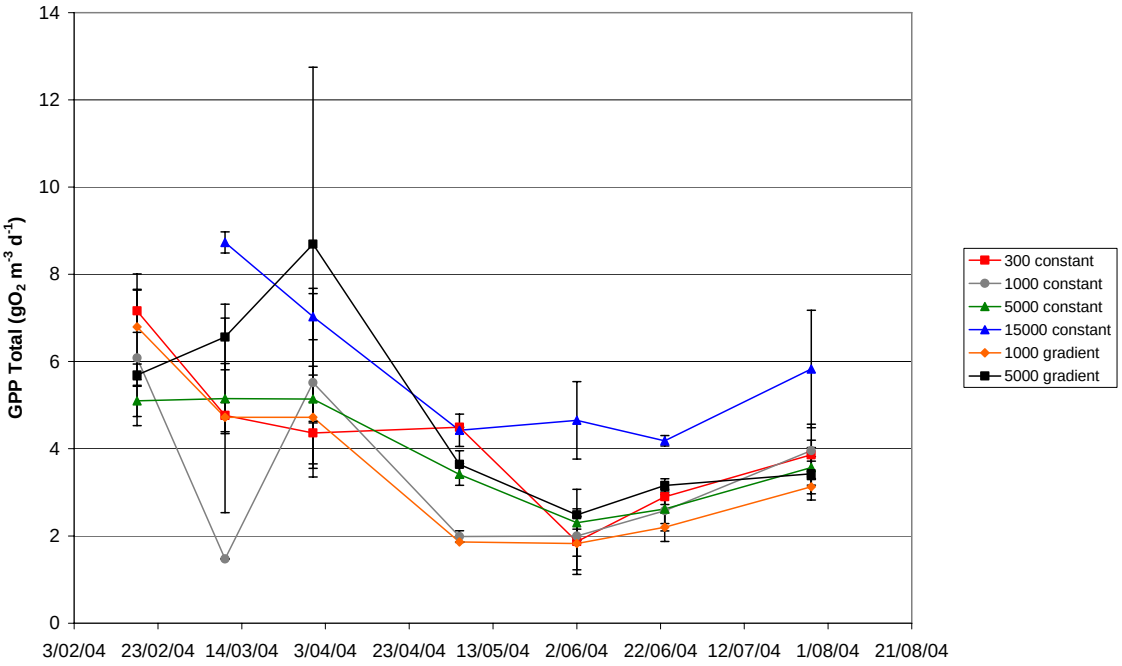

Figure 8: Average total mesocosm GPP in the first season.

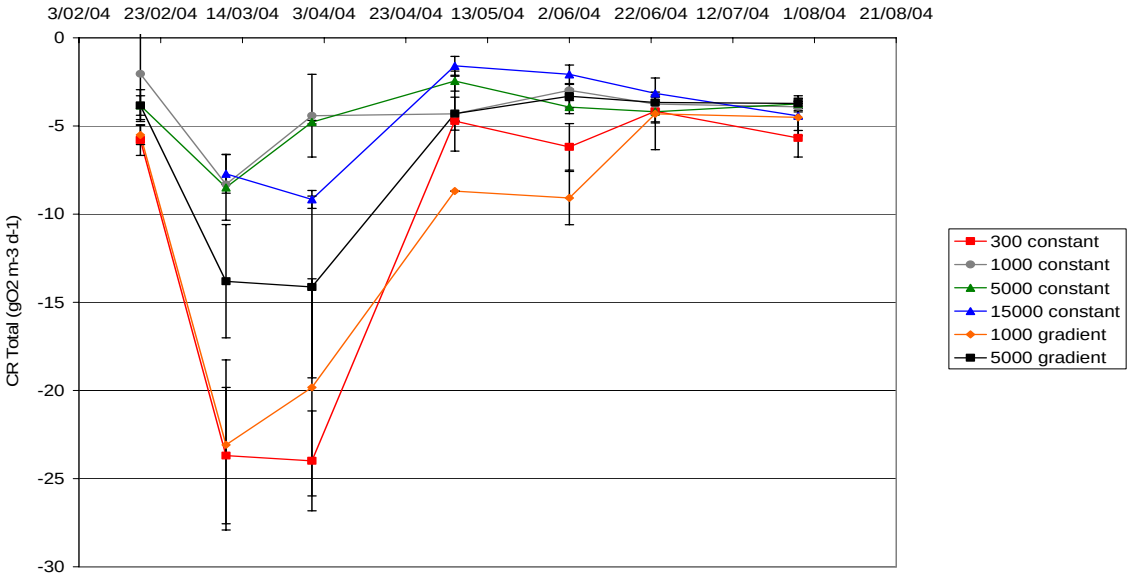

Figure 9: Average total mesocosm CR in the first season.

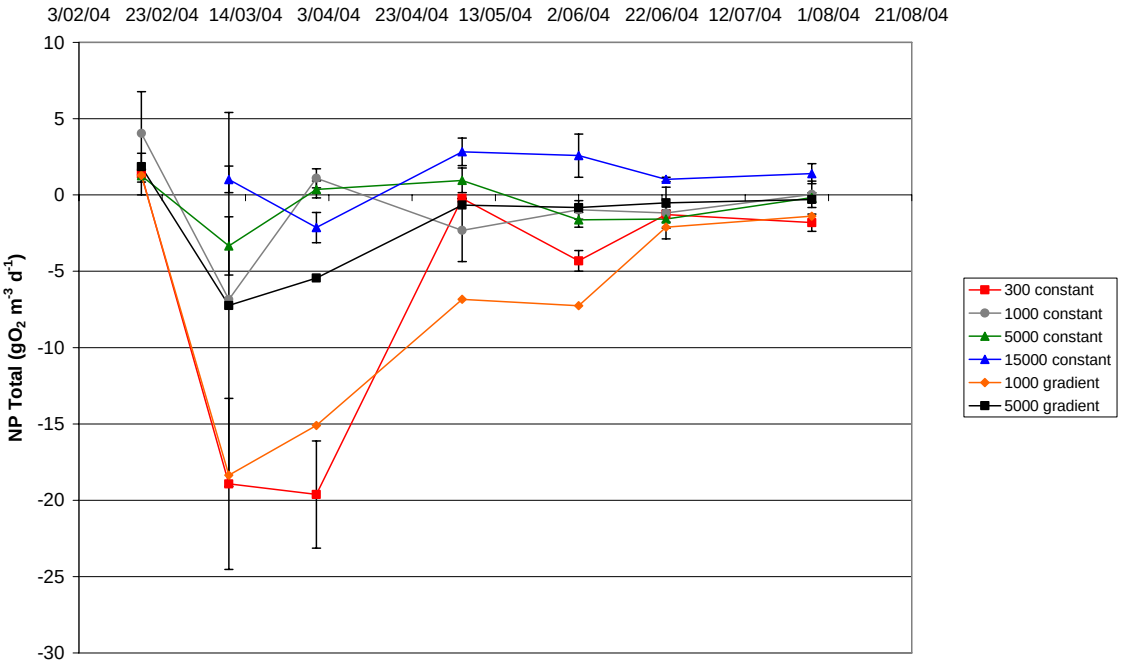

Figure 10: Average total mesocosm NP in the first season.

The interplay of GPP and CR caused differences in mesocosm NP between low and high salinity treatments (Figure 10). Lower salinities had negative NP over the season while higher salinities generally showed values near zero. The 15000 mg L^{-1} constant treatment was the only one to show a consistent positive NP. By the final sampling date of the first season the two lowest salinities (<300 constant and 1000 gradient treatments) had negative NP rates, mid-range salinities (1000 mg L^{-1} and 5000 mg L^{-1} constant, 5000 gradient treatments) had zero NP rates and the highest salinity had a positive NP rate.

