Determining environmental risks to Ewens Ponds in the South East

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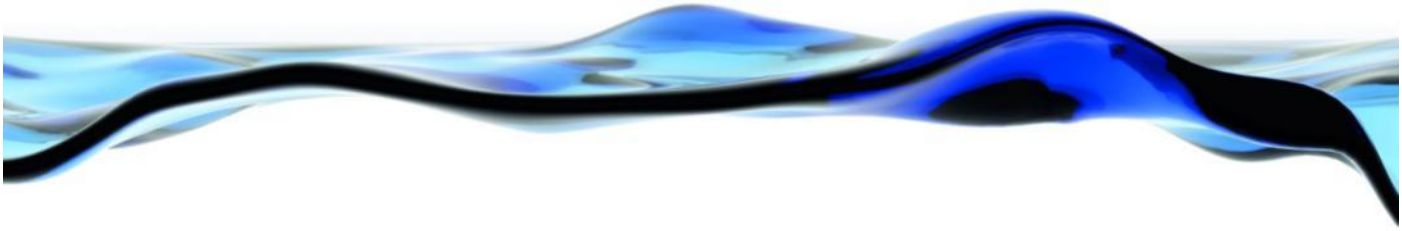

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Executive Summary

Ewens Ponds, located in the South East of South Australia, are exceptionally clear-water wetlands dominated by macrophytes, which provide critical habitat for protected aquatic species (Environment Australia, 2001, National Parks and Wildlife SA, 1999) including the critically endangered Glenelg Spiny Crayfish (*Euastacus bispinosus*).

Regional changes in land use from native vegetation to pasture, and alteration of the hydrology due to increased water extraction, decreased the quantity and quality of groundwater flowing into Ewens Ponds. Episodic occurrence of cyanobacterial blooms (Carmody, 2006) and epiphytic algal growth are initial warning signals of deteriorating water quality. Similar freshwater ecosystems have responded in a drastic way to increasing nutrients shifting from a clear-water macrophyte-dominated state to a phytoplankton-dominated state, with concomitant reduction in ecosystem health (Ibelings et al., 2007, Sheffer and van Nes, 2007, Bayley and Prather, 2003, Carpenter et al., 2011, Scheffer and Carpenter, 2003).

There is increasing concern that pelagic and epiphytic phytoplankton might outcompete macrophytes in Ewens Ponds causing habitat degradation and loss of endangered species. The uniqueness of the ponds and their regional and global importance are motivators for their protection and the maintenance of suitable water quality and flow.

The aims of this project were: a) to develop a water budget for the ponds and use this hydrological assessment with nutrient concentrations to quantify nutrient inputs; b) to evaluate how changes in nutrients and flow regimes affect algal growth and dilution to modify the light availability for rooted macrophytes.

The dominant flow and source of nutrients was the groundwater entering Pond 1 (~0.84 $m^3 s^{-1}$) with a second input of groundwater entering in Pond 3 (~0.36 $m^3 s^{-1}$). The total volume of the ponds was replaced in approximately 9.5 h. This rapid flow and short water residence time are likely to be the most important factors controlling the phytoplankton growth and water clarity. The flow recorded was about half of that noted in 1979 (Grandfield and Ashman, 1984). The age of the water entering the ponds was determined by analysis of CFCs at the groundwater inflow. It is estimated that this water entered the aquifer from rainfall between 1977 and 1988, indicating the travel time in the aquifer from the recharge zone to the ponds is 26-37 years on average. Relating aquifer residence time with the Australian fertilization trends over recent decades would indicate that a spike in nutrients entering the Ponds might be observed between 2026 and 2037.

Nitrogen is highly mobile in the groundwater but phosphorus significantly less mobile in the karst soils typical of the Limestone Coast. The ecosystem was clearly phosphorus limited with total nitrogen:total phosphorus (TN:TP) ratio >400 and high TN concentration, comprised predominately of nitrate (5.8 ± 0.5 mg L^{-1}). This was confirmed by bioassay experiments where nitrogen addition did not stimulate phytoplankton growth but phosphorus addition relieved the P-limitation and stimulated phytoplankton growth.

A phytoplankton growth and dilution model was developed to assess how nutrients, flow and growth rate would affect phytoplankton pelagic growth and the degree of shading this would represent for rooted macrophytes. A TP concentration > 0.02 mg L^{-1} would be sufficient to support pelagic algal growth up to 5.5 µg Chlorophyll-a L^{-1} in 20 days at relatively high growth rates (0.7 day^{-1}). This would increase light attenuation and restrict macrophyte distribution to about 1 m depth. However, the growth of the phytoplankton population is offset by the dilution from the high groundwater inputs. For large populations of pelagic algae to eventuate at abovementioned TP levels the flow rate would need to be

reduced considerably. In particular residence time would have to increase from current 9.5 h to 14 h considering as initial conditions the existing algal inoculum (~100 cells mL^{-1}) or to 12 h with an inoculum of 1000 cells mL^{-1} (~0.135 µg Chla L^{-1}).

An additional risk to macrophyte growth would be the expansion of epiphytic phytoplankton. Field experiments were conducted to estimate macrophyte primary productivity at different light intensities. Net primary productivity of macrophytes displayed considerable light limitation at a light intensity of 75 µmol photons m^{-2} s^{-1} showing that their development will be highly compromised at lower light conditions. The growth of epiphytic algae accelerated when P was artificially added to experimental samples.

A database of nutrient concentrations of South East wetlands was collated to determine the relative risk to these wetlands and place Ewens Ponds into a regional context. In 85% of the wetlands considered, TN concentration was greater than the 1 mg/L threshold guideline value (Australian and New Zealand Environment and Conservation Council, 2000) revealing effects of extensive agriculture and farming. The area close to Ewens Ponds is characterized by wetlands that are likely to be P-limited. Approximately 28% of all the sites considered in the database were P limited while 30% were N limited. The remaining sites were not limited by either of the nutrients.

Conservation planning for Ewens Ponds should focus on maintaining high flow and limiting phosphorus inputs.

Acknowledgements

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1. Introduction

Freshwater ecosystems can respond to changing conditions in very drastic and discontinuous ways, in particular when they have low resilience and when external conditions approach a critical level (Scheffer and Carpenter, 2003). A shift from clear-water, macrophyte dominated systems to phytoplankton dominated systems has been observed in lakes as nutrient concentrations have increased (Ibelings et al., 2007, Carpenter et al., 2011, Scheffer and Carpenter, 2003). This shift implies a loss of the macrophyte community and a decrease in water quality with consequent damage of the ecosystem function as habitat for vulnerable species.

Ewens Ponds, located in South Australia, are a clear-water, macrophyte dominated ecosystem of nationally recognized importance (Environment Australia, 2001) supporting endemic and threatened aquatic species such as Ewens pygmy perch (*Nannoperca variegata*), River blackfish (*Gadopsis marmoratus*), Glenelg Spiny Crayfish (*Euastacus bispinosus*), Burrowing crayfish (*Engaeus strictifrons*) and Freshwater crayfish (*Geocharax sp*.) (National Parks and Wildlife SA, 1999). The system is experiencing severe anthropogenic pressure due to changes in land use in the surrounding lands and increase of water extraction for agricultural purposes. Recent observations of algal blooms (Carmody, 2006) and increases in nitrogen concentrations indicate that the ecosystem is highly vulnerable and might be at risk of undergoing a regime shift toward a phytoplankton dominated state.

The hydrology of the South-East in South Australia has been extensively modified by a combination of drainage schemes, land clearance and water extraction. Extraction of groundwater across the region for industry and agriculture is thought to have resulted in a decline in groundwater discharge rates throughout the wetland complexes in the Lower Limestone Coast area (South East Natural Resources Management Board, 2013). Additionally, contamination of the unconfined groundwater aquifer by point and diffuse pollution sources in the region has been recognised (Emmett and Telfer, 1993) and an increase in nitrate concentrations has been observed over the last decades (unpublished data, Department of Environment Water and Natural Resources, DEWNR).

With the predominant land use in the surrounding region changing from native tea tree scrub to livestock (dairy and beef cattle and sheep) grazing pasture, Ewens Ponds has experienced a decline in both the quality and quantity of discharging spring water in the past four decades (unpublished data, EPA SA). Several episodes of cyanobacterial blooms were reported in the summer of 2004/05 and in the years of drought 2005/06 (Carmody, 2006). Growth of epiphytic and filamentous algae has been observed on the sloping banks of the three Ponds and on the aquatic vegetation such as *Triglochin procera*.

Modification of the hydrology and nutrient dynamics has raised concerns that a combination of decreased flow and increased in nutrients in Ewens Ponds might induce a shift from a clear-water and macrophytes dominated state to a turbid phytoplankton dominated state, which has been previously observed in many wetlands and shallow lakes (Ibelings et al., 2007, Sheffer and van Nes, 2007, Bayley and Prather, 2003).

The hypothesis is that increasing nutrient concentrations will favour phytoplankton growth and consequently decreasing light availability to the sediment-rooted macrophytes. It is critical to determine the level of risk associated to given conditions of nutrient enrichment and flow regime in order to be able to plan effective amelioration strategies and prevent a regime shift from a macrophyte dominated system to a phytoplankton dominated system. The value and uniqueness of the clear water Ewens Ponds for critical habitat and the outstanding

recreational experience that they offer demands they be conserved and preserved. It is critical to avoid the combination of events that could lead to a catastrophic shift of the ecosystem with no possible return to the previous state and a degradation of this environmental asset.

The aims of this project were to characterize the hydrology, hydrodynamics and biogeochemistry of Ewens ponds to identify the nutrient thresholds that will limit phytoplankton growth and maintain the water clarity necessary for macrophytes. The growth of phytoplankton communities is a function of nutrient and light availability. However in small lakes such as Ewens Ponds washout of the population can be a major determinant of the size of the resident community.

In this study water and nutrient balances were constructed from flow and nutrient concentrations. A growth and dilution model was developed to predict the size of the phytoplankton population and how this influenced light availability for macrophytes growing on the sediment. A preliminary evaluation of the light requirements of macrophytes was made with chambers measuring plant photosynthesis. Finally, a database on nutrient concentration in South-East wetlands was compiled to assess regional nutrient variability.

2. Methods

A combination of monitoring, laboratory and field experiments, and modelling was adopted in this study to characterize the environmental risk (Figure 1).

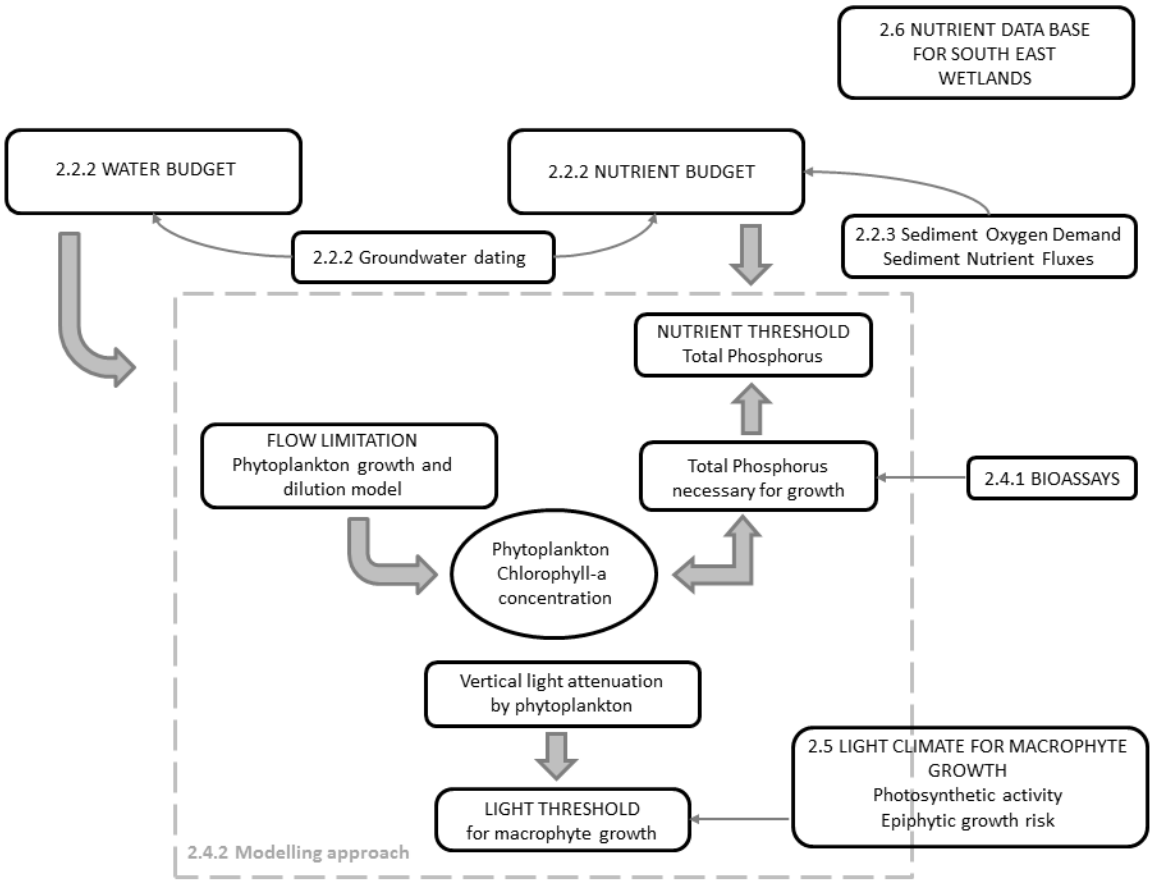

Figure 1. Risk assessment approach including experiments and modelling; numbers refers to the method sections of this report.

The hydrology of the system and the nutrient inputs were assessed by the development of water and nutrient budgets. Further information related to the hydrology was obtained through groundwater dating. Additionally, sediment incubation experiments were set up to evaluate possible internal inputs of nutrients (Section 2.2.3). Phytoplankton incubations (bioassays) at different nutrient levels allowed estimating nutrients limitation for pelagic algal growth (Section 2.4.1). A phytoplankton growth and dilution model allowed assessing conditions of flow and nutrients at which algal growth will shade macrophytes reducing their depth of colonization (Section 2.4.2). Moreover, experiments were conducted to evaluate changes in macrophyte photosynthetic activity at different light conditions and to evaluate the risk associated to epiphytic algal growth (Section 2.5).

