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TITLE OF THESIS :

THE DETERMINATION OF THE BIO- LIMITING FACTOR/S IN THE CONTROL OF ALGAL BLOOMS IN THE MARIBYRNONG RIVER ESTUARY.

COURSE: ENVIRONMENTAL BIOLOGY (HONOURS)

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ABSTRACT

A 3.5 kilometer stretch of the Maribyrnong river estuary was investigated in 1994 in order to monitor and record a <u>possible</u> outbreak of a phytoplankton bloom.

Nutrient analyses, physio-chemical monitoring, productivity experiments and kinetic nutrient experiments of the and a part of a Maribyrnong river estuary were assessed on weekly or fortnightly intervals from March 1994 to October 1994. An incubation method which considered tidal influences and column mixing along with variable light regimes was used to determine community primary productivity. Changes in dissolved oxygen concentrations in light/dark bottles were used as the indicator of community primary productivity which ranged from 7 mg C/L/H to 112 mg C/L/H. Pulses of Nitrogen in the form Ammonia which ranged from 2 mg NH4/L/H to 10 mg NH4/L/H increased community productivity which was expressed as Gross Photsypthesis in mg C/L/H in

surface layers.

Pulses of ammonium in conjunction with light and turbidity limited primary production in this shallow, partially stratified estuary.

The shallow river estuary was partially stratified into three layers. Each layer from surface to bottom had their own unique individual signature on temperature, conductivity, dissolved oxygen, pH and nutrient concentrations.

Variations in the physio-chemical parameters and nutrient concentrations were evident between the four sampling stations.

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INTRODUCTION

Phytoplankton are the primary producers of the worlds oceans and inland water bodies (Whittaker & Likens ,1975). Described as "floating plant life", these microscopic algae have an average size range from 0.2 to 200 µm (Clayton & King, 1994). Phytoplankton distribution within the water column is governed by light penetration (Cochlan, Price, Harrison, 1991). Turbidity and light availability are the limiting factors for phytoplankton primary productivity in most Australian estuaries.

Phytoplankton diversity is immense, encompassing the silicawalled diatoms (Bacillariophyta), the dinoflagellates (Dinophyta), the green flagellates (Chlorophyta and Euglenophyta), the golden- brown flagellates (Prymnesiophyta, Cryptophyta and Chrysophyta) and minute Cyanobacteria found distributed in almost all regions of the aquatic environment (Hostetter & Stoermer, 1971; Taylor, 1976; Jeffrey, 1976; Hallegraeff, 1980). Phytoplankton population explosions in freshwaters are primarily species of cyanobacteria whilst in seawater species of diatoms and dinoflagellates can cause the development of an algal bloom. The nutrient-limited phytoplankton communities, utilize the increased input-of dissolved nutrients into maximizing photosynthetic and biomass production rates. During the day, there is an increase in dissolved oxygen concentrations within the water body through increased photosynthetic rates (Lancelot, Mathot, 1987) along with a discolourization of the water with chlorophyll a.

The retardation of nutrients available for biomass production entering the estuarine environment along with other factors such as self shading, build up of toxic waste products and insufficient oxygen at night to support respiration, leads to the collapse of the bloom. The bacteria which graze on the decomposing algal biomass, require oxygen for survival. Such a process, deoxygenates the water body and makes it anoxic.

The drop in dissolved oxygen concentrations over an extended period of time, leads to the change in species diversity and abundance values.

Algal blooms have disastrous impacts on the economic, aesthetic and recreational values of the region. The management of nutrient discharges to estuarine environments is crucial to the stoppage of harmful algal blooms (Hallegraeff, 1992).

Light penetration is no longer a limiting parameter.

FACTORS CONTROLLING PHYTOPLANKTON POPULATIONS IN AQUATIC SYSTEMS.

Phytoplankton abundance of estuaries are generally contolided by a variety of interacting factors (Christian, Boyer, Stanley, 1991). Nutrients, available sunlight, turbidity, pH, salinity gradients, temperature and oxygen availability govern the response of individual phytoplankton species prior to population explosions (Anderson & Stolzenbach, 1985; Collos, 1986; Sciandra & Amara, 1994; Rudek et.al, 1991). Individual species of phytoplankton have been found to respond differently to the parameters mentioned above (Eppley et.al, 1968; Heaney & Eppley, 1981). Individual responses of phytoplankton species to one factor can be influenced by others (Heaney & Eppley, 1981). For instance, diatom blooms in seawater is controlled by the availability of inorganic nutrients such as nitrate, phosphate and silicate in conjunction with the availability of sunlight and favourable hydrological conditions (Clayton & King, 1994). It is generally known that estuaries have high nutrient availability, especially at the end of winter (Fichez et.al, 1992). Phytoplankton blooms tend to develop in early spring or summer and is light dependent (Ryther & Dunstan, 1972; Thayer, 1974; Boynton et.al, 1982 . In clear, nutrient rich waters, bloom development can be expected on warm, clear days where light penetration is no longer a limiting parameter.