Planktonic

Planktonic GPP in the constant salinity treatments was measured over the two seasons while gradient treatments were only measured in the first season. In the first season GPP showed patterns in accord with the phytoplankton biomass differences total GPP measurements between salinity treatments. Planktonic GPP was above 1 mg L^{-1} d^{-1} in the two high salinity treatments but in the low salinity treatments it declined below the level of detection. This contrasts with the total GPP measurements which showed reduced GPP levels for all treatments but rates did not approach zero. Only the 15000 mg L^{-1} constant treatment was noticeably higher than others in the total GPP measurements while in the planktonic measurements both the 15000 mg L^{-1} and 5000 mg L^{-1} treatments were higher.

The ratio of the planktonic to total GPP is an approximate indicator of the planktonic contribution, assuming that measurements made at 30cm depth reflect those of the total mesocosm. This is reasonable first assumption as the average irradiance in the mixing mesocosms occurred at ca. 30cm, but more detailed analyses of photosynthesis-light

responses and respiration rates will be required to improve the accuracy of these comparisons. Major differences are likely to be reliable and indicate that planktonic phototrophs made an insignificant contribution to GPP in the low salinity treatments (≤ 1000 mg L^{-1}) during the first season. In the higher treatments the contribution is more significant, particularly in the 5000c treatment were the majority of GPP was associated with the plankton (Figure 11).

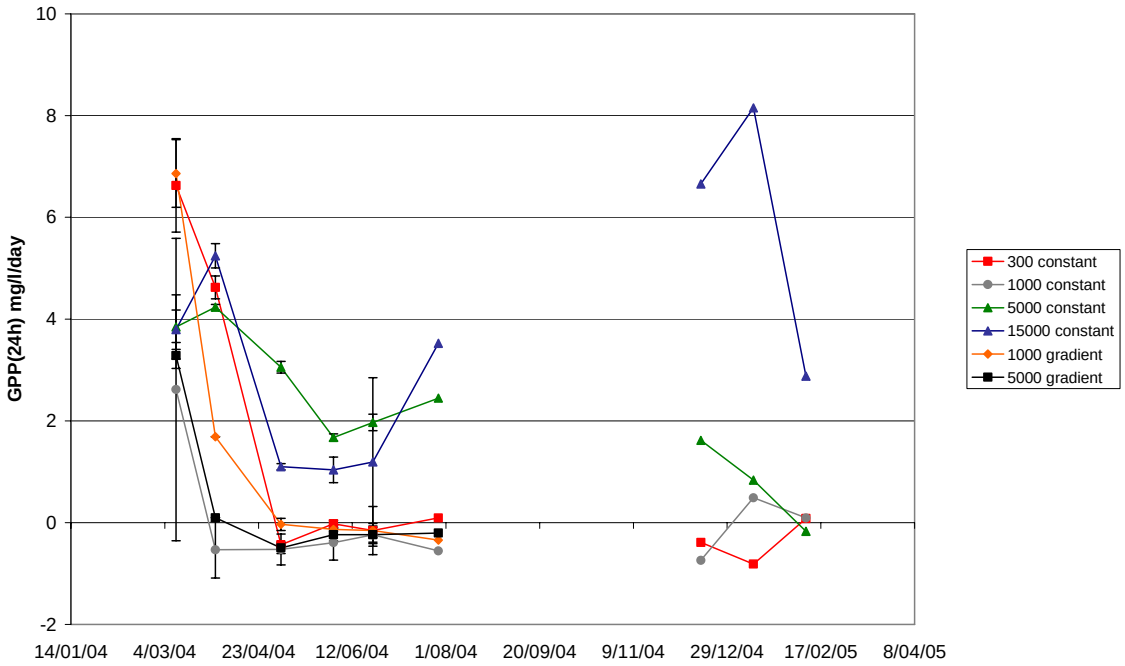

Figure 11: Planktonic GPP

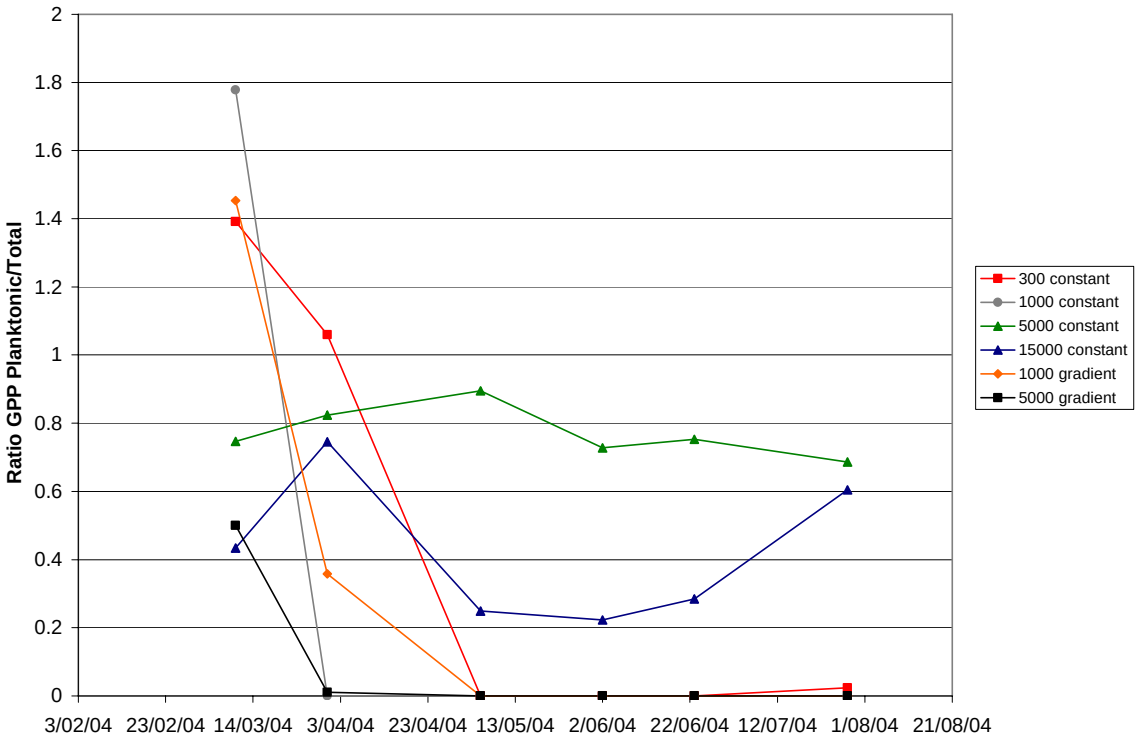

Figure 12: Ratio of planktonic to total GPP in the first season.

In the second season GPP remained high for the 15000 mg L^{-1} constant treatment but was near zero for all other treatments including the 5000 mg L^{-1} constant treatment.

Planktonic respiration rates were initially large (negative) in all treatments then declined until late in the season when the two higher salinity treatments increased again. In the latter part of the first season respiration rates were four times larger in the two highest salinity treatments than in the others. This reflects patterns in phytoplankton biomass and planktonic GPP patterns, although unlike GPP which was near zero for low salinities, measurable respiration rates were nearly always observed. As with GPP the planktonic to total CR ratio provides an estimate of the planktonic contribution. Respiration rates are based on night time measurements and so are less influenced by incubation location within the mesocosms. The pattern of respiration ratios is broadly similar to that of the GPP ratios, with plankton in the two higher salinity treatments making more of a contribution than those in the lower salinities (Figure 12).