2.1 Study site

Ewens Ponds are located in the Lower Limestone Coast of South Australia approximately 30 km south of Mt Gambier (Figure 2). They consist of three karst wetlands connected by channels that feed into Eight Mile Creek discharging to the sea approximately 2.5 km downstream. Ewens Ponds ecosystem is dependent upon underground water sourced from two distinct aquifers: the upper unconfined Tertiary Limestone Aquifer and the lower Tertiary Confined Sand Aquifer. Water can be observed bubbling into the Ponds through the bottom sediments. The three Ponds (Ponds 1, 2 and 3) have depths of about 11, 6 and 9 m, respectively, and volumes of approximately 28000, 11000, and 4400 m^3 (Grandfield and Ashman, 1984). Ewens Ponds are recognised and afforded particular protection under the Lower Limestone Coast Water Allocation Plan together with two adjacent karst wetlands systems: Piccaninnie Ponds and Crescent Pond (South East Natural Resources Management Board, 2013). Both Ewens and Piccaninnie Ponds are listed on the Register of the National Estate for their biological significance (Environment Australia, 2001). Piccaninnie Ponds was included on the Ramsar List of Wetlands of International Importance in 2013. The unique feature of Ewens Ponds supports several vulnerable and endangered species of aquatic fauna and flora (National Parks and Wildlife SA, 1999).

Figure 2. On the left: location and map of Ewens Ponds, modified from Grandfield, 1984. On the right: locations of bores, flow meter and surface water sampling points at Ewens Ponds.

2.2 Water and nutrient balances

2.2.1 Sample collection and analysis

Regular monitoring occurred from May 2014 to January 2015, (May, July, September, December, and January). Flow measurements were taken at the outflow of each Pond at the start of the study period using a StreamPro (Teledyne RDI; Poway, California); an Acoustic Doppler Current Profiler (ADCP) downward mounted on a floating platform with the capability of recording cross-sectional velocity and depth transects. A salinity probe (TROLL loggers, In-Situ, Fort Collins, Colorado) was deployed in each Pond at a depth of 2 m on chains attached to buoys and in the channel downstream of Pond 3, and recorded data every 30 minutes. Barometric pressure probes (transducers) were installed at the outflow of Pond 3 to monitor changes in water level.

A flow meter ultrasonic Doppler (Starflow, Flow Recorder Model 6526) was deployed at the outflow of Pond 3 and recorded flow velocity every 30 min (Figure 2). There was no direct surface water inflow evident. Thermistors were deployed initially in each of the Ponds and later at different depths in Pond 3 to detect differences in temperature between the Ponds and in the water column.

Water samples were collected periodically at each of the three Ponds (surface and 4 m depth) and from two bores up-gradient of the Ewens Ponds in the unconfined aquifer. Groundwater samples in the Ponds were collected in May 2014 and in January 2015. Samples from additional bores (Table 1, Figure 2) were taken at the end of September 2014 after a rainfall event. Water samples were analysed by the accredited Environmental Analysis Laboratory (Southern Cross University) using standard methods (APHA, 2005). Total Nitrogen (TN), total phosphorus (TP), nitrate (NO_3), nitrite (NO_2), phosphate (PO_4) and ammonia (NH_4) were determined.

Table 1. Names and locations of the bores sampled in proximity to Ewens Ponds, see also Figure 2.

Obswell n./Name	Loc. Easting	Loc. Northing
MAC 045	479734.8	5791715.36
MAC 093/094	481609	5791307
MAC 030	481375.79	5791393.42
CAR 004	484079.81	5792195.38
MAC 025 –Earl's cave	479743.78	5792479.41

To test differences between nutrient concentrations measured in different Ponds we conducted one-way analysis of variance (ANOVA) with IBM SPSS software. Analysis of variance is robust to departures from normality, although the data should be symmetric, so the groups should come from populations with equal variances. To test this assumption, the Levenes' homogeneity-of-variance test was used. Tukeys' post-hoc test was used to determine sources of significant differences between parameters. For all analysis *p* values less than 0.05 indicated significant differences. When the homogeneity-of-variance assumption was violated, an alternative non parametric test was used, the Friedman test. If significance values resulting from the Friedman test are lower than 0.05 then there is an overall statistically significant difference between the mean ranks of the related groups.

2.2.2 Water budget, nutrient budget and groundwater dating

The water budget was calculated by adopting the mass balance approach accounting for the major inputs and outputs (e.g. Owen, 1995, Yin and Nicholson, 1998, Windolf et al., 1996):

$$V(t) - V(t\text{-}1) = SI(t)+GI(t)+P(t)-E(t)-GO(t)-SO(t)$$

where V(t) is the lake volume at time t, SI is surface inflow, GI is groundwater inflow, P is precipitation, E is evaporation, GO is groundwater outflow and SO is surface outflow.

Meteorological data used were from the Mount Gambier station (Bureau of Meteorology, Australian Government). Outflow data were obtained by Doppler measurement of flow. Daily budgets were calculated for each sampling date as snapshots for different seasons. Loss of water from the ponds to the groundwater is unknown and it was assumed to be negligible, given the high flow of groundwater into the Ponds. Additional assumptions were that $V(t) - V(t\text{-}1) = 0$ is valid for the short time period modelled here, SI(Pond1) is equal to zero, SI(Pond2) =SO(Pond1) and SI(Pond3)=SO(Pond2), thus unknown groundwater inflows can be estimated.

The nutrient budget was calculated following Nõges et al. (1998), where the net budget is sum of the internal and external budget:

$$External\ buget = external\ loading\ (riverine + atmospheric + groundwater) - outflow$$

$$Internal\ budget = C_{t2} * V_{t2} - C_{t1} * V_{t1}$$

where C_{t1} is concentration at time 1 and V_{t1} is volume at time 1.

Ewens Ponds is a well oxygenated, high flow system and dominated by groundwater input which enabled two assumptions to be made when calculating the nutrient budget. Due to the low retention time and oxygenation the internal nutrient load at daily time scale was assumed to be zero, so the net nutrient input is equal to the external nutrient input. The atmospheric contribution is considered negligible and the riverine contribution was zero for Pond 1 and equal to the outflow of the preceding Pond for Ponds 2 and 3. The net budget is calculated using groundwater flows estimated from the water budget. The mean nutrient concentration observed in the Ponds was used for the nutrient budget as, due to the high groundwater flow and mixing, it was the most representative of the daily input. Outflow nutrient concentration, considering the low water retention time of the Ponds, was assumed to be the same as that measured in Pond 3.

Water age was estimated by analysing Chlorofluorocarbons (CFCs) and sulphur hexafluoride (SF_6), two environmental tracers commonly used to determine water ages of between 1 and 100 years old (Busemberg and Plummer, 1992, Cook et al., 1995). CFCs are stable, synthetic compounds that have been released into the atmosphere since the 1930s, and for which the atmospheric mixing ratios have been reconstructed over the past 50 years. SF_6 is an anthropogenic and naturally occurring compound that has been released into the atmosphere by humans since the 1960's (Busemberg and Plummer, 2008). The atmospheric concentration of SF_6, unlike CFCs, is expected to continue increasing over time. Water carries small amounts of these gases, which can be extracted to determine the estimated time from when the water entered the aquifer. Samples were collected for CFCs and SF_6 where water was observed to be discharging into Ponds 1, 2, and 3 from the groundwater, in the channel between Ponds 1 & 2, between Ponds 2 & 3, and at the outflow from Pond 3. Within the Ponds, the samples were collected by opening the glass sample bottles under water at the

desired location, then evacuating the bottle using nylon tubing connected to a glass syringe to obtain a clean sample. Samples were also collected at groundwater bores MAC45 and MAC94, using a submersible pump connected to nylon tubing. Bore water was pumped into a chamber at the surface containing the sample bottle, allowed to continuously overflow the bottle until a clean sample was obtained, and capped under water. Samples were collected between 26 and 30 May 2014 and analysed at GNS Science (Lower Hutt, New Zealand) using Gas Chromatography with Electron Capture Detection.

2.2.3 Sediment oxygen demand and sediment nutrient fluxes

Sediment oxygen demand (SOD) and sediment nutrient fluxes were determined to evaluate what could be the effect of nutrient release if flow decreased and sediment would be anoxic. Sediment cores were collected on the 29 of May 2014. At Pond 1 three replicates at 4 m depth and two at deeper levels (8 m) were collected. At Pond 2 two replicates were collected at 4m depth and two at 4.5 m depth. At Pond 3 two replicate at 4m depth and two at deeper levels (4.5 and 8m) were collected. Intact sediment cores were collected using cylindrical chambers with an internal diameter of 5.8 cm. Unsealed cores were attached to a pole and pushed 10-15 cm into the sediment, sealed and extracted. Cores deeper than 4 m were collected manually by divers. Cores were then sealed and transported to the laboratory for incubation in the dark at 25ºC to determine sediment oxygen demand.

Additional sediment samples were collected on the 21 January 2015 at Pond 1, three replicates at 4 m and 8 m depth to determine TP concentration in soil. Samples were analysed by Environmental Analysis Laboratory at Southern Cross University using standard methods (APHA, 2005).

Incubations were initialized replacing the original overlying water in the cores with Ewens Ponds surface water. One core containing only water was used as control. Cores were sealed and placed in dark at 20°C; overlying water was mixed through magnetic stirrers as in Brookes (2008) and dissolved oxygen (DO) was measured every 30 min with a dissolved oxygen probe (Model WP-82 Dissolved Oxygen-temperature meter). Initial and final oxygen concentration was measured for all the cores. Five cores, one for each Pond collected at 4m depth and two collected at 8m depth from Pond 1 and Pond 3 were measured continuously for 5 days in addition to the control, which just contained water. Sediment oxygen demand was calculated as the change in DO (mass) per unit surface area, per unit time over the first 24 hours. Rates were corrected for changes in DO levels within the control chambers.

Nutrient flux rates were determined as the change in concentration over five days in the overlying water. Nutrient concentrations were measured at initial condition and at the end of the five-day incubation following methods in section *2.2.1*. To test differences between SOD and nutrient fluxes measured in different Ponds, a statistical analysis analogous to the one adopted in section *2.2.1* was used.

2.4 Modelling changes in the ecosystem conditions

2.4.1 Phytoplankton growth under different nutrient conditions, bioassays

Bioassays were conducted to assess whether phosphorus or nitrogen was the limiting nutrient and to estimate the pelagic phytoplankton growth rate at different nutrient concentrations. Incubation experiments were performed in August 2014 during an 11 day period, using water collected from Ewen Ponds at controlled light, 100 µmoles m^{-2} s^{-1} (cool

white, fluorescent lamps) with a light-dark cycle of 12:12 h, and temperature of 20°C. As the phytoplankton concentration was close to zero a pure culture of green algae, *Ankistrodesmus falcatus*, was used as inoculum. The initial cell density of all treatments was adjusted to approximately 1000 cells mL^{-1}.

In order to reduce the effect of nutrients accumulated in the cultured cells, algae were maintained in nitrate- and phosphate-free BG 11 media for five days before the experiment. Four nutrient treatments were used: no nitrate and phosphate added (control); dipotassium hydrogen orthophosphate (K_2HPO_4) added at 100 µmol L^{-1} (P); sodium nitrate ($NaNO_3$) added at 1000 µmol L^{-1} (N); and both K_2HPO_4 and $NaNO_3$ added at the abovementioned level (N+P). Additionally, algae growth was examined using seven levels of PO_4^{3-}P addition (0.1, 0.2, 0.5, 1, 2, 5, 10 µmol L^{-1}), all with fixed NO_3^--N (1000 µmol L^{-1}). Nutrients other than phosphorus and nitrogen for phytoplankton growth during the bioassay were provided by adding stock solutions following the formula of BG-11 media (Stanier et al., 1971).

Chlorophyll-a was measured spectro-photometrically (spectrophotometer: Libra S22 Biochrom, Cambridge, UK) from hot ethanol extracts of GF/C filtered samples. Cell counting was undertaken with an OLYMPUS BX40F4 optical microscope (Olympus, Tokyo, Japan) following standard procedures (APHA, 2005).

Phytoplankton growth rates were calculated on chlorophyll-*a* concentration (μ_{chla}) and cell numbers (μ_{cell}) using the following equation

$$\mu = ln(X_t/X_0)/t$$

where X_t is final chlorophyll-a concentration or cell number, X_0 is initial Chl-a or cell number, and t is the duration of incubation.

Nonlinear regression was used to fit growth rates with phosphate concentration following growth kinetics by Monod (1950). Statistics were performed using SPSS 19.0 (IBM, Armonk, NY, USA), values were logarithmically transformed to meet the requirements for parametric tests when necessary. One-way analysis of variance (ANOVA) was used to test for differences in data between cultures with variable nutrient supply patterns. Tukey's post-hoc tests were used evaluate differences between treatments. Estimate of nonlinear fitting parameters was determined using OriginPro 9.0 (OriginLab, Northampton, MA, USA).

2.4.2 Phytoplankton growth model in Ewens Ponds

The modelling aim was to identify a nutrient threshold that would limit phytoplankton growth and maintain water clarity satisfying the light requirements for macrophyte development. The development of phytoplankton communities in small lakes such as Ewens Ponds is a function of nutrient availability, light availability and washout of the population by flow. Modelling the growth of phytoplankton under different nutrient concentrations and flow conditions will allow an estimation of the nutrient thresholds to maintain water clarity at the desired level (see modelling approach scheme in Figure 1).

Considering the observed low residence time of the Ponds (about 9 hours) the most suitable approach was adopting a phytoplankton growth model accounting for growth and dilution. Other fully coupled hydrodynamic models, as GLM-FAMB (Hipsey and Bush, 2012) are only suitable for systems with higher residence time (e.g. 4 days up to over 1 year).

Increasing phytoplankton cells and consequently increasing Chlorophyll-a concentration will increase light attenuation and decrease light at the macrophyte colonization level. It was postulated that if the light dose that currently reaches the maximum depth of colonization only reached 1 m above it, then the plants would not receive sufficient light to maintain photosynthesis and the depth of colonization would shift up one metre. Two scenarios were simulated using different growth rates and flow rates: the light availability to maintain the growth of macrophyte at the present level and a change in light attenuation reducing by one metre the maximum depth of colonization.

Light attenuation

The most common macrophyte observed in Ewens Ponds are Angiosperms (*Triglochin procera*; *Hydrocotyle verticillata*) and Charophyta Ranunculaceae and Apiaceae (e.g. *Ranunculus inundatus*; *Triglochin striata*; *Lilaeopsis polyantha*). The maximum depth of colonization observed in Ewens Ponds for these species were respectively 1.5 m for *T.procera*; 4 m for *H. verticillata*, *R. inundatus*, *T. striata*; and 5 m for *L. polyantha* (Grandfield and Ashman, 1984). The maximum depth of colonization (z_{col}) is an important parameter to assess light requirements (Kirk, 2011). Similar z_{col} of about 4 m were observed by Middelbow and Markager (1997). Light availability at different z_{col} was calculated following the Lambert-Beer law:

$$I_z = I_0 e^{-k_d * z}$$

where I_z is the radiation at a particular depth (z); I_0 is radiation at surface and k_d is the extinction coefficient.

Average daily light radiation (wavelength range of 300 - 400 nm) measured in Ewens Ponds in January 2015 (380 µmol photons m^{-2} s^{-1}) was used as a reference with an extinction coefficient of 0.33 (m^{-1}) estimated from irradiance profiles measurements in the field.

Two levels of limiting light (1) to maintain the present state and (2) shift the macrophyte growth 1 m closer to the surface were established based on *L. polyantha*, the species that grows at greatest depth (5 m) and at average light conditions of approximately 75 µmol m^{-2} s^{-1}.

Chlorophyll-a concentration thresholds corresponding to the two light levels were calculated considering vertical light attenuation by phytoplankton as follow:

$k_i = k_w + (k_1 * C_1)$ and $C_1 = (k_i - k_w)/k_1$

where k_i is the calculated light extinction coefficient; k_w is the light extinction coefficient for clear water (m^{-1}); C_1 is the concentration of the algal group 1 (µg Chla L^{-1}) and k_1 is the specific extinction coefficient for algal group 1 (m^{-1} *[µg Chla L^{-1}]$^{-1}$). Typical ranges of k_1 for different phytoplankton groups are: 0.015 to 0.025 (Hamilton and Schladow, 1997); 0.01 to 0.03 (D. Hamilton personal communication modelling Tarawera lake, NZ); 0.01 to 0.02 (Reynolds, 2006).

Following Reynolds (2006) and considering a generic phytoplankton group, the extinction coefficient k_1 was set equal to 0.015 m^{-1} *[µg Chla L^{-1}]$^{-1}$

Phytoplankton growth and dilution model

Phytoplankton cell concentration was calculated as follows considering different growth rates (r_i) and dilution or flushing rates (D_i):

$$C_{t_i} = C_{t_{i-1}} * e^{r_i * (t_i - t_{i-1})} - C_{t_{i-1}} * D_i$$

where C_{ti} is the phytoplankton concentration in cells/mL at time t_i . The cell development was calculated for a 20 day period with a time step of 1 day. The growth rates were varied within different model runs from 0.1 to 1.2 day^{-1} spanning a wide range of phytoplankton species. Maximum specific growth rates higher than 1.2 day^{-1} were only observed in laboratory culture under continuous light and maximum resource availability and unlikely to happen in the Ponds. The dilution rates considered (e.g. dilution rates from 75% to 250%) and corresponding residence times and flow rates are specified in Table 2. These compare with an estimated current residence time of about 0.44 day for Pond 1 and 0.5 day for the three Ponds.

Table 2. Dilution rates and correspondent residence times and flows adopted in the model

Dilution rate	Residence time (day)	Flow ($m^3 s^{-1}$)
75%	1.33	0.2431
100%	1.00	0.3241
170%	0.59	0.5509
200%	0.50	0.6481
250%	0.40	0.8102

Two different initial conditions were considered: an inoculum of 100 cells mL^{-1} and an inoculum of 1000 cells mL^{-1}. These correspond to Chla concentrations of non-diatomaceous phytoplankton of respectively 0.0135 and 0.135 µg Chla L^{-1} (Reynolds (2006). Cell concentrations obtained from the model not flushed via the outflow were converted to Chla concentrations and used to estimate light attenuation. An estimated value of 1.37 µg Chla cell^{-1} was adopted considering cell volumes of approximately 30 µm^3 representing green algae approximately the size of *Ankistrodesmus* as observed in Myponga reservoir (South Australia) and a cell ratio C:Chla of 50:1 (Reynolds, 2006).

Nutrient thresholds

The total phosphorus (TP) required to support the growth of phytoplankton cells in the system was estimated from the Chlorophyll-a concentration obtained as the sum of the flushed and unflushed phytoplankton cells predicted by the model. Chlorophyll-a and total phosphorus (TP) relationships have been identified by previous works and are summarized in Table 3. The equation by Dillon and Rigler (1974) was adopted as it was obtained including a variety of ecosystems and had a higher r^2 value compare to others.

Table 3. References for Chlorophyll-a and total phosphorus relationships. Units, equations and r^2 are provided

Units	Formula	Source
Chla (mg m^{-3}) and TP (mg m^{-3})	log10 [Chla] = 1.583 log10 [TP] - 1.134 r^2=0.975	(Dillon and Rigler, 1974)
Chla (mg m^{-3}) and TP (mg m^{-3})	log10 [Chla] = 1.062 log10 [TP] – 0.509 r^2=0.63	(Knowlton et al., 1984)
Mean epilimnetic TP (µg L^{-1}) and Chla(µg L^{-1})	Log10[TP]=1.774 + 0.250 log10 [Chla] r^2 =0.55	(Mc Queen et al., 1986)
Max Chla (µg L^{-1}) and max TP (mgL^{-1})	[Chla]= 195.57 [TP] + 1.71 r^2=0.78	(Linden et al., 2004)
Chla (µg L^{-1}) and TP (µgL^{-1})	log10 [Chla] = 1.026 log10 [TP] – 0.455 r^2=0.78	(Phillips et al., 2008)

2.5 Light climate for macrophyte growth

2.5.1 Photosynthetic activity at different light conditions, field experiment

Field incubations in chambers were used to estimate primary productivity of macrophyte at different light conditions. Photosynthetically active radiation was recorded every 5 minutes at surface and underwater, at the chambers depth, using a data logger LI-1400 and an Odyssey logger, respectively. Three clear Perspex chambers of 35 cm diameter with lids were positioned on top of the sediment colonized by macrophytes in Pond 1 at approximately 4 m depth. Chambers were deployed at 12:30 pm the 20th of January 2015 and retrieved at 4:30 pm of the following day. The water inside the chamber was mixed with a submersible pump and changes in dissolved oxygen were measured at one minute intervals with an optical dissolved oxygen sensor (D-02 D-opto Logger). Chambers were flushed twice during the day to avoid super saturation of oxygen. Incubations were carried out for one day and one night in order to calculate respiration during the dark period and net productivity during the light period. Respiration was calculated as the difference between DO concentration at the beginning and end of dark period hourly (mg O_2 L^{-1} h^{-1}) and gross primary productivity was the difference during light periods. Net productivity was estimated for each hour as difference between gross productivity and respiration averaged during the night (Noel et al., 2010). To standardize the results the net productivity was corrected for unit of biomass (mg O_2 L^{-1} h^{-1} g^{-1}). Macrophytes biomass growing over the surface area covered by the chamber was collected and measured as dry weight. Finally, hourly average light intensity was related to hourly averaged macrophyte net productivity.

2.5.2 Assessing risk related to epiphytic growth

Mesocosm experiments were conducted to evaluate the rate of epiphytic algal growth. River red gum (*Eucalyptus camaldulensis*) 13x12 cm wood blocks were used as a substrate for epiphytic algae that were observed to develop on macrophyte leaves in Ewens Ponds. Epiphytic algae were cultured at ambient light and temperature conditions in a greenhouse for 4 weeks using two treatments: a) no nutrient addition, natural conditions using water collected at Ewens Ponds and b) nutrients over saturation, continuous release of nitrogen and phosphorus using Osmocote® fertilizer. In the second treatment it can be assumed that there is no limitation of nutrients availability for plants. Five replicates were cultured for each treatment. Initial conditions with similar inoculum of epiphytic algae were obtained deploying wooden blocks at Ewens Ponds at about 10 cm depth for approximately 4 weeks. Epiphytic algal biomass as Chlorophyll-a was measured at initial and final conditions spectrophotometrically from hot ethanol extracts following standard methods (APHA, 2005).

Additionally, the light attenuation of epiphytic algae was estimated with optical experiments (Sand-Jensen and Søndergaard, 1981). Epiphytic algae were scraped from a known area of the wood blocks used in the mesocosm experiment. For each replicate, light attenuation and algal biomass were measured. Portion of light passing through a Petri dish filled with 10 mL of algal solution obtained from 1/5 of the epiphytic algae scraped from the block surface was measured with a light data logger LI-1400. Light at source was 1200 µmoles m^{-2} s^{-1}. Chlorophyll-a was determined spectrophotometrically as previously specified.

2.6 Nutrient data base for South East Australian Wetlands

Many wetlands in the South East are undergoing pressures similar to Ewens Ponds, such as increasing nutrients and flow reduction. For example, several highly valued wetlands, which are groundwater dependent, were identified as being located within groundwater development risk zones in the South East (Harding, 2012). In order to contextualize the environmental risk for Ewens Ponds and identify possible nutrient concentration hot-spots, the aim was to collate information available on nutrient concentrations observed in South East wetlands and represent the data spatially. When available, nutrient concentrations observed at drains flowing in and out the wetlands were also included in the data set.

The data base included site name, location (northing and easting coordinates), date, nutrient concentration. The main sources were: data collected by the Department of Water, Land and Biodiversity Conservation, Government of South Australia in 2008 (Hobbs and Stratman, 2008), data collected by the Environmental Protection Authority (EPA) in 2009 (Goonan et al., 2011), data collected during a previous Goyder Institute project in 2010 (Aldridge et al., 2011), data collected by the Environmental Protection Authority in 2014 (EPA, unpublished). While creating the database it was noticed that data on conductivity and pH was more extensively available compared to nutrient (e.g.Taylor, 2006, Baldwin et al., 2012), although this was not the focus of this analysis. Nutrient data were available only for a relatively short period of time and on many occasions sampling points changed. Total nitrogen (TN) and total phosphorus (TP) were most frequently available at different sites, so they were selected for the database collation.

Data were reorganized based on coordinates and site names. In the majority of cases sites were sampled only once between 2008 and 2014; when sampled more than once the average was calculated. All the data for TP and TN were plotted using ESRI ArcGIS 10.2 software. Symbols of different sizes were used to represent 5 classes grouped using quartiles. The ratio between TN and TP was calculated for each site and it can be used to identify if the primary productivity in the system is limited by phosphorus or nitrogen. Ratios lower than 20 normally indicate that the system is N limited, while values higher than 50 suggest that the system is P limited (Dzialowski et al., 2005, Guilford and Heckey, 2000).

3. Results

3.1 Water and nutrient balance, groundwater dating

Water balance results

Surface areas of the three ponds were estimated at 3133 m^2, 2423 m^2 and 977 m^2 using geographical information system (GIS) mapping, and these values were used to calculate daily precipitation and evaporation for the water budget.

The relative amount of groundwater flow coming from the different ponds was estimated from the ADCP measurements at the start of the study period (May 2014): the average flow rates were 0.733 $m^3 s^{-1}$, 0.729 $m^3 s^{-1}$ and 1.052 $m^3 s^{-1}$ for ponds 1, 2 and 3, respectively. Thus, the estimated flush rates from Pond 1 to 3 were 0.44, 0.19 and 0.07 days, respectively. The flush rate for the whole system is about 0.48 days, so in less than 12 hours all the water is replaced in the system.

Surface water inflow was considered negligible. The groundwater inflow, as measured in May, was mostly entering Pond 1 (70% of the flow). No groundwater input was observed in Pond 2 and 30% of the groundwater inflow came from Pond 3. This proportion

was assumed constant, which was proved to be reasonable by the results obtained from the mass balance analysis of the salinity data as follows. Assuming (i) a simplified closed system where water enters at two locations in Ponds 1 and 3 and leaves only via the Pond 3 outfall (ii) the salinity of groundwater sources entering Pond 1 and Pond 3 were not the same (iii) the salinity of groundwater entering both Ponds 1 and 3 remained relatively constant and given that observed salinity in Pond 1 was effectively equal to Pond 2 during the whole study period, the ratio of the salinity between Pond 3 outflow and Pond 1 was indicative of the ratio between the relative flow entering Pond 1 and Pond 3. This ratio was constant, as found for measurements taken in May, and it was used both for the water budget and nutrient budget calculations. Water budget snapshots were calculated for each monitoring date and later used in calculating nutrient budget snapshots. The total outflows varied from 81,000 to 133,000 m^3 day^{-1} throughout the year and measured outflows varied less than 0.04% from measured groundwater inflows entering the Ponds (Table 4). The contribution of precipitation and evaporation was minimal compared to the groundwater inflow and the main inflow was coming from the bottom of Pond 1.

Table 4. Ewens Ponds water balance and nutrient balance. Groundwater inflow form Pond 2 was negligible.

Water balance					
	Total Outflow (m^3 day^{-1})	Groundwater inflow (m^3 day^{-1})		Total Groundwater inflow (m^3/day)	
		Pond 1	Pond 3		
May 2014	90892.8	63324.9	27560.6	90885.5	
July 2014	115776	81037.6	34730.8	115768.4	
Sept 2014	80818.6	56594.1	24249.9	80844	
Dec 2014	133056	92464.3	40613.1	133077.4	
Jan 2015	103680	72588.5	31107.9	103696.4	
Nutrient balance					
	TP (mg day^{-1})				
	Total Outlet	Groundwater inlet		Total groundwater inlet	Net budget
		Pond 1	Pond 3		
May 2014	1.109	0.773	0.336	1.109	-8.5E-07
July 2014	3.531	2.472	1.059	3.531	0
Sept 2014	2.272	1.591	0.682	2.273	0.0007
Dec 2014	2.661	1.849	0.812	2.662	0.0004
Jan 2015	1.866	1.307	0.560	1.867	0.0003
	TN (mg day^{-1})				
	Total Outlet	Groundwater inlet		Total groundwater inlet	Net budget
		Pond 1	Pond 3		
May 2014	496.0	345.6	150.4	495.9	-0.040
July 2014	714.8	500.3	214.4	714.8	-0.047
Sept 2014	468.9	328.3	140.7	469.0	0.147
Dec 2014	860.4	597.9	262.6	860.5	0.138
Jan 2015	556.9	389.9	167.1	557.0	0.088

Total outflow data from the Starflow Doppler were available for each monitoring period, but occasionally they were not obtained in continuum due to the instrument limitation of measurement in extremely clear water. Outflow rates were estimated as 1.34 $m^3 s^{-1}$ on the 29th of July 2014, 0.935 $m^3 s^{-1}$ on the 29th of September 2014, 1.54 $m^3 s^{-1}$ on the 9th of December 2014 and 1.2 $m^3 s^{-1}$the 19th of January 2015. The high flow rates observed in December and January were related to rainfall events from October to December 2014 (e.g. a rainfall event of 8.8 mm the 4th of December) and to a rainfall event of 32.6 mm the 13th of Jan 2015.