Planktonic NP rates were generally closer to zero than the total NP rates, but were similar in that positive NP rates occurred in the higher salinities. The very large negative NP rates of the total systems did not occur in the plankton. In the second season the NP was positive only in the 15000 mg L^{-1} constant treatment with all other constant salinity treatments giving negative NP values.

Measurements of total mesocosm respiration and photosynthesis were not correlated with the planktonic chlorophyll-a concentration indicating that other components were playing an important role, as indicated by the ratios of planktonic to total measurements (Figure

13, Figure 14; Figure 15.). Even in the high salinity samples where the planktonic metabolism made a major contribution to overall metabolism, correlations with phytoplankton biomass were weak. In contrast the planktonic respiration rates in most mesocosms were strongly correlated with the phytoplankton biomass except in the 5000 mg L^{-1} constant treatments. This was largely driven by the early reduction in the phytoplankton biomass and the concurrent reduction in planktonic community respiration rate. In contrast, there were no correlations between phytoplankton biomass and planktonic GPP, largely because in most mesocosms the planktonic GPP reduced to levels below detection.

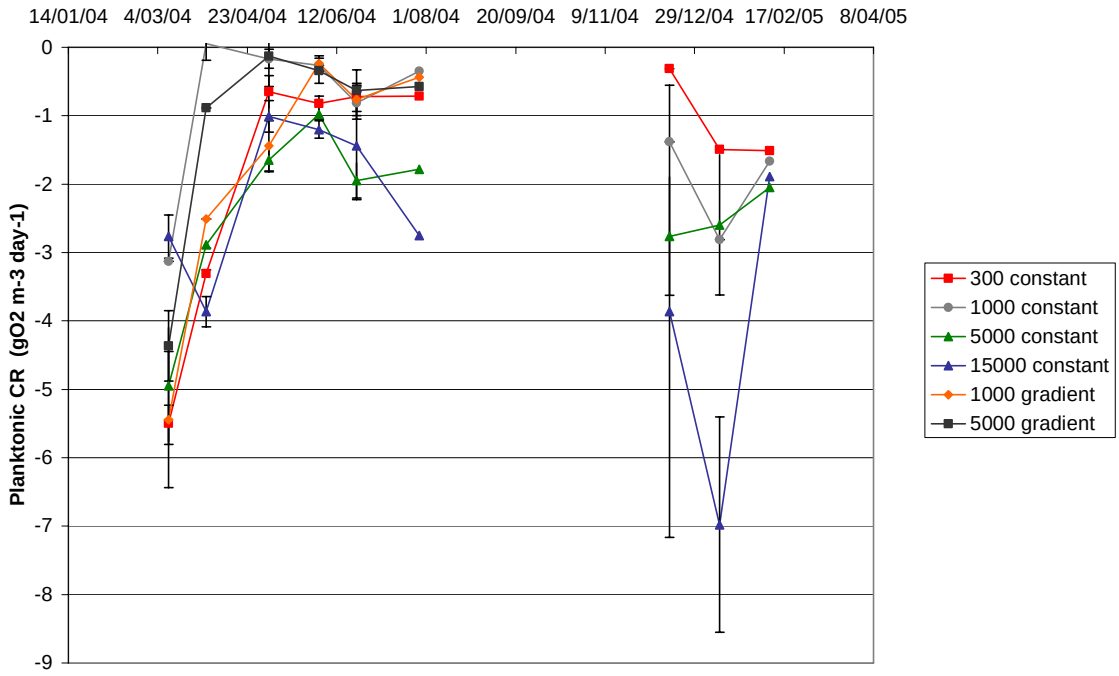

Figure 13:Planktonic respiration rates.

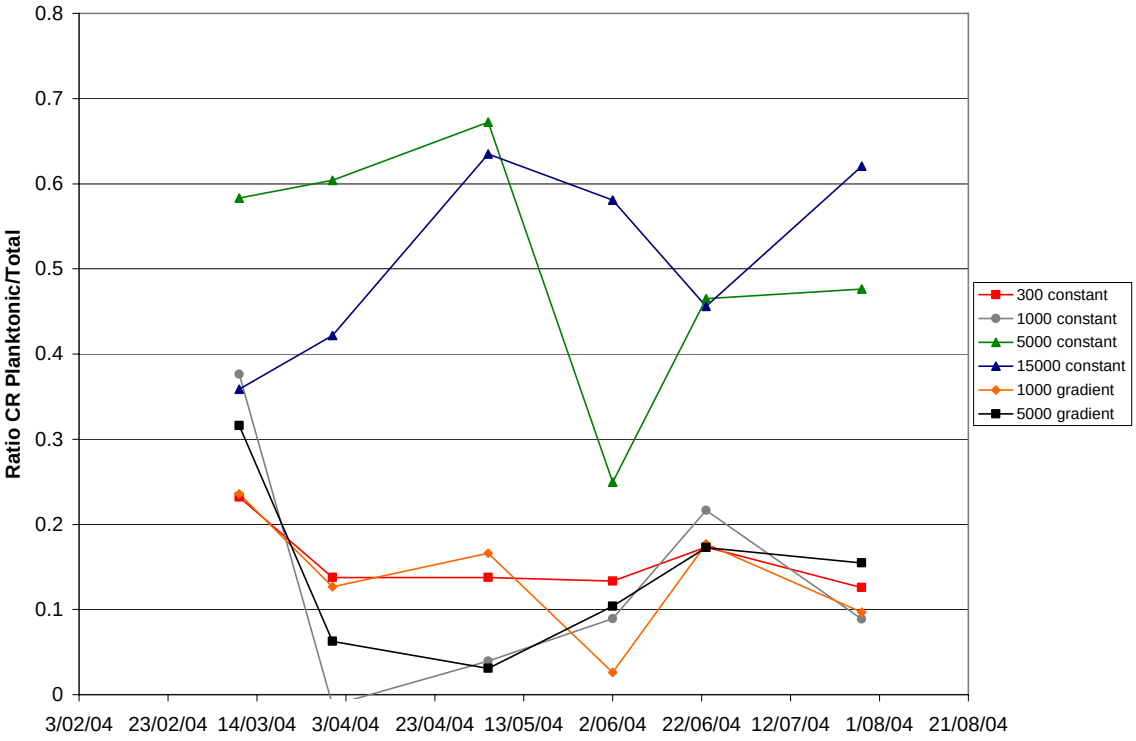

Figure 14: Ratio of planktonic to total CR for the first season.