Water level was almost constant: barometric probes indicated a decrease of about 0.1 m from May to September and then a similar increase from September to December. This was consistent with total outflow measurements that detected a higher flow in December than in September. This is in agreement with previous historical data showing surface level variation was almost constant in Ewens Ponds from 2004 to 2013 with yearly oscillation of less than 0.2 m (DEWNR, unpublished data).

Nutrient balance results

Temperature profiles, thermistor data and oxygen profiles showed the water column in the Ponds was well mixed and well oxygenated throughout the year. Temperature was almost constant at about 15°C and no temperature difference was observed between the three Ponds.

Observed nutrient concentrations are in Table 5. The average concentrations of TP and TN in Ewens Ponds during the study period were respectively 0.022 mg L-1 and 5.8 mg L^{-1}. The TN concentration was extremely high and above the guidelines of 1 mg L^{-1} for inland water bodies of South Australia. On the other hand, TP fell below the guidelines of 0.1 mg L^{-1} (Australian and New Zealand Environment and Conservation Council, 2000). This supported the hypothesis that Ewens Ponds is a P limited system; a concept that was later tested in the bioassay experiment. For the majority of the sampling dates there was no statistically significant difference in nutrient concentration (TP and TN) between Ponds or between surface and deeper layers. A difference was observed between Ponds in September 2014 and January 2015 only for TN, but it was not significant with respect to the TN budget and the average TN in the water column was used.

Results of the nutrient budget showed that most of the nutrients entering via the groundwater inflows were flushed out of the system. A high variability of TP and TN inputs was observed during the study period (Table 4). The nutrient budget suggested that the input of nutrient from the sediment was not significant. This was analysed in detail in the following section on sediment oxygen demand and sediment fluxes.

No correlation was found between nutrient concentrations at the two bores in the unconfined aquifer (MAC094 and MAC045) and at the Ponds. Additionally, samples taken from bores from the unconfined aquifer in a radius of about 2 km in September 2014 showed high spatial variability and different concentrations from those observed in the Ponds (Table 5). Thus, the groundwater entering the Ponds was not coming from a single source and might come either from a combination of water of different origins or from a different aquifer.

Groundwater dating results

Dissolved concentrations of CFC-11, CFC-12, CFC-113, and SF_6 in water samples are usually measured in grams per kilogram of water, which are converted to equivalent atmospheric partial pressures based on the gas solubility at an assumed recharge temperature

and pressure (Cook et al., 1995). Equivalent atmospheric partial pressures are reported as parts per thousand volume (pptv) and are converted to apparent recharge years using the historic concentrations of these trace gases in the atmosphere. In reality, groundwater represents a mixing of waters that have recharged over time; therefore, the recharge year represents an apparent groundwater age only. In Table 6, 1GW, 2GW, 3GW are the samples collected where water was observed bubbling into Ponds 1, 2, and 3 respectively. OUTF1, OUTF2, and OUTF3 are the samples collected between Ponds 1 & 2, Ponds 2 & 3, and at the outflow of Pond 3. MAC45 and MAC94 are the samples collected from bores (Table 6). The last four samples represent average values from duplicate analysis. A "C" in the table indicates that the sample was at a higher concentration than could be explained by equilibrium with modern air and therefore deemed to be 'contaminated'.

Table 5. (A) Nutrient concentrations: Average concentration and standard error deviation (± SE) of 6 to 9 replicate samples for: the three Ponds (Ewens Ponds); groundwater samples collected by divers at the bubbling points indicating groundwater discharge in Ponds 1 and 2 (Groundwater); average concentration of water sampled at observation wells MAC045 and MAC094 in the unconfined aquifer (Bores). (B) chemical analyses from selected observation wells within 2km from the ponds in the unconfined aquifer.

(A)						
	TP (mg L^{-1})					
	Ewens Ponds	± SE	Groundwater	± SE	Bores	± SE
May 2014	0.012	0.0016	0.013	0	0.01	0.004
July 2014	0.03	0.0021			0.04	0.016
Sept 2014	0.028	0.0054			0.03	0.018
Dec 2014	0.02	0			0.027	0.008
Jan 2015	0.018	0.0008	0.021	0.0026	0.025	0.003
	TN (mg L^{-1})					
	Ewens Ponds	± SE	Groundwater	± SE	Bores	± SE
May 2014	5.46	0.42	3.9	0.96	3.20	2.51
July 2014	6.17	0.34			4.57	1.38
Sept 2014	5.80	0.31			3.91	0.11
Dec 2014	6.47	0.36			4.52	0.30
Jan 2015	5.37	0.49	5.07	0.65	3.64	1.03
(B)						
	Sept 2014					
Bores	TN (mg L^{-1})	TP (mg L^{-1})	Nitrate (mg L^{-1})	Nitrite (mg L^{-1})	Orthophosphate (mg L^{-1})	Ammonia (mg L^{-1})
MAC 045	3.98	0.02	3.256	0.006	0.01	0.001
MAC 093/094	3.83	0.045	3.603	0.002	0.012	0
MAC 030	6.42	0.027	4.949	0.021	0.014	0.005
CAR 004	14.72	0.062	14.275	0.004	0.011	0.002
MAC 025 – Earl's cave	6.76	0.032	6.409	0.004	0.009	0.001

Among the CFC analyses, CFC-12 provides the most reliable groundwater dating results, as it is more stable than CFC-11 and CFC-113 in subsurface environments, and present at much higher concentrations than SF_6. Using this tracer, the apparent recharge years

for all samples fell within a relatively narrow range of years from 1977 to 1988. No trend in age difference was apparent between the Ponds. The age of the groundwater at the bore closest to the Ponds (MAC 094) was about 10 years younger than the groundwater discharge at the Pond 1 and 3 and suggested, supporting results obtained with nutrients analysis, that there was not a single and direct flow of this aquifer to the Ponds.

In general, the concentrations of SF_6 in the Ewens Ponds samples were very high, suggesting that the sampled waters are not in equilibrium with modern air, thus SF_6 was not a reliable tracer of groundwater ages in the Ewens Ponds.

Table 6. Groundwater dating results: 1GW, 2GW, 3GW are the samples collected where water was observed bubbling into Ponds 1, 2, and 3 respectively. OUTF1, OUTF2, and OUTF3 are the samples collected between Ponds 1 & 2, Ponds 2 & 3, and at the outflow of Pond 3. MAC45 and MAC94 are the samples collected from bores.

	Atmospheric Partial Pressure								Apparent recharge year			
Site	CFC-11 pptv	± CFC-11 pptv	CFC-12 pptv	± CFC-12 pptv	CFC-113 pptv	± CFC-113 pptv	SF_6 pptv	± SF_6 pptv	CFC-11	CFC-12	CFD-113	SF_6
1GW	189	19	422	37	35.9	5.2	690	132	1984.0	1987.5	1985.5	C
	177	17	379	31	25.0	4.4	-	-	1982.5	1985.5	1982.5	-
2GW	293	38	421	49	37.4	6.4	628	120	C	1987.5	1985.5	C
	186	23	400	46	31.3	5.6	-	-	1983.5	1986.5	1984.0	-
3GW	191	24	332	38	31.3	5.6	642	123	1984.0	1982.5	1984.0	C
	129	16	192	23	9.8	4.1	-	-	1977.5	1975.0	1975.5	-
OUTF1	193	24	396	45	27.4	5.3	1431	274	1984.5	1986.0	1983.0	C
	219	20	414	35	28.2	5.0	-	-	1987.0	1987.0	1983.0	-
OUTF2	189	18	391	32	26.6	4.7	867	75	1984.0	1986.0	1983.0	C
OUTF3	197	17	405	30	25.5	4.4	5.34	0.45	1985.0	1986.5	1982.5	2004.5
MAC045	67.5	7.9	430	39	28.5	5.2	143	12	1972.0	1988.0	1983.5	C
MAC094	81.7	7.3	248	20	11.7	3.1	4.48	0.42	1973.5	1977.5	1976.5	2000.5

Future trends in nutrient concentrations in Ewens Ponds can be estimated from the apparent groundwater ages if long-term trends in fertiliser use are known. Trends in fertiliser use (N and P) in Australia (Food and agriculture organization of the United Nations, 2014, World Bank, 2014) are apparent from historical data, available from 1962 through 2012. Beginning around 1991, fertiliser use rose considerably each year before plateauing in 1998 and then decreasing slightly in the 21st century. In a review of fertiliser use in Australia, the Fertilizer Industry Federation of Australia (2011) reports a flat trend in nitrogen sales since 1990, while sales of phosphorus have nearly tripled.

For samples collected in 2014, groundwater entering Ewens Ponds recharged the aquifer between 1977 and 1988, implying a mean residence time of 26-37 years. Thus, based on the fertiliser use trends, higher levels of nutrients would be expected to enter the Ponds via groundwater inflows in the future, with peak nutrient loads occurring in 2026 -2037.

3.1.2 Sediment oxygen demand and sediment nutrient fluxes

The dissolved oxygen concentration of the water overlying the incubated sediment cores dropped in the first 24 hours from about 5 PPM to 2.5 PPM as shown in Table 7. The average sediment dissolved oxygen demand (24h), for Ewens Ponds was 415 mg O_2m^{-2} d^{-1} with a standard deviation of 215 mg O_2m^{-2} d^{-1}, showing high variability between samples. No

statistical difference (p>0.05) was identified between oxygen demand calculated at different Ponds or at different depths.

Table 7. Initial and final dissolved oxygen (DO) concentrations measured on the overlying water of the incubated cores. DO final 24h is showed only for cores for which continuous data were collected.

Cores	Collected depth (m)	Sediment level (m)	DO initial (PPM)	DO final 5 days (PPM)	DO final 24h (PPM)	O2 mg m^{-2} in 5days	O2 mg m^{-2} in 24h
1A	4	0.095	5.15	3.05		493.5	
1B	4	0.135	4.33	3.8	3.68	103.35	126.75
1C	4	0.12	5.8	3.35		514.5	
1D	8	0.22	3.9	1.32		283.8	
1E	8	0.175	4.97	2.44	2.3	392.15	413.85
2A	4	0.155	5.89	2.98	3.91	509.25	346.5
2B	4	0.135	4.7	2.81		368.55	
2C	4.5	0.215	4.8	1.67		359.95	
2D	4.5	0.13	4.99	4.1		178	
3A	4	0.17	5.46	2.53	2.57	468.8	462.4
3B	4	0.125	5.67	3.37		471.5	
3C	8	0.12	4.25	0.38	0.8	812.7	724.5
3D	4.5	0.175	4.05	1.58		382.85	
Control			4.57	1.93	2.71		

Positive nutrient flux values denoted a flux from the sediments to the water and negative indicated flux from the water to the sediments. Standard errors were calculated taking into account the standard deviation of the nutrient concentration measured at the initial conditions (19 replicates taken for different Ponds). Differences between initial and final concentrations for TN were not significant $\chi^2(2) = 3.769$, $p = 0.052$, so the flux for TN was considered negligible. Nitrite flux was extremely low (average < 1 mg $m^{-2}day^{-1}$) and could be considered negligible. Negative values obtained for nitrate indicated not a flux towards sediment, but a change in concentration as nitrate was being reduced.

A release of TP was observed from the sediment at anoxic conditions: rate of about 2.4 mg P m^{-2} d^{-1}. For TP and phosphate the fluxes were not significantly different between sites (p = 0.135) varying between 1 to 8 mg P m^{-2} d^{-1}, while there was significant difference between samples collected above and below 4 m depth (Figure 3). This might be related to the different sediment types that were richer in sand closer to the water surface and higher in clay at the lower depths.

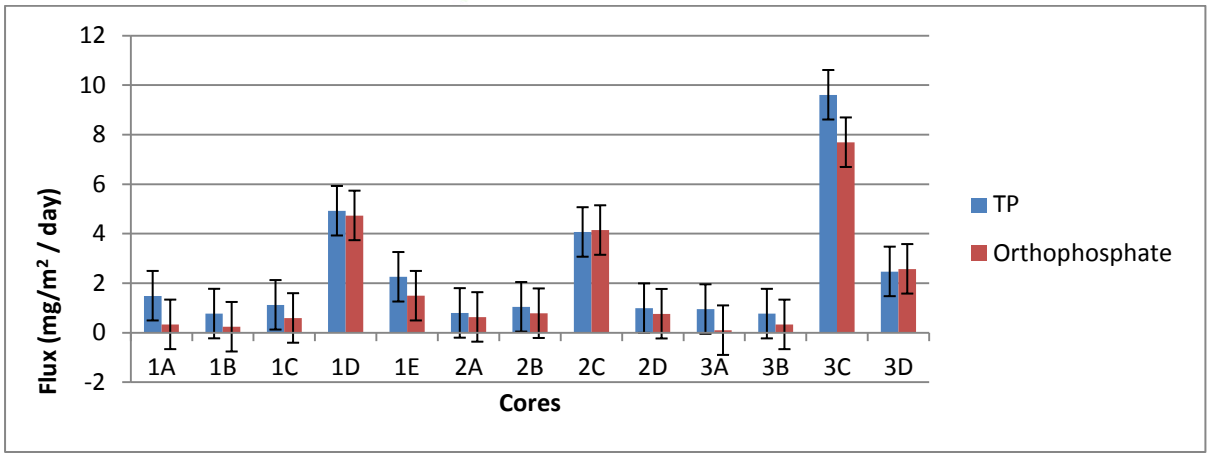

Figure 3. Total Phosphorus and Orthophosphate fluxes estimated for different cores collected in Ewens Ponds.

The TP flux rates calculated with incubation experiments can be used to estimate the risk of the development of phytoplankton growth under stratified-anoxic conditions and reduced water flow. Water column stratification has not been observed, even in the summer period. Although, a worst case scenario would be represented by low flushing rates, at which anoxic conditions might develop, resulting in increased phosphorus concentration in the water column and pelagic algal growth. Here, it was assumed that all the phosphorus released was available for uptake and algal growth. If flow was to be highly reduced, and anoxic conditions occurred at the bottom, the phosphorus released from the sediment would be sufficient to generate a significant increase in algal biomass (about 13 µg Chla L^{-1} in 20 days). Following Lambert-Beer law, this would result in a decrease of light availability for macrophyte growth of about 50%.