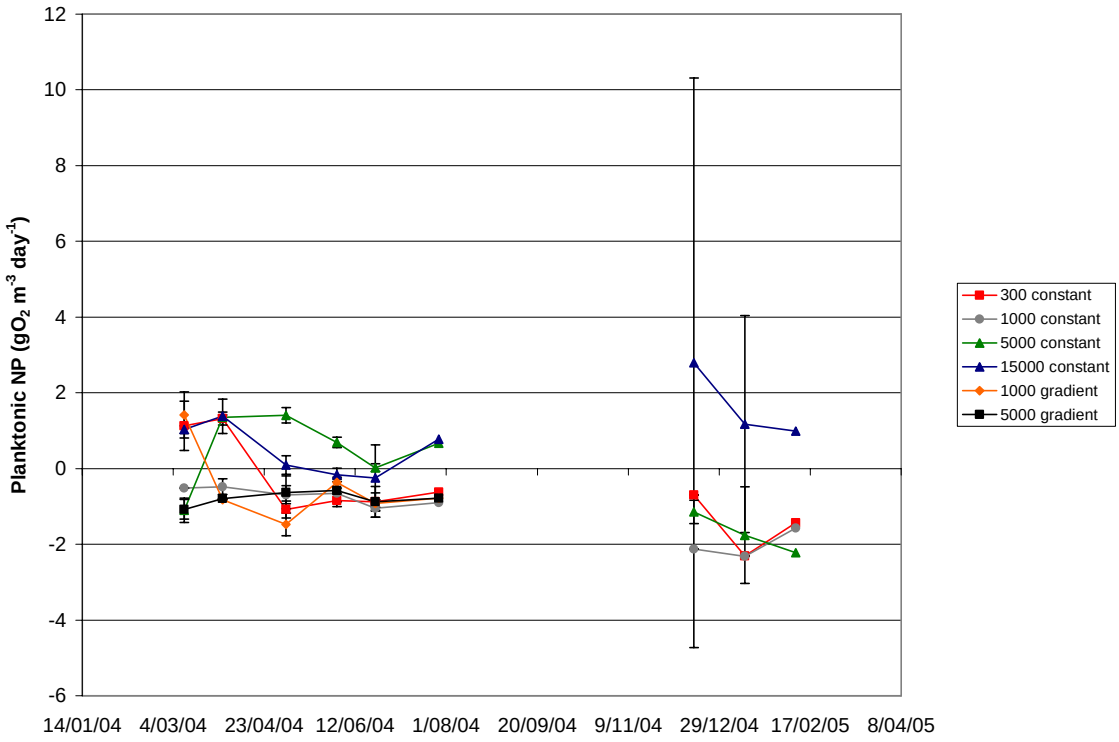

Figure 15: Planktonic NP.

Aquatic Plant Communities

A total of 42 plant taxa germinated from the damp pots, 25 from the flooded pots and 10 taxa from the bottom sediment (Table 1)

Table 1: Total number of aquatic plant taxa recorded from each treatment across whole experiment. (Note that this includes some species that germinated but died before establishment in the 15000 mg L^{-1} treatment)

		Number of taxa		
Salinity (mg L^{-1})	Treatment	Damp pots	Flooded pots	Bottom sediment
<300	constant	35	17	10
1000	constant	31	18	10
5000	constant	11	16	9
15000	constant	3	7	2
1000	gradient	30	14	9
5000	gradient	24	17	10

Overall there was no significant difference in the number of plants germinating between the fresh and 1000 mg L^{-1} (constant and gradient) treatments. Both these treatments had more plants than the 5000 mg L^{-1} (constant and gradient) treatments ($P<0.001$) which had more than the 15000 mg L^{-1} treatment ($P<0.001$).

There were more plants germinating in the damp pots than in the flooded pots ($P<0.001$) and both of these had significantly more plants germinating than from the bottom sediment ($P<0.001$).

Overall there was a differences in the number of species germinating among all salinity treatments with most species germinating in the fresh such that 300 > 1000> 5000 > 15000 mg L^{-1} (Figure 16).

Although initially more species germinated along the 1000 mg L^{-1} and 5000 mg L^{-1} salinity gradient treatments compared to the corresponding constant salinity treatments these differences reduced as the salinity gradients reached maximum levels and plant establishment consolidated over time (Figure 16). By the last score date at 13 months these differences were much reduced in the 1000 mg L^{-1} treatments and also reducing in the 5000 mg L^{-1} treatments under both flooded and damp conditions (Figure 17).

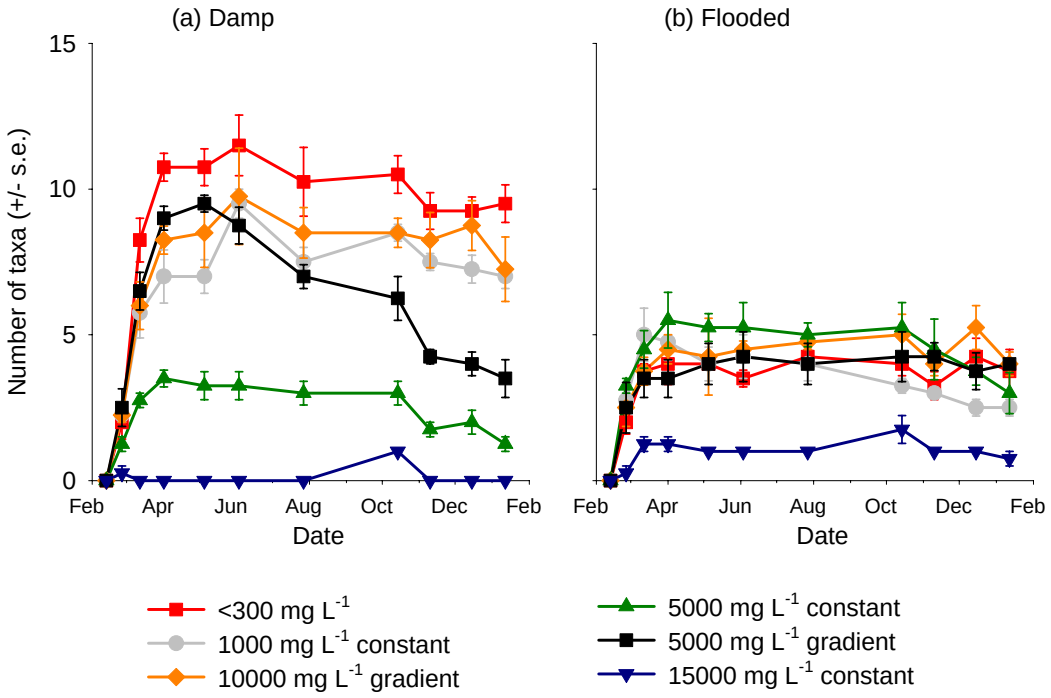

Figure 16: Number of aquatic plant taxa taxa present in each treatment at monthly intervals during the 13 month mesocosm trial in pots under a) damp . and b) flooded conditions

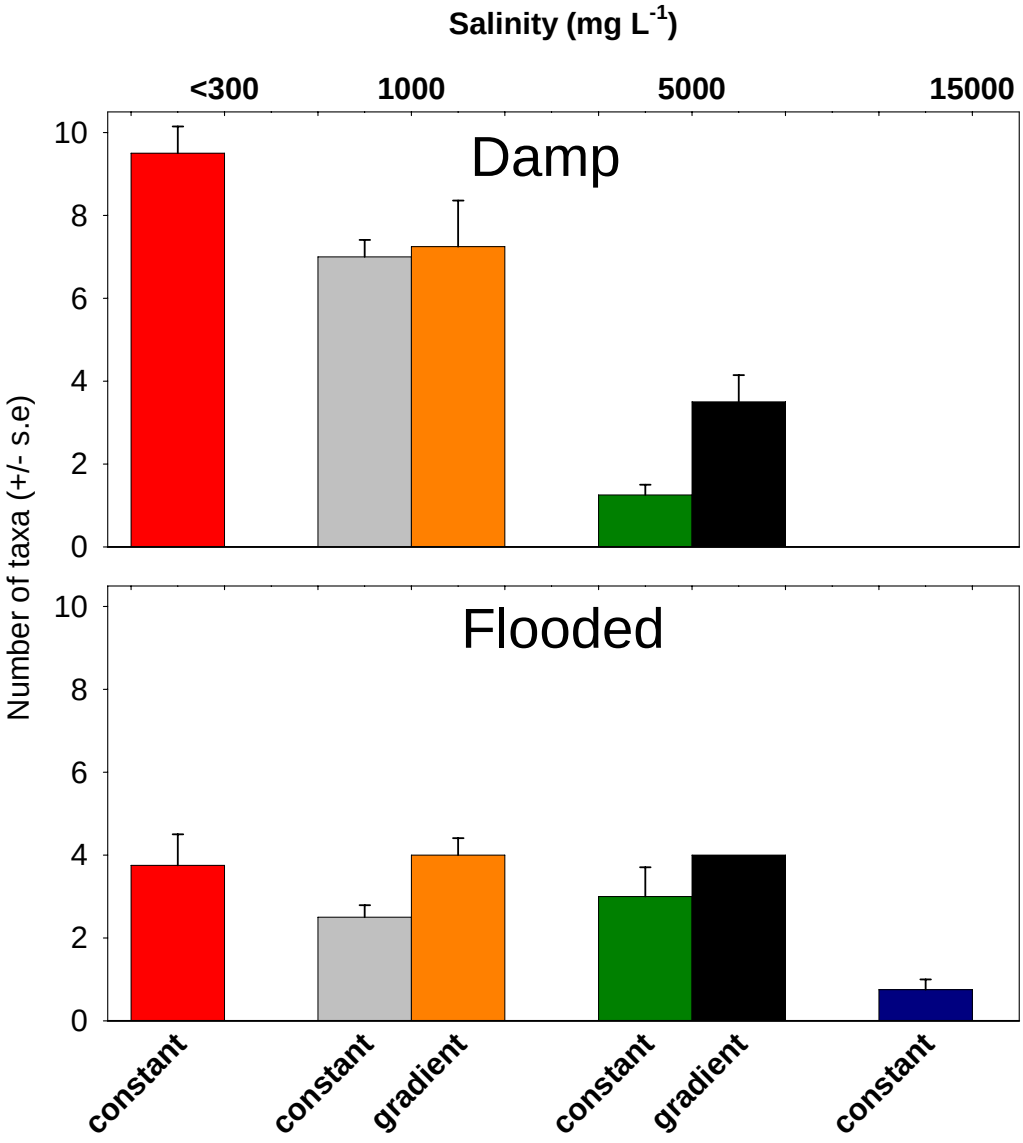

Figure 17: Number of aquatic plant taxa present in each treatment at final score date after 13 months of experimental conditions

Zooplankton Communities

A total of 46 zooplankton taxa were recorded from all treatments (38 rotifer taxa, 8 microcrustacean taxa). Of these, the majority of taxa were found in either the fresh (<300 mg L^{-1}) or the 2 gradient treatments and the least number of taxa were recorded from the 15000 mg L^{-1} constant treatment (Table 2).

Table 2: Total number of zooplankton taxa recorded from each treatment across the whole experiment. (Note that this includes some species that emerged but died before establishment in the 15000 mg L^{-1} treatment)

Salinity (mg L^{-1})	Treatment	Number of taxa
<300	constant	37
1000	constant	30
1000	gradient	38
5000	constant	25
5000	gradient	35
15000	constant	19

In the fresh (<300 mg L^{-1}) 1000 mg L^{-1} constant and the two gradient treatments, emergence and community development was rapid with these treatments becoming taxon rich (Figure 18). After the initial pulse of emergence all three treatments reduce in the numbers of taxa. By the end of the 12 month experimental period the richness of the fresh (<300 mg L^{-1}) and 1000 mg L^{-1} (constant and gradient) treatments were similar, but the 5000 mg L^{-1} gradient treatment was substantially reduced in taxa and similar to that of the 5000 mg L^{-1} constant treatment (Figure 19). Both the 5000 mg L^{-1} and 15000 mg L^{-1} treatments remained taxon poor with less taxa occurring in the higher salinity treatment.

Overall there was no significant difference in the number zooplankton emerging between the fresh and 1000 mg L^{-1} (constant and gradient) treatments. Both these treatments had more zooplankton than the 5000 mg L^{-1} (constant and gradient) treatments which had more than the 15000 mg L^{-1} treatment ($P<0.001$).

Overall there was a differences in the number of taxa emerging among all salinity treatments with most species emerging in the fresh such that 300 > 1000> 5000 > 15000 mg L^{-1} (Figure 18).

Although initially more species emerged along the 1000 mg L^{-1} and 5000 mg L^{-1} salinity gradient treatments compared to the corresponding constant salinity treatments these differences reduced as the salinity gradients reached maximum levels and populations consolidated over time (Figure 18). By the sampling date at 13 months these differences were much reduced in both the 1000mg L^{-1} 5000 mg L^{-1} treatments (Figure 19).

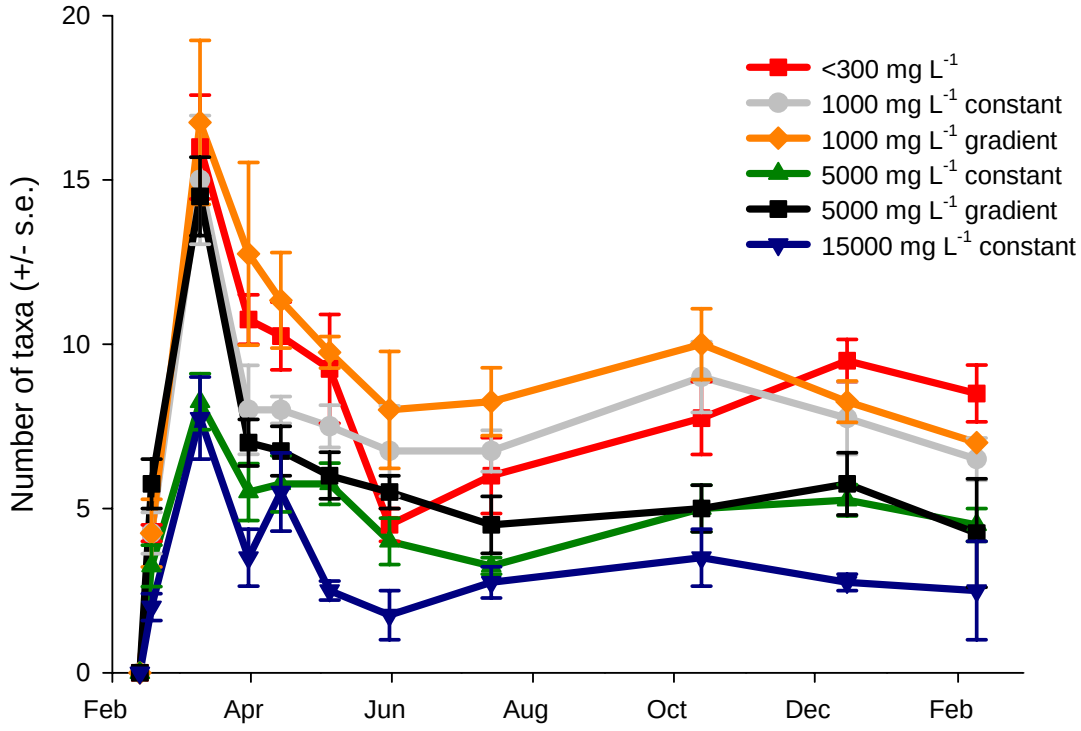

Figure 18: Number of zooplankton taxa present in each treatment at monthly intervals during the 13 month mesocosm trial