3.2 Modelling changes in the ecosystem conditions

3.2.1 Phytoplankton growth under different nutrient conditions, bioassays

Nutrient addition to water samples significantly increased the phytoplankton cultures chlorophyll-*a* (Chl-a) content and cell number with respect to the control ($p<0.01$). In excess of both phosphorus and nitrogen (P+N) the highest Chl-a and cell concentrations were obtained (Figure 4). The total Chl-a developed at the end of the treatment was significantly higher when adding phosphorus than nitrogen. The increase in cell number obtained with addition of P alone was the same as that obtained by adding both P and N, showing that P was the controlling factor for growth (Figure 4).

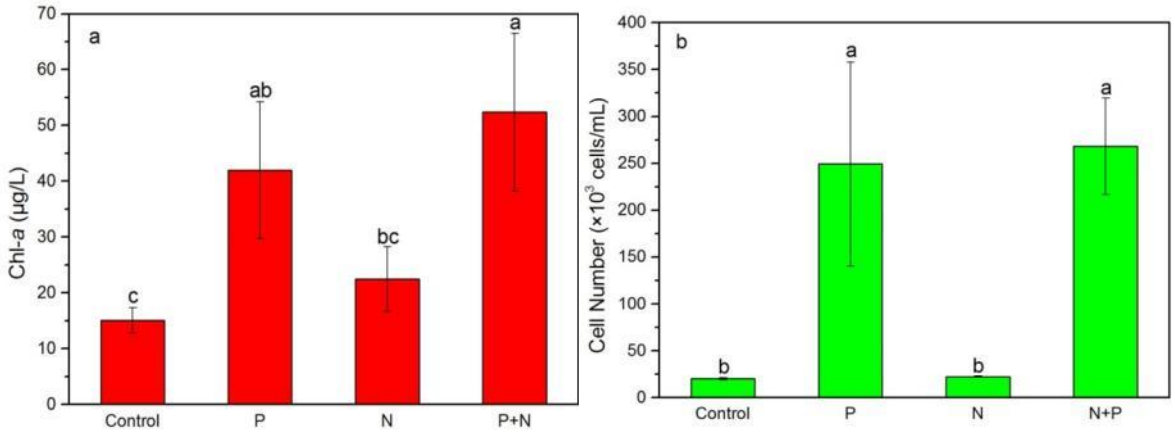

Figure 4. a) Chlorophyll-a content and b) cell number at the end of the incubation experiments in samples with addition of excess of phosphorus (P), nitrogen (N) and both (P+N). Bars are standard deviations, columns labelled with different letters are significantly different ($p<0.05$).

The bioassays confirmed the initial hypothesis that phosphorus was the limiting nutrient in Ewens Ponds. Thus, the risk of phytoplankton development is closely associated with phosphorus increase and nitrogen was present at concentrations that were excess to demand.

The second bioassay experiment allowed the estimation of phytoplankton growth rates at different P concentrations in excess of nitrogen. Algae growth rate increased consistently until the P concentration reached about 0.035 mg L^{-1} (Figure 5 and Figure 6). Maximum growth rates, obtained from fitting results with the Monod equation, were respectively 0.3 d^{-1} and 0.43 d^{-1} when accounting for biomass change as chlorophyll-*a* or cell number. Half-saturation constants were respectively 0.016 mg P L^{-1} for chlorophyll-*a* and 0.019 mg P L^{-1} for cell number.

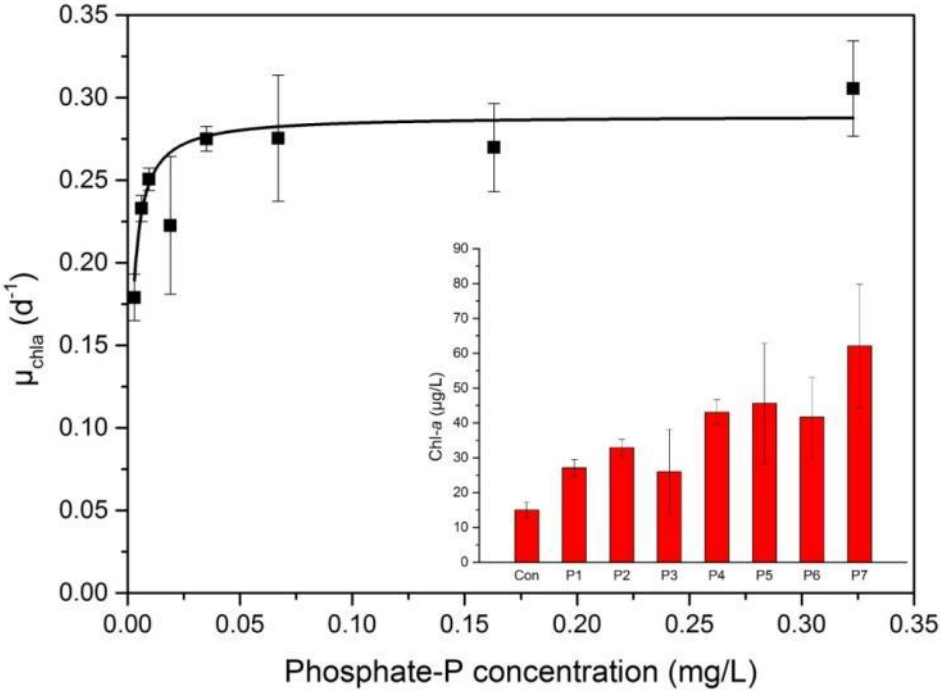

Figure 5. Relationship between growth rates calculated from chlorophyll-a content and initial phosphate concentrations for samples treated with different phosphate additions, bars are standard deviations. The curve is the Monod function fit to the data.

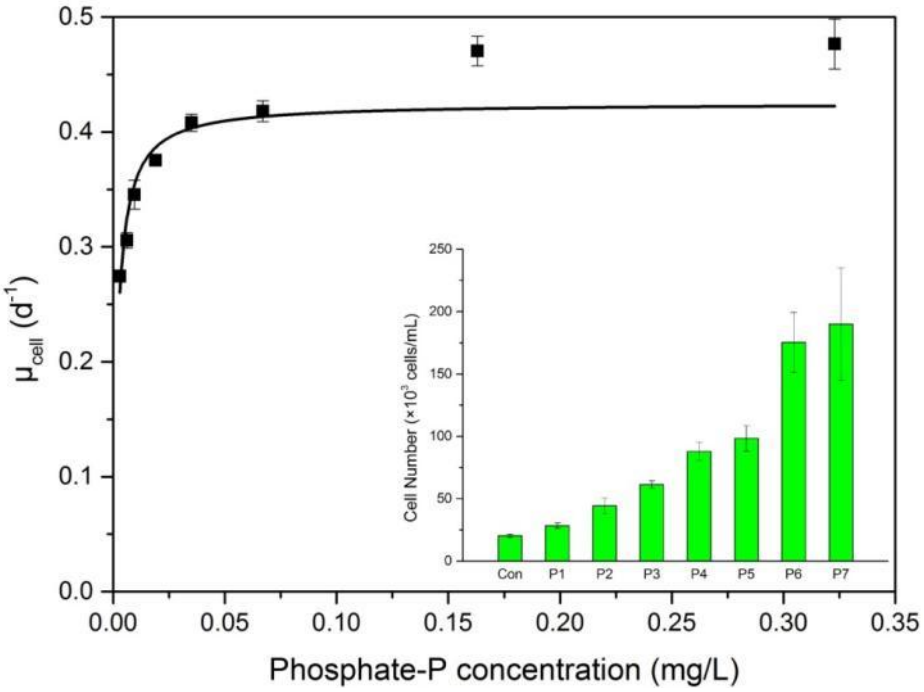

Figure 6. Relationship between growth rates calculated from cell number and initial phosphate concentrations for samples treated with different phosphate additions, bars are standard deviations. The curve is the Monod function fit to the data.

Cultured green algae, *Ankistrodesmus falcatus*, was used as inoculum for the bioassay experiment. This species only partially represents the behavioural characteristic of a phytoplankton community that might develop in Ewens Ponds. Algal concentration in Ewens Ponds were close to the limit of detection (<0.01 µg Chla L^{-1}) and species observed included *Cryptomonas* spp., *Rhodomonas* spp., and diatom *Fragilaria* spp. Different species of algae have different kinetics of P uptake and growth (Holm and Armstrong, 1981, Gotham and Rhee, 1981, Tilman and Kilham, 1976). Species with low half saturation constant (K_s) and high maximum growth rate (μ_{max}) have a tendency to dominate in natural phytoplankton assemblages under P limitation (Tilman and Kilham, 1976, Holm and Armstrong, 1981). In Ewens Ponds, enhancement in P concentration increases the possibility of pelagic algal development, although this development will be controlled also by outflow rates through cell flushing.

3.2.2 Phytoplankton growth model in Ewens Ponds

The growth of pelagic phytoplankton, affecting macrophyte development in Ewens Ponds, is mainly dependent on nutrient availability and water flow. Through modelling it was possible to estimate the conditions at which the increase in phytoplankton biomass (or Chl-a concentration) would alter the light availability for macrophyte growth in 20 days.

Two light limiting levels were considered: 73 µmoles m^{-2} s^{-1} sufficient to maintain the present light conditions and 48 µmoles m^{-2} s^{-1} that would decrease the maximum depth of colonization for macrophytes by 1 m. The corresponding Chlorophyll-a concentration allowed in the system to maintain these two light levels were respectively 0.5 µg Chla L^{-1}, and 5.5 µg Chla L^{-1}. Such concentrations could be supported by the system if the TP in the water column was at least respectively 0.004 and 0.02 mg L^{-1}. Additionally, such

concentrations of algal biomass could develop only under particular combinations of dilution rates and phytoplankton growth rates. These are showed in Table 8.

Five different dilution rates were adopted in the model ranging from observed dilution rates of about 250% (close to the observed present flow, e.g. 0.8 $m^3 s^{-1}$) to 75%. Lowest dilution rates of 75% correspond to a flow of 0.24 $m^3 s^{-1}$ that is about ¼ of that observed during this study.

Table 8. Model results summary. Phytoplankton biomass required to create conditions satisfying modelled macrophyte light requirement scenarios. Limit 1 is sufficient to maintain present light conditions while Limit 2 would decrease macrophyte depth of colonization by 1 m. TP is the total phosphorus and *r* is the phytoplankton growth rate (day^{-1}).

	Limit 1	**Limit 2**
Light	73 µmoles $m^{-2} s^{-1}$	48 µmoles $m^{-2} s^{-1}$
Phytoplankton biomass	0.5 µChla L^{-1}	5.5 µChla L^{-1}
Conditions		
Inoculum 100 cells/mL (0.0135 µChla L^{-1})		
	TP < 0.004 mg L^{-1}	**TP < 0.02 mg L^{-1}**
Dilution rate		
75%	*r*<0.7	*r* <0.8
100%	*r* <0.8	*r* <0.9
170%	*r* <1.2	*r* <1.2
200%	*r* <1.2	-
250%	-	-
Inoculum 1000 cells/mL (0.135 µChla L^{-1})		
	TP < 0.004 mg L^{-1}	**TP < 0.02 mg L^{-1}**
Dilution rate		
75%	*r* <0.6	*r* <0.7
100%	*r* <0.8	*r* <0.8
170%	*r* <1.1	*r* <1.1
200%	*r* <1.2	*r* <1.2
250%	-	-

A large range of growth rate values was used, 0.1 to 1.2 day^{-1}, accounting for species with different adaptations. Growth rates higher than 1.2 day^{-1} were observed in laboratory culture under continuous light and maximum resource availability, but are unlikely to occur in the Ponds based on the known variation in current conditions. Pelagic phytoplankton growth would occur only if the population had high a growth rate (0.6 – 0.8 day^{-1}) and did not occur at lower growth rates, even when reducing the Ponds dilution rate from 250% to 75%, or increasing the length of simulation up to a month. The phytoplankton developing in the Ponds would be more likely characterized by high growth rate, so possibly Chlorophyte and Diatom species would be more abundant than Cyanobacteria.

A significant light reduction could occur as a result of TP abundance supported at TP> 0.02 mg L^{-1}, if accompanied by reducing dilution rate from 250% to 170% - that is, a reduction in flow rate from about 0.8 to about 0.55 $m^3 s^{-1}$. This level of TP was observed in September and December 2014, indicating that the main factor controlling phytoplankton biomass at present is flushing. Maintaining TP under the cited value would be a first precautionary measure to reduce risk. Based on the observed variability it is not likely that

the flow rate would decrease to almost half of the present condition, although, if there was an increase in phytoplankton cell inoculum (e.g. 1000 cells/mL) a similar light reduction for macrophytes could be reached at dilution rate of 200% (0.65 m^3 s^{-1}), that is a flow reduction of about 20% from present.

3.3 Light climate for macrophyte growth

3.3.1 Photosynthetic activity at different light conditions, field experiment

Chambers set up and deployment is shown in Figure 7. Changes in DO recorded at the different domes are shown in Figure 8 and were used to calculate hourly net primary productivity. Hours when chambers were flushed (respectively 4, 5, 22 and 25 hours from the deployment) were excluded from calculations.

Figure 7. Submerged domes deployment in Ewens Ponds for the photosynthetic activity assessment.

Productivity was averaged by the macrophyte biomass (Charophytes, *Ranunculus inundatus*) in each dome. Macrophytes dry weight in domes 1 to 3 was respectively 20.7, 9.3 and 39.8 g. Photosynthetically active radiation measured underwater every 5 min, was averaged hourly at the same time intervals used for the calculation of net primary production.

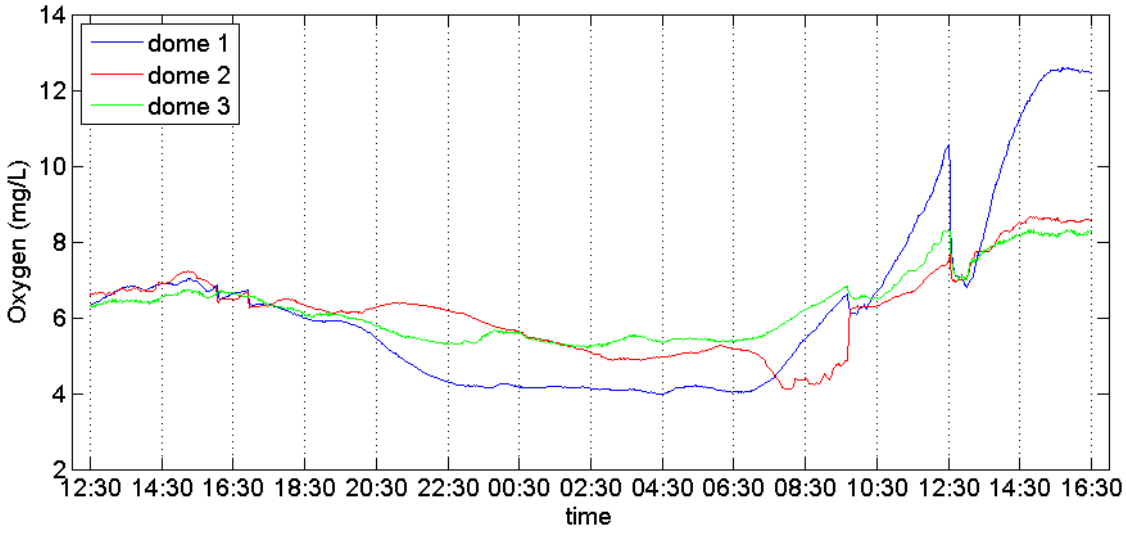

Figure 8. Dissolved oxygen concentration recorded at each of the submerged domes during the macrophyte photosynthetic activity experiment from the 20th Jan 2015 at 12:30 to the 21st at 16:30.

Net primary productivity ranged from values close to zero at light lower than 25 µmoles m^{-2} s^{-1}, to approximately 0.1 mg O_2 g^{-1} dw h^{-1} at high light intensity of about 340 µmoles m^{-2} s^{-1} (Figure 9).

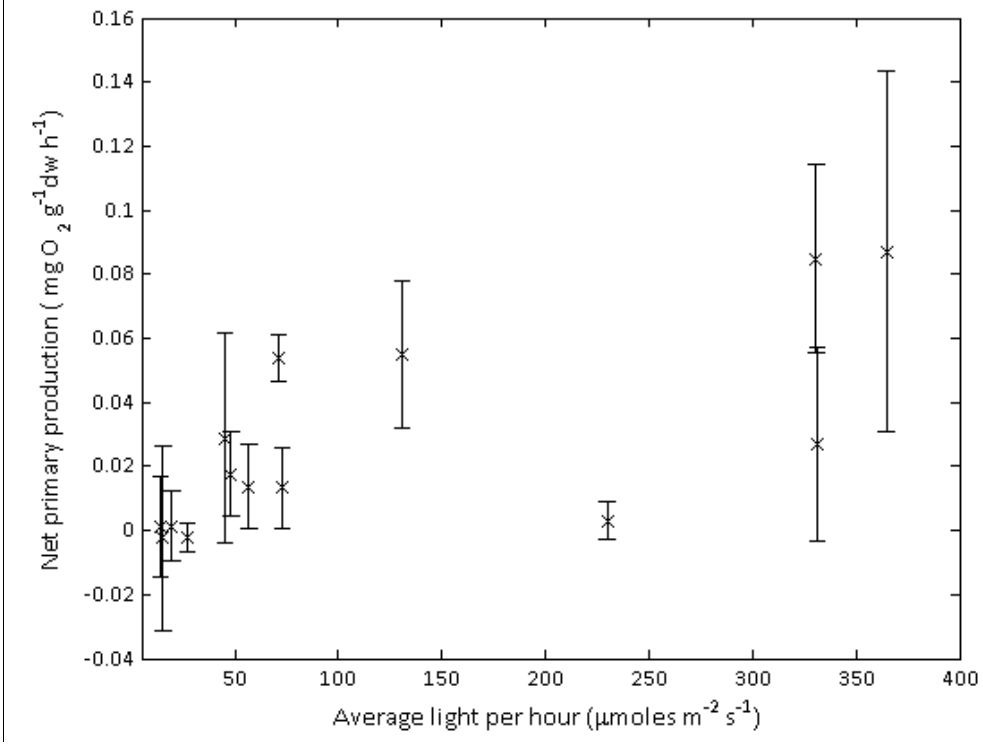

Figure 9. Relationship between light intensity and hourly net primary production of macrophytes. Units of primary productivity are milligrams of oxygen per hour per gram of dry weight. Bars represent standard deviations calculated using data from the three domes.

Productivity showed a large variation between domes and at different light conditions. Light intensity was a poor predictor of net primary production. Productivity steeply increased with light up to about 120 µmoles m^{-2} s^{-1} when it became more stable. These results are in

agreement with the limiting light levels adopted in the phytoplankton growth model, where it was considered that macrophyte development would be compromised at light values lower than approximately 75 µmoles m^{-2} s^{-1}.

3.3.2 Assessing risk related to epiphytic growth

A preliminary evaluation of the risk related to epiphytic growth was obtained by incubating epiphytic algae using water collected at Ewens Ponds (no treatment) and maintaining high constant nutrient concentration using a fertilizer (nutrient addition). Initial and final concentrations for the two treatments are shown in Figure 10. A high biomass increase of up to 110 mg Chl-a m^{-2} was observed when nutrients were not a liming factor, but when no nutrients were added there was no significant difference between initial and final epiphytic biomass (Figure 10).

Under an assumption of exponential growth, the estimated growth rate for epiphytic algae was 0.02 day^{-1} with no addition of nutrients and 0.12 day^{-1} with fertilizer addition at no nutrient limitation. The experiment was conducted for 28 days, but a rapid accumulation of biomass on the blocks was observed in the first 10 days, so calculating the growth rate using the full period might be an underestimate. More realistic growth rates calculated using an interval of 10 days will be approximately 0.06 and 0.31 day^{-1}. These preliminary results indicate that epiphytic algae could develop 5 times faster than present if phosphorus was not limiting their growth in the Ponds.

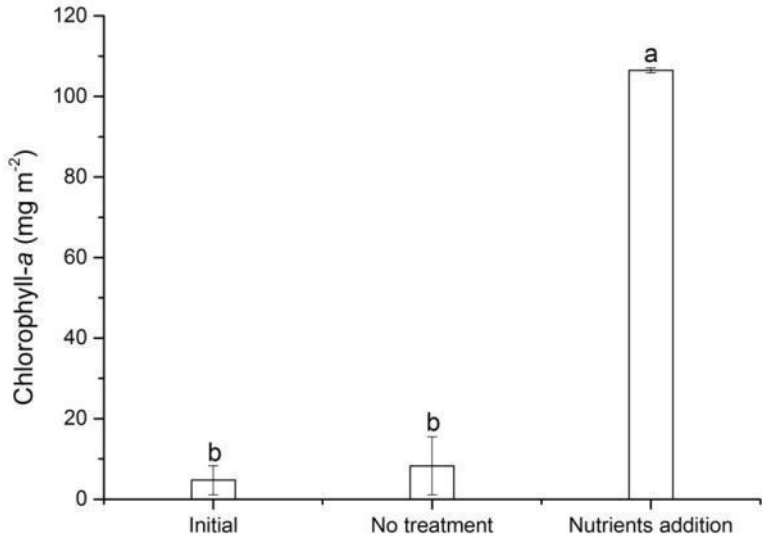

Figure 10. Epiphytic algal concentration at initial conditions and at the end of the 28 days incubation with no treatment or with nutrient addition (constant release of fertilizer). Bars are standard deviations, columns labelled with different letters are significantly different ($p<0.05$).

Linear decrease in light transmission was observed with increasing epiphyte biomass concentration (Figure 11). An increase of epiphytic biomass of about 20 mg Chl-a m^{-2} that could occur at the observed nutrient concentrations, would decrease light availability for macrophytes of about 20%. At no nutrient limitation the potential epiphytic biomass resulting would mean that the light reaching a macrophyte leaf would be reduced up to 80%. Additional experiments should be done to assess more in detail the risk related to epiphytic growth at different nutrient conditions as they might represent a risk greater than pelagic algae.

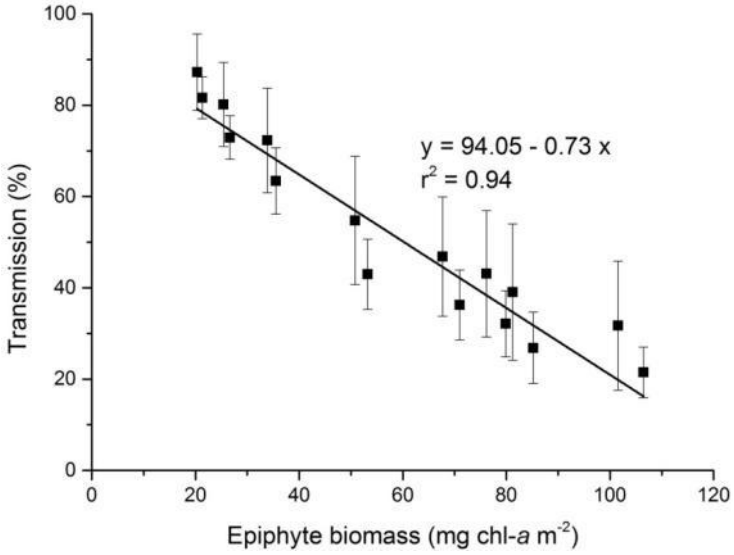

Figure 11. Relationship between increase in epiphyte biomass and changes in light transmission.

3.4 Nutrients in wetlands in the South East

A data base on TP and TN concentration in South East wetlands was collated, see Appendix A, Table 9. Figure 12 shows the spatial distribution of TP and TN concentrations in the South East. Maps showed a high spatial variability of both variables and no evidence of main hot-spots. In general, nutrients showed higher concentrations close to the main towns (Bordertown, Naracoorte, and Beachport) and close to Lake Bonney. The TP, instead, was low in the wetlands south of Mount Gambier where high TN values were observed. The concentration observed were compared with the Australian and New Zealand Guidelines for fresh and marine water quality (Australian and New Zealand Environment and Conservation Council, 2000), respectively 0.1 mg/L of TP and 1 mg/L of TN for South Australian inland waterbodies. Observed values were above the guideline levels in 34% of the sites for TP, and in 84.7% of the sites for TN.

The spatial distribution of the TN/TP ratio available for the different wetlands in the South East region and the values of TN/TP ratio versus the Euclidean distance to Ewens Ponds are showed in Figure 13. Wetlands close to Ewens Ponds are located in a region characterized by P limited systems. About 28.2% of the sites were P limited and approximately 30.5% were N limited. The rest of the sites, about 40%, were not limited by either nutrient.

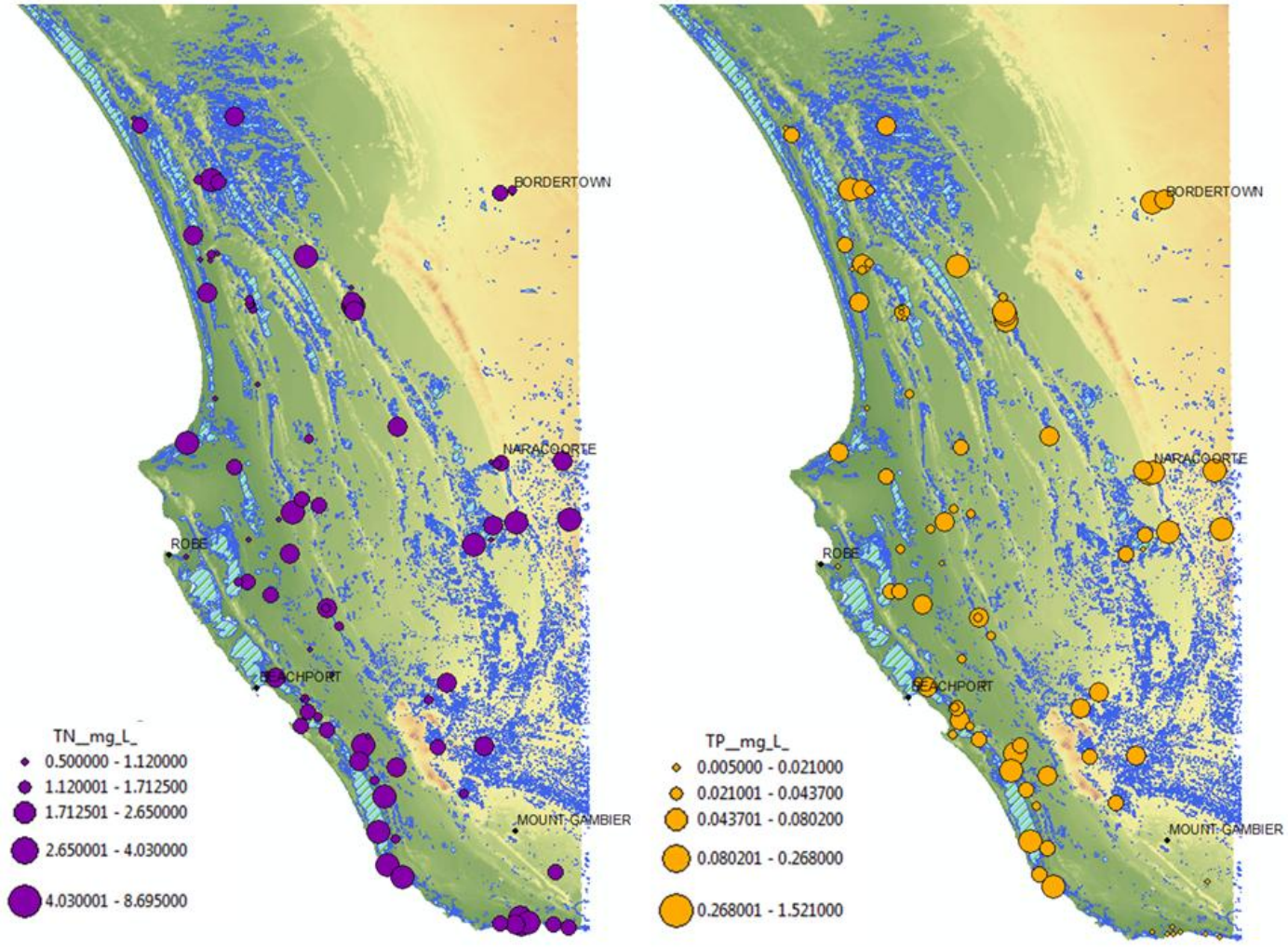

Figure 12. Map of the total nitrogen (TN mg/L) and total phosphorus (TP mg/L) in the South East wetlands and drains.