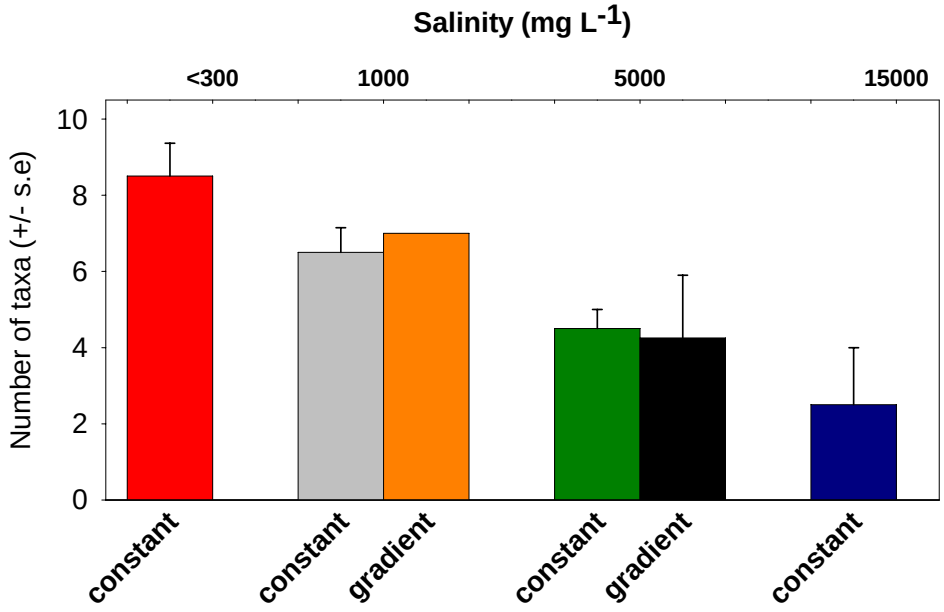

Figure 19: Number of zooplankton taxa present in each treatment at final score date after 13 months of experimental conditions

Planktonic Algae

Composition of phytoplankton

<300 mg L^{-1} Constant

A similar algal history was observed in each of these mesocosms:

An initial pulse of chlorophyll a of 45-140 ug L^{-1} was followed by a decline to less than 10 ug L^{-1}or less during April (but with Tank 9 increasing again to 45 ug L^{-1} on 28 July).

A variable covering of the aquatic fern *Azolla* was removed from many tanks across the entire experiment on 20 May 2004. *Azolla* removal from the 300 mg L^{-1} tanks was of 100% cover from Tanks 9, 10, and 23, but only 18 plants from Tank 4.

The initial algal pulse seen in all tanks was comprised primarily of Green flagellates and Euglenophyceae. The subsequent population in Tank 4 (less than 10 µg L^{-1} chorophyll-a) was examined in detail in terms of algal biovolumes, and found to be a mixture of Chlorophyta, Euglenophyta, Pennate diatoms, Green flagellates and Cyanobacteria.

1,000 mg L^{-1} Gradient

A similar algal history was observed in each of these tanks:

An initial pulse of Green flagellates led to chlorophyll-a values of 40-110 ug L^{-1}, but this was followed by a marked drop in algal biomass during March/April to less than 5 ug L^{-1} chlorophyll-a (Figure 20).

A 100% cover of *Azolla* was removed from Tanks 1, 2 and 19 on 20 May, and a 25% cover from Tank 24. A change in algal composition was then noted in the more closely examined Tank 1 (Figure 21), but in all four tanks algal numbers remained low to the end of the experiment with a mixture of algae being present.

1,000 mg L^{-1} Constant

Three of the tanks had a similar algal history (11, 15, 18) – an initial pulse comprising Green flagellates and Euglenophyceae (*Euglena* and *Trachelomonas*) giving chlorophyll a values of 30-130 ug L^{-1}, followed by a drop in biomass in March/April to less than 10 µg/L chlorophyll-a, with the more detailed biovolume examination of Tank 11 showing a subsequent population dominated by Centric diatoms, Pennate diatoms and Euglenophyceae. The cover of *Azolla* removed from the tanks on 20 May was 100% for Tank 11, 75% for Tank 15, and 80% for Tank 18.

Tank 6 was different, with chlorophyll-a values persisting at 140-215 ug L^{-1} for the duration of the experiment (Feb-July 2004), an initial pulse of Green flagellates and Euglenophyceae being followed by a mixture of algae including Pennate diatoms and Green non-flagellates. A 50% cover of *Azolla* was removed from Tank 6 on 20 May.

5,000 mg L^{-1} Gradient

A similar algal history was observed in each tank – an initial pulse of chlorophyll-a of 30-80 µg L^{-1} followed by a decline to 5 ug L^{-1} or less in March/April. A peak of 47 ug L^{-1} chlorophyll-a in Tank 12 on 2 June was not supported by the counts (on 31 May).

Azolla removal on 20 May was of 100 plants only from Tanks 5 and 16, 20 plants from Tank 12, and 10 plants from Tank 14.

The initial algal pulse comprised Green flagellates and Euglenophyceae, with some Green non-flagellate *Scenedesmus* in Tank 14 and *Monoraphidium* in Tank 16. The later and much lower populations of Tank 14 were dominated by Pennate diatoms, with some Euglenophyceae, Green flagellates and Cyanobacteria.

5,000 mg L^{-1} Constant

For this treatment chlorophyll-a values remained high in all tanks. Coincidentally, *Azolla* occurrence was minor, with 30 plants being removed from Tank 20 on 20 May, less than 10 from Tanks 13 and 17, and none from Tank 21.

The percentage contribution of the various algal groups to overall biovolume was examined in detail for Tanks 13 and 20. After an initial population of Euglenophyceae, Tank 13 was dominated by Green non-flagellates (mostly *Monoraphidium*) but with flagellates also contributing in mid April and Euglenophytes in mid May. In Tank 20, an initial dominance of green flagellates was replaced by Pennate diatoms (*Nitzschia* and *Fragilaria*), Green non-flagellates (*Monoraphidium*), Euglenophyceae and Green flagellates.

15,000 mg L^{-1} constant

Once again, higher chlorophyll-a values persisted for the duration of the experiment (Feb – July 2004) (Figure 22). Coincidentally, no Azolla developed in this treatment.

The population of Tank 8 was deemed to be typical of the treatment– an initial population of Green flagellates was replaced by Centric diatoms (*Cyclotella*) with a contribution at the end of May by pennate diatoms (*Nitzschia* and *Fragilaria*), and the dominance at the end of the trial by a small Green flagellate (Figure 23).

The pattern Green flagellates, then Centric and Pennate diatoms, then a contribution by Green flagellates in June/July was unexpectedly consistent across all tanks in this treatment, apart from the non-occurrence of Green flagellates in Tank 22 in June/July.

Analysis

Multivariate analysis using nMDS and ANOSIM confirmed there were significant differences ($P < 0.05$) between:

- The size/composition of the phytoplankton populations in the 15,000 mg L^{-1} constant treatment, and all other treatments
- The size/composition of the phytoplankton populations in the 5,000 mg L^{-1} constant treatment, and all other treatments
- The algal composition (presence/absence) of the 15,000 mg L^{-1} constant treatment and all other treatments
- The algal composition (presence/absence) of the 5,000 mg L^{-1} constant treatment, and the 15,000 mg L^{-1} constant, 1,000 mg L^{-1} constant and 1,000 mg L^{-1} gradient treatments