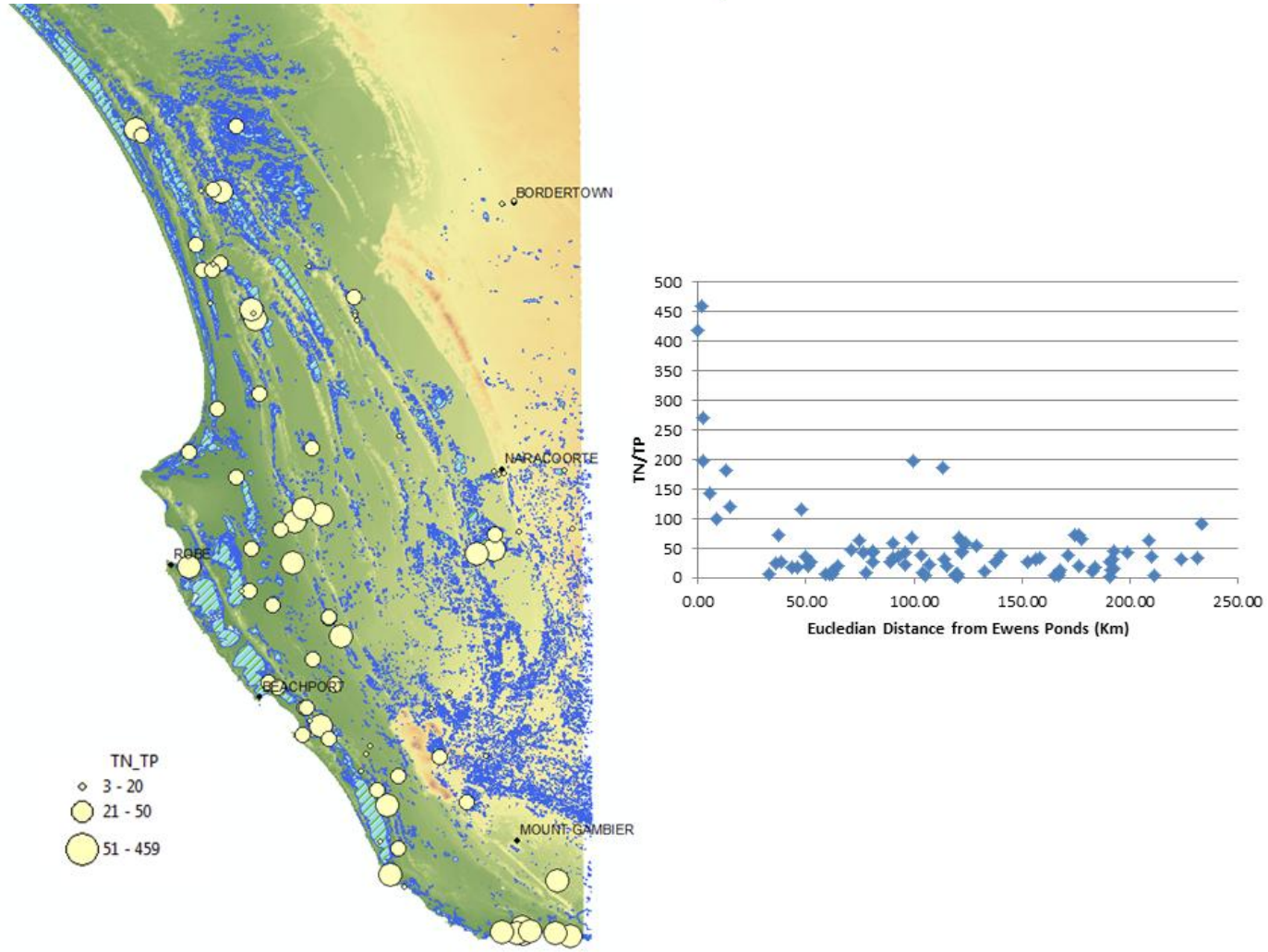

Figure 13. Map of TN/TP ratio in the South East region and TN/TP ratios values represented as function of distance from Ewens Ponds (TN/TP= 420).

4. Discussion

Water balance, nutrient balance and groundwater dating

The water in Ewens Ponds is replaced quickly in a system characterized by a residence time of approximately 9.5 hours. The average total flow exiting Pond 3 was about 1.2 m^3s^{-1} during the monitoring period (July 2014- Jan 2015). The magnitude of the flow was close to the flow estimated in previous work (Wood, 2011). In 2011, it was suggested that most of the groundwater inflow was coming from Pond 3. In contrast, our measurements indicated that most of the groundwater flow (about 70%) was coming from Pond 1, 30% was coming from Pond 3, and the flux was negligible in Pond 2. Additionally, the present average flow is remarkably low compared to historical flow values of about 2.28 m^3 s^{-1} observed in 1979 (Grandfield and Ashman, 1984). In the 1980s the flow recorded in the first and second channel were respectively 0.9 and 1.3 m^3s^{-1} suggesting that there was a groundwater inflow entering Pond 2 that was no longer observed in 2014.

Nutrient inputs to Ewens Ponds are coming from groundwater. The average influx calculated during the monitoring period was approximately 2.3 mg d^{-1} for TP and 619 mg d^{-1} for TN. The nutrient budget suggested that most of these inputs were flushed out of the system with limited accumulation in the sediments or assimilation into biomass. The average TP and TN concentrations during the monitoring period were respectively 0.022 ± 0.007 and 5.8 ± 0.5 mg L^{-1} and in most of the cases no significant difference was observed between surface and deeper levels or between the Ponds. The age of the groundwater entering Ewens Ponds is relatively old: a time lag of about 26-37 years between the time at which the surface water recharged the limestone aquifer and when it entered the Ponds was estimated. This was in line with previous results that estimated an age of about 23 years for Pond 1 and Pond 3 water using analogue techniques (Wood, 2011). Previously, measuring tritium concentrations, the groundwater was estimated to be more than 15 years old (Allison and Holmes, 1973). Considering that trends in fertiliser use in the last decades were rising until about 1998 (Food and agriculture organization of the United Nations, 2014, World Bank, 2014, Fertilizer Industry Federation of Australia, 2011), an increase of nutrients entering the Ponds is expected in the future, with peaks in 2026-2037.

Sediment fluxes and sediment oxygen demand into context

An additional source of nutrients in the Ponds could come from the sediments. This was assessed analysing the sediment oxygen demand (SOD) and sediment fluxes. The average SOD demand in Ewens Ponds was 415 ± 215 mg O_2m^{-2} d^{-1} that is in the range of oligotrophic and mesotrophic systems (0.05 to 0.3 g O_2 m^{-2} d^{-1}) (Hu et al., 2001, Beutel, 2003). The SOD in Ewens Ponds was comparable to the one observed in other freshwater systems in the South East, for example in Lake Bonney SOD was found to be 667.2 ± 376.8 mg O_2 m^{-2} d^{-1} (Aldridge and Brookes, 2009) and in Lake George was varying from about 19.6 to 471.8 mg O_2 m^{-2} d^{-1} (Brookes and Aldridge, 2007). Higher SOD values (1 to 4 g O_2 m^{-2} d^{-1}) have been observed in eutrophic systems (Hu et al., 2001) or in highly urbanized systems such as Patawalonga Lake (0.9 to 5 g O_2 m^{-2} d^{-1}) (Aldridge and Brookes, 2006).

The flux of TN from the sediment was negligible in Ewens Ponds while a release of TP was observed at anoxic conditions at a rate of about 2.4 mg P m^{-2} d^{-1}. This is comparable to rates observed in Lake Bonney, varying from 0.96 to 2.4 mg P m^{-2} d^{-1} (Aldridge and Brookes, 2009) and Lake George, varying from 0.5 to 5.3 mg P m^{-2} d^{-1} (Brookes and Aldridge, 2007). Ewens Ponds TP flux rate is lower than annual fluxes observed in eutrophic systems in previous studies, although nutrient fluxes show high variability with season, thermal stratification and flow velocity (Reddy et al., 1999). For example, TP sediment flux range from -17 to 19 mg P m^{-2} d^{-1} in shallow eutrophic lake Loch Leven (Scotland) (Spears et al., 2008), from 2 and 6 mg P m^{-2} d^{-1} in shallow eutrophic Danish lakes (Sondergaard et al., 2001), from -0.8 to 9.2 mg P m^{-2} d^{-1} in eutrophic wetlands in USA (Reddy et al., 1999). In two oligotrophic lakes in USA which depth was >14m, Lake et al. (2007) observed lower fluxes of about 0.0016 mg P m^{-2} d^{-1}.

At present, due to the low residence time, anoxic conditions are unlikely to develop, although, at reduced flow the TP released by the sediment would be able to support the growth of phytoplankton up to 13 µg Chla L^{-1} in 20 days, decreasing macrophyte light availability by 50%.

Bioassay

Nutrient enrichment bioassays were conducted to characterize nutrient limitation in Ewens Ponds. Nitrogen concentration has been increasing in Ewens Ponds and limitation by nitrogen has been previously associated with eutrophic lakes (Lewis and Wurtsbaugh, 2008, Suttle and Harrison, 1988), so we expected phosphorus to be the limiting factor in the system. The phosphorus limitation in Ewens Ponds is manifested by a TN:TP ratio of about 400 (Maberly et al., 2002, Ptacnik et al., 2010). Nitrate concentration in Ewens Ponds was already close to 5 mg L^{-1} in the early 1980s (SA EPA, unpublished data), higher than eutrophic range of 0.50 to 1.00 mg L^{-1} (Lin et al., 2008). Bioassay results confirmed that low phosphorus availability is constraining phytoplankton growth. Addition of nitrogen alone had no relevant effects on the algal biomass. Phosphate concentration no longer limited phytoplankton growth in incubations when reaching about 0.040 mg L^{-1}.

Modelling approach

The modelling approach established in this study allowed a first estimation of the risk related to phytoplankton growth development in Ewens Ponds related to flushing rates and nutrient limitation. Similar model approaches accounting for primary production and flushing rates were typically applied in estuaries and adapted for different turbidity conditions (see Ferreira et al., 2005, Muylaert et al., 2005). The flow rate was the most important factor controlling the pelagic algal growth in Ewens Ponds. The other two main factors were the phytoplankton growth rate and the phosphorus availability, as nitrogen was abundant. A TP concentration higher than 0.02 mg L^{-1} would be sufficient to support Chlorophyll-a development of up to 5.5 µg Chla L^{-1} in 20 days at relatively high growth rates (0.7 day^{-1}) reducing depth of light penetration by about 1 m. However, this would only occur if the flow rate were substantially reduced; e.g. dilution rates decreased from current 250% to 170% with the existing algal inoculum (~100 cells mL^{-1}) or 200% with an inoculum of 1000 cells mL^{-1}.

Reasonable and straight forward risk estimation was obtained through modelling. This approach has some limitations as some assumptions had to be made when calculating the TP to support algal growth from the predicted level of Chlorophyll-a concentration. Firstly, a

constant carbon cell content was adopted representing green algae, but the phytoplankton cell size - and consequently its carbon content – might be highly variable between groups and between species. For example, using approximations suggested by Reynolds (2006), Cyanobacteria *Anabaena*, green algae *Ankistrodesmus*, green algae *Chlorella* and Diatoms *Cyclotella* cellular carbon content will be respectively about 29, 6.7, 3.4 and 112 pg C/cell. This might lead to a partial overestimation or underestimation of the TP necessary to support algal growth depending on the group that will develop in the Ponds. Secondly, an approximately constant Carbon:Chlorophyll-a ratio in the cells was assumed, but again this can vary depending on the phylogenetic group developing (Menden-Deuer and Lessard, 2000).

This modelling approach can be easily adapted to other wetlands, where information is scarce, collecting a reduced amount of data on flow, nutrients and total phytoplankton biomass. The model provides useful figures that are helpful for ecosystem management purposes such as risk assessment.

Light climate for macrophytes

Additional information on the risk associated to a decrease of light availability for macrophytes was obtained evaluating their change in productivity at different light levels. Light intensity was a poor predictor of net primary production for *Ranunculus indundatus* in Ewens Ponds, although, a rise of net primary productivity with light availability was observed up to ~ 120 µmoles m^{-2} s^{-1}. Then the productivity was almost constant increasing light up to ~370 µmoles m^{-2} s^{-1}. This is consistent with previous works on Charophytes where the maximum growth rate was reached at about 110 µmoles m^{-2} s^{-1} (Sand-Jensen and Madsen, 1991). Irradiance saturation point for growth might be relatively variable depending on species. For example, considering nine species of macrophytes in shallow coastal Danish water, Middelboe et al. (2006) observed saturation points ranging between 100 and 200 µmoles m^{-2} s^{-1}. A range even wider was observed by Küster et al. (2004) studying Charophytes in the Baltic sea where the irradiance saturation for growth was estimated to vary from 70 µmoles m^{-2} s^{-1} (*C. baltica*) to 380 µmoles m^{-2} s^{-1} (*C. canescens*). Additional experiments should be carried out assessing primary productivity for other macrophytes species in the Ponds.

Previous works estimated the net primary productivity for dense macroalgae mat (*Chaetomorpha linum*) developing in shallow coastal areas through laboratory experiment being close to 0.096 mg O_2 g^{-1} dw h^{-1} at light intensity of about 120 µmoles m^{-2} s^{-1}(Krause-Jensen et al., 1996). This is similar to the productivity obtained in this study, between 0.06 and 0.08 mg O_2 g^{-1} dw h^{-1}, at analogous light intensities. To convert values from C to O_2 in order to make comparisons an O_2/C molar ratio of 1.2 was adopted (Sand-Jensen and Madsen 1991).

Other studies found higher Charophyte primary productivity than in Ewens Ponds and showed a high variability in net productivity between species. For example, a net daily primary production of about 26 mg mg O_2 g^{-1} dw was estimated for *Chara rudis* in shallow lakes, that is approximately 1 mg O_2 g^{-1} dw h^{-1}(Kufel and Kufel, 2002). In a lotic habitat in South eastern Brazil, maximum net photosynthetic rates measured at 20°C were extremely variable depending on Charophyte species: lower than 1 mg O_2 g^{-1} dw h^{-1} for *Chara guairensis* and up to 8 mg O_2 g^{-1} dw h^{-1} for *Nitella sp.*(Viera and Necchi, 2003). Low productivity in Ewens Ponds could be related to the low environmental temperature (15°C) and to the low P availability. Although, it has to be considered that productivity values are

not directly comparable because different stressors will act in different systems and the net primary production is influenced not just by light intensity but also other factors (e.g. salinity, temperature and nutrients).