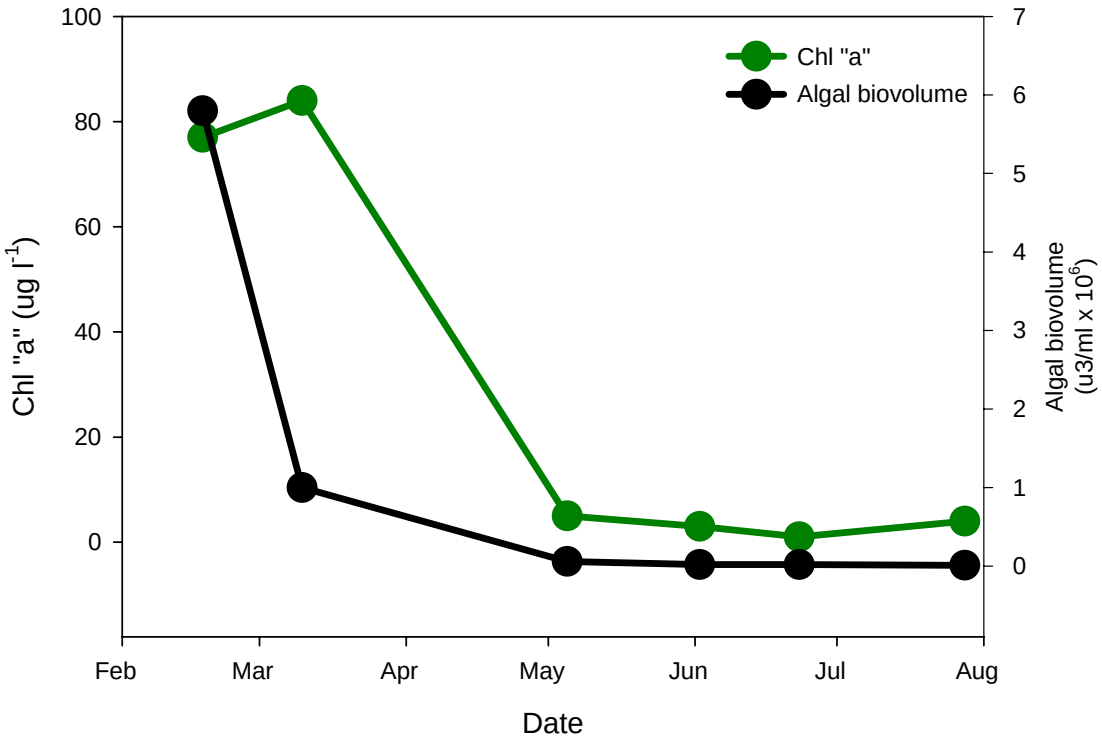

Figure 20: Chlorophyll-a and algal biovolume over time in Tank 1

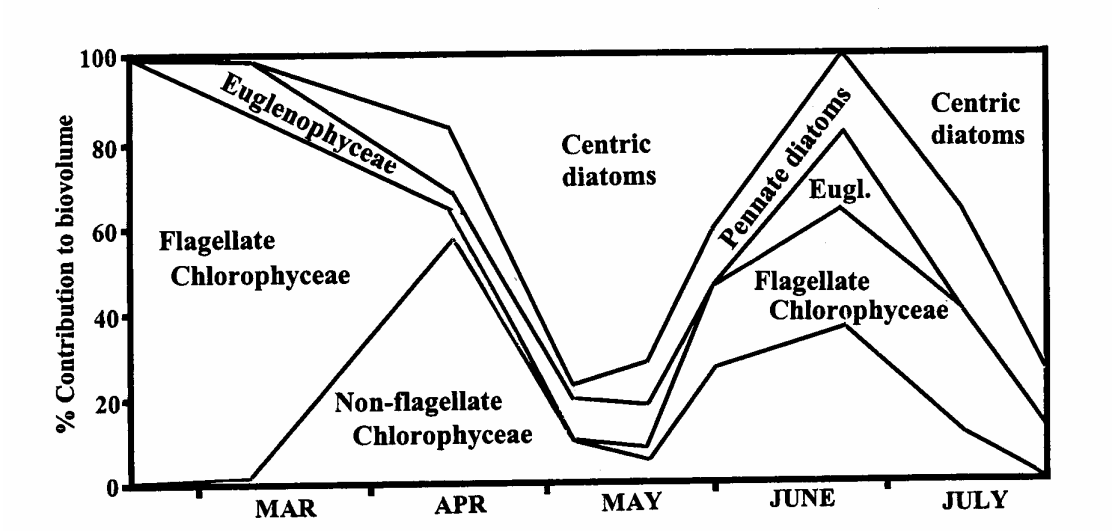

Figure 21: Percentage contribution of various algal groups to total biovolume in Tank 1

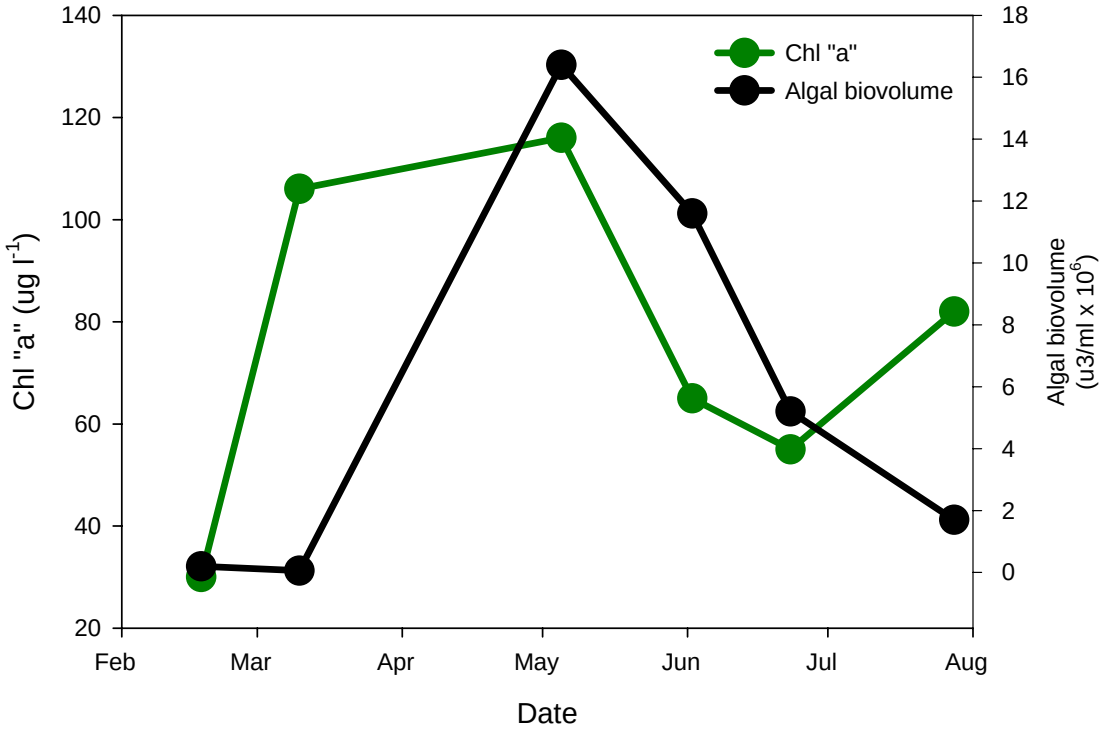

Figure 22: Chlorophyll-a and algal biovolume over time in Tank 8

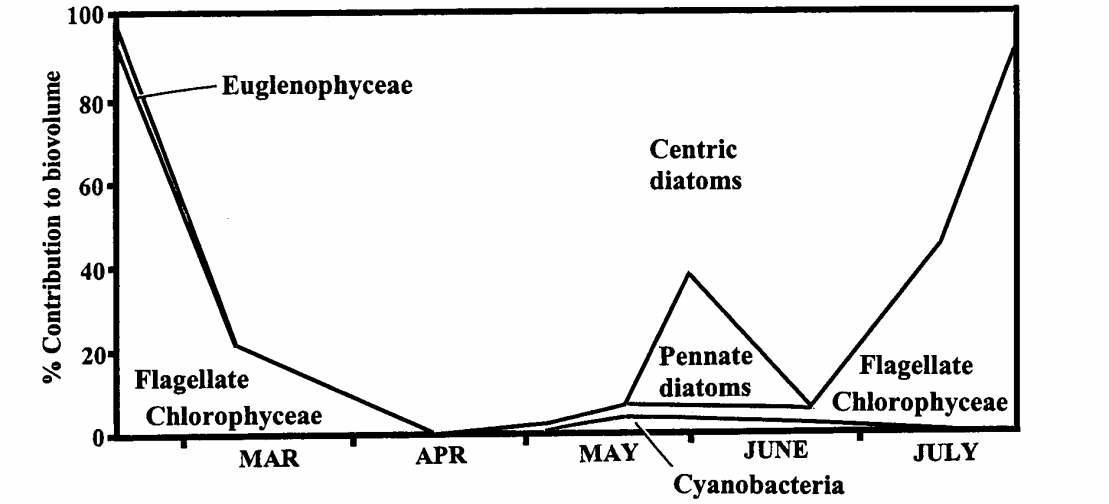

Figure 23. Percentage contribution of various algal groups to total biovolume in Tank 8

Discussion

Biogeochemistry

The overall microbial community in the sediments were relatively resilient to salt additions in our experiment. Ordinations did show that the community structure at the highest salinity was starting diverge from those at the lower salinities and we expect that this would continue with time. It is clear, however, that an acute salt addition would take several months to have a major effect on the overall community.

The changes within different microbial groups do show that salt effects are still very important. In our study, the prevalence of sulfate-reducing bacteria will have a major effect on the way nutrients are moved through the system, particularly iron and phosphorus. A further implication is that these sediments would then potentially be targeted as having the potential to produce acid if the sediments underwent a cycle of wetting and drying.

Microbial community was affected by time, with the greatest changes dependent on sample time, rather than salinity. The most noted example was immediately after setup of the tanks, with this peak coinciding with first wetting and an initial flush of nutrients from the sediments. The community change was coincident with the changes in production and activity measured with algal metabolisms .

Microbial activity in the water column was significantly affected by salt additions, indicating the water column processes would be more sensitive to Salinization. The highest salt additions had the highest microbial activities. These also were correlated with algal biomass, indicating that "microbial processes" may become more important at the salinities measured in our studies. The importance and dominance of microbial processes is well known for extremely saline environments, such as solar ponds, but the changes at the salinites we have examined have not been examined previously.

Algal Productivity

Biomass and metabolic responses of the phytoplankton were substantially different in high salinities of 5000 and 15000 compared to lower salinities. Phytoplankton biomass was larger in the higher salinities and this was associated with the salinity difference as confirmed in the 5000 gradient treatments which showed low biomass in the first season when salinity was increasing, but high biomass in the second season once high salinity was established. Both planktonic respiration and GPP were larger in the higher salinity treatments in the first season but this was only maintained in the 15000c in the second season.

All of these responses suggest that there is a significant enhancement of phytoplankton success as salinities increase to between 1000 and 5000 mg L^{-1}. The experimental design does not allow for a more precise indication of the critical salinity levels than this. The reasons for these changes are still to be fully unravelled but the following points are indicative.

Phytoplankton made a significant contribution to total mesocosm GPP at the higher salinity levels indicating that competing plants, both macrophytes and attached algae, had

reduced in importance compared to the lower salinity treatments. Presumably this is associated with reductions in their biomass as the differences in mesocosm GPP did not reflect changes in phytoplankton biomass and was not correlated with it.

At low salinities the phytoplankton biomass dropped during the first season to low values and planktonic GPP fell below detection. This could be explained either by competition for resources or increased grazing pressure. A comparison of nutrient conditions and invertebrate concentrations might help identify potential causes.

There was also a change in phytoplankton community composition at the higher salinities but whether this was driven by the salinity differences or by resource availability or grazing pressure requires further analyses.

The total mesocsom results indicate that following an initial period of re-adjustment all treatments ended up with quite similar respiration rates and GPP rates, except for the 15000c which had substantially higher GPP rate driven largely by phytoplankton. The mesocosms went through an initial period of high respiration rates requiring a source of organic carbon that may have come from the sediments or been generated through photosynthesis. The capacity for this organic carbon to be utililzed was a function of salinity with far lower respiration rates observed in the high salinity treatments. This suggests a change in microbial activity and perhaps community composition. Integration of the respiration rates over time will give an indication of the total organic carbon supply utilized. Part of the organic carbon supply may have come from the high initial phytoplankton biomass, but this was similar between treatments and does not account for the salinity influences on respiration rates. Also planktonic respiration accounted for only a small proportion of respiration in the low salinity treatments, but mesocosm respiration may have been driven by phytoplankton losses to the sediments rather than metabolism in the water column.

Scenario 1: flooding of soil released nutrients that stimulated phytoplankton growth. Initially in low salinity treatments planktonic respiration relatively high but drops quickly along with phytoplankton biomass. Mesocosm respiration increases to maximum during peak of phytoplankton biomass then falls quickly at phytoplankton biomass declines. In the high salinity treatments planktonic respiration plays larger role but total mesocosm respiration is less even though phytoplankton biomass remains high. Appears that phytoplankton rapidly metabolised in lower salinity tanks perhaps because of higher grazing pressure and bacterial activity driving respiration which is largely in the sediments. What is the role of macrophytes?

Scenario 2: Flooding releases nutrients but sediments also have substantial organic carbon reserves, These are utilised rapidly but more so in the low salinity tanks than in the high salinity tanks because conditions more suitable for microbial activity. Organic carbon supplies utilised. Phytoplankton increase due to nutrients but then decline as nutrient limitation sets in. But why limited in lower salinities and not higher is unclear.

Community Structure

The affect of the constant salinities on the plant and zooplankton communities is consistent with previous findings where as salinity increases there is a corresponding decrease in the numbers and richness of wetland plants (Nielsen *et al.* 2003; Brock *et al.*

2005). Not unsurprisingly the community found associated with the higher salinity of 15000 mg L^{-1} was depauperate in numbers and species when compared with the other treatments

These results show that in the two treatments used in this mesocosm trial:

- More species and a higher numbers of individuals germinate and establish in damp conditions that flooded conditions.
- Salinity of 15000 mg L^{-1} reduced plant germination and zooplankton emergence markedly in all water levels. *Typha* sp. was the only plant to germinate and establish in this salinity
- For amphibious and semi-terrestrial plants growing under damp conditions salinity reduced the number of plants and the number of taxa germinating above 1000mg/L (Figure 1).
- When salinity is delivered as a gradient from fresh to the maximum level (1000 mg L^{-1} or 5000 mg L^{-1}) initial emergence and germination parallels that found in the fresher treatments. However this initial greater establishment under fresher conditions does not lead to ongoing establishment once higher salinities are reached. After 12 months the gradient and constant treatments had converged for both 1000 and 5000 mg L^{-1} treatments (Figure 16, Figure 17, Figure 18 & Figure 19).
- Along a gradient of salinity different taxa will be lost at different salinity levels. From our data we may be able to predict which taxa will be eliminated first and what species may remain to dominate salinizing communities
- Along a gradient of salinity different species will be lost at different salinity levels. From our data we may be able to predict which species will be eliminated first and what species may remain to dominate salinizing communities
- Taxa differ in their responses to salinity. We may be able to predict what plant and zooplankton communities will look like if left to emerge from freshwater wetland sediment exposed to elevated salinity
- For submerged plants, species numbers were too low to substantiate differences among salinity treatments of <300, 1000 and 5000 mg L^{-1} (Figure 16).
- There was an initial and substantial pulse of chlorophyll-a in all tanks, due to the presence of Green flagellates and Euglenophyceae.
- declined in all treatments (except for the 5000 mg L^{-1} constant and 15000 mg L^{-1} constant treatments) with a subsequent mixture of algal taxa (the one exception was Tank 6).
- Chlorophyll-a in the 5,000 mg L^{-1} constant treatment remained high, with communities dominated by Green non-flagellate algae, or having a mixture of algae.
- Chlorophyll-a in the 15000 mg L^{-1} constant treatment remained high, with communities dominated by Centric and Pennate Diatoms and a Green flagellate.

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