5. Conclusions

At present, the ecosystem conditions in Ewens Ponds are mainly controlled by flow. Change in water residence time has been identified as the most important factor controlling future environmental risk. A low residence time of about 9.5 h (flow rate ~1.2 $m^3 s^{-1}$) flushes out of the system pelagic phytoplankton cells that might develop at the surface. Additionally, the high flow rate avoids stratification, even during the summer, maintaining high oxygenation in the water column. In this highly oxygenated environment there is almost no internal release of phosphorus from the sediment.

In combination with flow, another factor controlling environmental risk in the Ponds is indeed phosphorus availability. Nutrient monitoring and bioassay experiments showed that the system is phosphorus limited (e.g. TN:TP ratio of about 420) and nitrogen is extremely high 5.8 ± 0.5 mg L^{-1}. However, sediment flux experiments revealed that there is sufficient total phosphorus available in the sediment to represent a risk. If the flow rate decrease and anoxic conditions develop in the bottom layers, the TP released in 20 days would be able to support pelagic algal growth and reduce light availability for macrophytes of about 50% at their maximum depth of colonization.

The combined effect of TP and flow on pelagic phytoplankton development and consequently on light availability for macrophyte growth, were evaluated by modelling. This allowed estimating TP and flushing rate thresholds to maintain clear water and preserve the macrophyte community. If phytoplankton concentrations are kept under 1 µg Chla L^{-1} there will be almost no changes in light conditions for the macrophytes. Instead, the maximum depth of colonization of macrophytes will be reduced by about one metre if phytoplankton concentration were to reach approximately 5.5 µg Chla L^{-1}. This biomass could be sustained by a relatively low TP concentration of 0.02 mg L^{-1} in the water column, but it would only happen if the residence time will be increased from about 9.5 h to about14 h under the present initial conditions (~100 cells/mL; 0.0135 µg Chla L^{-1}) or to about 12 h with a higher inoculum (e.g. 1000 cells/mL; 0.135 µg Chla L^{-1}).

The risk in Ewens Ponds is also associated with a possible increase of TP groundwater input in the future. It has been estimated that a spike in nutrients entering the Ponds might be observed between 2026 and 2037. This was obtained relating the calculated water age of the Ponds with the Australian trends in fertilizer use in the last decades. This risk might still be mitigated by flow rates, because, even with increasing TP, pelagic phytoplankton growth will be unlikely to occur if the present flushing rate is maintained.

An additional risk for the clear water system conservation is represented by epiphytic algal growth. The development of epiphytic algae might occur at higher flow rate than pelagic algae and it might cover the macrophyte shading the light necessary for their development and compromise ecosystem function. Preliminary experiments have been carried out and results suggest that additional investigation should be conducted to carefully evaluate this aspect. With no limitation of phosphorus the epiphytic algal growth rate could drastically increase up to 5 times the current rates. An increase of epiphytic biomass of about 20 mg Chl-a m^{-2} on macrophyte leaves could decrease light availability by about 20%. If this did occur at the present maximum depth of colonization (about 5 m) macrophyte productivity would be close to zero and they could only survive in shallower areas.

Other wetlands in the South East are undergoing similar pressures as Ewens Ponds, in particular related to nutrient increase. The collated data on nutrients concentration in South East wetlands did not identify main hot-spot areas but showed that commonly nitrogen was highly available, as per a TN:TP ratio >20 in about 70% of the systems. The majority of the systems (>80%) sampled between 2008 and 2014 presented TN concentrations above the Australian and New Zealand fresh water quality guidelines. This reflects the pressure on freshwater systems coming from extensive land use for agriculture and farming in the South East.

The model approach adopted in this work could be easily applied to other South East wetlands, on which there is limited information available. Measuring few primary variables as flow, nutrients and total phytoplankton biomass, it would be possible to obtain useful insights for ecosystem conservation and management.

Considering the conclusions of this work, several recommendations are suggested that should be taken into account for future management plans:

- Develop long term monitoring programs on nutrients and flow in Ewens Ponds: the two main variables controlling environmental risk should be monitored regularly to detect seasonal and long term variations affecting the ecosystem.
- Carry out specific evaluation of the main pressures affecting the system: in particular, it would be meaningful a) investigating which areas are contributing the most as nutrient inputs for groundwater, b) determining the recharge areas and the sources of groundwater entering the Ponds and c) quantifying number of users and water demand that might alter water extraction from the aquifer.
- Initiate additional analysis of the risk associated with epiphytic growth: filamentous epiphytic algae have been developing even at high flow rate and their growth at increased phosphorus concentration might result in severe consequence for the macrophyte development.

When planning any mitigating action for nutrients, managers should take into account that the water residence time in the aquifer is about 26-37 years therefore effects will be only observed in the long term. Stakeholders' awareness on the fragility of the ecosystem should be improved and they should be involved in decisions regarding land management and water allocation.

Appendix A. Collated data base on TP and TN concentration in the South East wetlands.

Table 9. Collated data set: site name, location, years when sampling was conducted, average total nitrogen and total phosphorus values and TN/TP ratio.

Site Name	Easting	Northing	Years	Samples	TN (mg/L)	TP (mg/L)	TN/TP
8 Mile Creek - Ewens Ponds	481981	5790878	2010-2014	4	5.69	0.01	418.79
Avenue Flat-K Drain, near Lucindale	429200	5899275	2009	1	2.65	0.04	67.95
Baker Range Drain, near Mount Burr	462722	5852503	2009	1	3.76	0.19	20.00
Benara Creek, Lake Bonney SE	449246	5811528	2014	1	1.50	0.06	27.33
Big Reedy	438097	5951673	2008-2009-2010	5	4.99	0.60	8.34
Big Telowie	402398	5965295	2008-2009-2010	5	0.98	0.03	31.81
Biscuit Flat Drain, Biscuit Flat	416336	5875592	2009	1	2.61	0.12	22.60
Blackford Drain, near Kingston SE	401796	5927170	2009-2014	2	0.71	0.02	33.90
Blackford Drain, near Mount Scott Conservation Park	412918	5930921	2009-2014	2	0.89	0.03	31.66
Bloomfield	449722	5919817	2010	1	3.16	0.27	11.79
Bool - Drain	469892	5888894	2010	1	4.13	0.06	67.15
Bray Drain, near Lake Hawdon South	410300	5879125	2009-2014	2	1.92	0.06	32.53
Bucks	447123	5804660	2010	1	5.60	0.08	71.79
Bunbury	406866	6001280	2010	1	3.05	0.10	31.09
Butchers Gap Drain, near Butchers Gap Conservation Park	394413	5915593	2009-2014	2	4.05	0.16	26.10
Cortina Lakes	400677	5984673	2008-2009	4	8.07	0.22	37.00
Deep Creek, near Riddock Bay	480650	5789125	2009-2014	2	3.65	0.01	270.56
Didicoolum Drain, near Marcollat Hall	437638	5956312	2014	1	0.82	0.02	37.45
Dine	493110	5910857	2010	1	3.63	0.63	5.75
Drain 31, Millicent	442050	5838550	2009-2014	2	0.66	0.06	10.46
Drain 31, near Millicent	440871	5836328	2009	1	8.70	1.52	5.72
Drain 44, near northern end of Lake Bonney SE	439600	5831850	2009-2014	2	3.81	0.67	5.66
Drain 57, near Snuggery	449372	5830478	2014	1	3.35	0.16	20.65
Drain at Bevilaqua Ford, south from Rendelsham	431150	5840175	2009-2014	2	2.35	0.05	47.42
Drain K, between Lucindale and Robe	424522	5900778	2009-2014	2	1.82	0.03	59.52

Drain L, east from Lake Hawdon North	410725	5890150	2009	1	1.03	0.02	43.62
Drain L, near Robe	394322	5885628	2009-2014	2	1.05	0.02	54.52
Drain L, north from Biscuit Flat	418500	5895400	2009	1	1.12	0.03	44.80
Drain M, near Beachport	415250	5855100	2009-2014	2	1.10	0.03	35.12
Drain M, near Kangaroo Inn	434371	5867328	2009	1	1.48	0.03	59.20
Drain M, north-east from Beachport	426836	5861203	2009-2014	2	0.82	0.03	27.68
Eight Mile Creek, Riddock Bay	482200	5789075	2009-2014	2	6.08	0.01	459.06
Glencoe Drain, south from Kalangadoo	472400	5835900	2009-2014	2	3.57	0.21	17.20
Hacks	474854	5893916	2010	1	3.05	0.08	38.03
Henry Creek	400688	5965085	2008-2009	4	1.61	0.10	16.12
Henry Creek, south from the Tilley Swamp Conservation Park	400450	5963500	2009-2014	2	0.67	0.03	26.59
Hitchcox Main Drain, near Brown Bay	484122	5789678	2009-2014	2	4.10	0.02	197.47
Honans	467280	5823537	2010	1	1.46	0.06	24.01
Jackie White Drain, near Avenue Flat	426543	5916755	2009-2014	2	1.71	0.06	26.76
Jerusalem Creek, east from Port MacDonnell	476646	5789460	2009-2014	2	2.26	0.02	143.17
K-C Road	397368	5984593	2010	1	1.70	0.41	4.19
Kingston Main Drain, south-east from Kingston SE	406801	5909146	2009	1	2.12	0.05	39.26
Lake Bonney	444698	5813354	2010	1	6.49	0.34	18.87
Lake Frome North Drain, near Southend	426100	5845050	2009-2014	2	1.96	0.26	7.67
Lake Frome Outlet Drain, Southend	424300	5841350	2009	1	1.81	0.04	42.09
Lake George	446348	5822691	2010	1	5.03	0.04	115.10
Lake Hawdon South	408061	5879144	2010	1	1.26	0.06	19.53
Little Reedy	438097	5951673	2010	1	4.03	0.89	4.55
Mandina	402671	5984196	2010	1	1.75	0.03	63.18
Marcollat Watercourse at Jip Jip Waterhole, near Jip Jip Conservation Park	425696	5964603	2009-2014	2	5.01	0.48	10.46
Marshes	460235	5835637	2010	1	2.15	0.06	35.19
Morella	381960	5998983	2008-2009	4	2.13	0.06	33.95
Morella site 2	380413	6000704	2010	1	0.90	0.01	91.39
Mosquito Creek, east from Joanna near the SA/Victorian border	495121	5895478	2009	1	4.49	0.99	4.56

Mosquito Creek, Struan	481100	5894650	2009-2014	2	4.60	0.49	9.40
Mullins	424832	5848568	2010	1	0.99	0.02	45.86
Naracoorte Creek, Naracoorte	477100	5910225	2009-2014	2	2.25	0.32	7.08
Naracoorte Creek, west from Naracoorte	474395	5910671	2009	1	0.50	0.19	2.58
Naracoorte Creek, western edge of Naracoorte	475900	5909900	2009	1	1.49	0.41	3.64
Narrow Neck Drain, near Rendelsham	428897	5843584	2009-2014	2	1.70	0.03	64.30
Pelican Point Drain, near Carpenter Rocks	450950	5801450	2009	1	5.42	0.79	6.87
Picanninie Ponds	491235	5802885	2010	1	2.16	0.02	120.00
Piccaninnie Blue Lake Outlet, Piccaninnie Ponds Conservation Park	494600	5788300	2009-2014	2	2.18	0.01	181.88
Picks Swamp Outlet Drain, west from Piccaninnie Ponds Conservation Park	490695	5789095	2009-2010-2014	3	1.99	0.02	100.67
Reedy Creek Drain, near Mount Burr	458072	5848228	2009	1	1.38	0.21	6.70
Reedy Creek-K Drain, between Robe and Lucindale	422125	5897300	2009	1	5.21	0.09	60.58
Reedy Creek-Mount Hope Drain, Near Hogan's Lane Regulator	432566	5854670	2014	1	0.89	0.02	42.38
Reedy Creek-Mount Hope Drain, near Mullins Swamp	425400	5848350	2009-2014	2	1.71	0.06	26.76
Reedy Creek-Wilmot Drain, near Greenways	431075	5872075	2009	1	1.22	0.03	43.39
Reedy Creek-Wilmot Drain, near Reedy Creek Conservation Park	431197	5872253	2009	1	3.06	0.14	22.01
Rocky	410988	5952186	2010	1	1.28	0.02	72.73
Rocky Swamp(Parakie)	411181	5952188	2008-2009	4	1.35	0.07	20.45
Seymour-Robertson Drain, Bool Lagoon	474423	5890061	2009	1	0.99	0.01	198.00
Smiths	411737	5950637	2010	1	1.45	0.02	72.14
Snuggery	410753	5953252	2010	1	1.27	0.02	64.80
Stony Creek, near eastern edge of Lake Bonney SE	443722	5826878	2009-2014	2	1.71	0.06	26.76
Sutherland Drain, near Beachport	417700	5853875	2009-2014	2	2.77	0.08	34.39
Taratap	399776	5954948	2008-2009	4	2.79	0.16	17.71
Taratap Drain, south from Tilley Swamp	397808	5963693	2009	1	0.73	0.02	45.63

Conservation Park							
Tatiara Creek, Bordertown	479815	5981860	2009	1	1.34	0.11	12.76
Tatiara Creek, east from Bordertown	476711	5981155	2009-2014	2	1.77	0.64	2.75
Unnamed Drain, near Tilley Swamp	396064	5970054	2009	1	2.72	0.06	43.87
Willalooka North	437874	5952583	2010	1	3.78	0.28	13.36
Willalooka South	438409	5950301	2010	1	3.59	0.81	4.42
Wilmot Drain, near Earth Quake Springs	421500	5886550	2009	1	3.35	0.02	186.11

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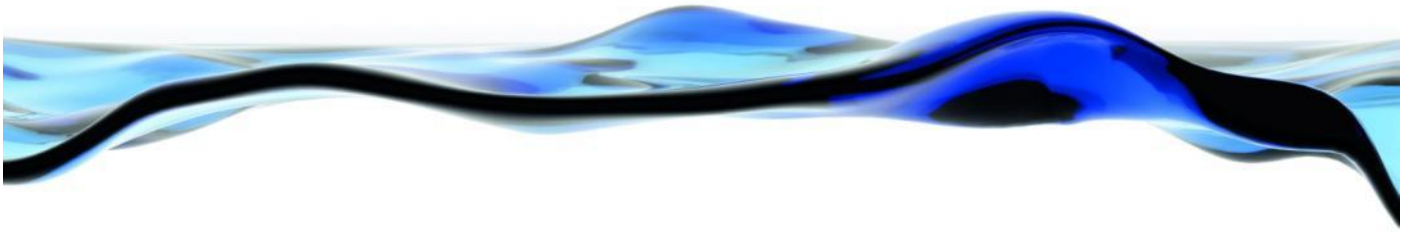

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