In turbid estuaries, productivity of phytoplankton populations is related to critical depth (Sverdrup, 1953; Grobbelaar, 1985). If there is a fluctuating light regime, phytoplankton move rapidly up and down the water column, photosynthesizing in the light regions and respiring in the dark. For example, the cyanobacteria which have gas vacuoles, regulate their buoyancy and their position in the water column. This migration of phytoplankton vertically , helps to maintain primary productivity (Yoder & Bishop, 1985). As a consequence, phytoplankton populations are maintained hence allowing time for bloom development.

Nutrient availability and light penetration depth tend to play a key role in the development of algal blooms.

The following sections will introduce and discuss the various key factors which play a major role in the control of algal blooms.

FACTORS RESPONSIBLE FOR THE DISTRIBUTION AND SEASONAL

SUCCESSION OF PHYTOPLANKTON (LUND, 1965).

1) PHYSICAL FACTORS:

 a) TEMPERATURE - Within growth range; affects growth rates, nutrient demands and on enzymatic processes; thermal stratification;

b) LIGHT - Lenght and brightness of day; inhibitory or lethal
light intesities; IR absorption; UV effects;

c) WATER MOVEMENTS - Allows vertical and horizontal migrations of phytoplankton populations;

d) DENSITY DISTRIBUTION - Effects of salinity, temperature, metabolism, in relation to the sinking or rising of the phytoplankton.

2) CHEMICAL FACTORS:

a) INORGANIC SUBSTANCES - Iron; phosphates; silicates;
sulphides; nitrogenous compounds; trace metals; oxygen; ionic
ratios and salinity; pH;

b) ORGANIC SUBSTANCES - Vitamins; acids; chelates; humus; natural chelates and most extracellular compounds. 3) BIOLOGICAL FACTORS:

a) Inhibitory or stimulatory substances;

b) Intrinsic factors;

c) Life histories;

d) Symbiotic relationships;

e) Grazing - Qualitative and Quantitative effects;

f) Parasitism by viruses and grazing bacterioplankton and zooplankton;

g) Rate of sinking, weight variations amongs phytoplankton.

According to studies conducted by Anderson and Stolzenbach (1985), individual species of phytoplankton respond differently to these parameters. Individual phytoplankton responses to one factor is influenced by others (Heaney & Eppley, 1981). As stated earlier, the iniation of diatom blooms in seawater is contolled by the availability of inorganic nutrients whilst cyanobacteria blooms in freshwater is initated by the availability of organic nutrients in the form of sewage.

In European waters the spring diatom blooms are initiated by increased illumination and stratification of surface waters following deep winter mixing (Sverdrup, 1953). Such a response by diatoms in seawater illustrates the point that individual phytoplankton species responses to one factor is influenced by others (Heaney & Eppley, 1981).

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Nutrient distribution within an estuary is determined by a variety of factors (Christian et.al, 1991). Nutrient concentrations at the head and mouth set initial conditions. The magnitude of water entering the estuary at high tide and the concentrations of nutrients within that influx of water determines the extent of nutrient additions. Within the body of the estuary itself, physical, chemical and biological processes determine nutrient concentrations

(Imberger et.al, 1983; Peterson et.al, 1985). Flushing and vertical mixing act similarly on dissolved nutrients or on particulate forms.

According to Eppley et.al (1979), phytoplankton growth is driven by the addition of nitrate in the euphotic zone. Further studies have also indicated that many regenerative processes within the euphotic zone (from microbial decomposition and animal excretions) result in the formation of ammonium ions and urea which makes up about 50% of the total nitrogen assimilated (McCarthy and Goldman, 1979). Therefore, it can be stated that the presence of inorganic nutrients such as nitrate and phosphate does not imply that the water body is fertile because one must consider the contributions of nutrients by regenerative processes (ie fluxes not amounts are important).

Studies carried out in Californian coastal waters indicate that phytoplankton prefer ammonium ions and urea. Therefore, it seems to be that phytoplankton population dynamics are limited or controlled by the concentrations of ammonium ions and urea in the water body for coastal/marine waters. Phosphorus is the other major biolimiting nutrient for phytoplankton growth (O'Neill, 1990). However, low inorganic phosphate concentrations in the water body may not limit phytoplankton growth (Clayton, King, 1993). The presence of adequate surface and extracellular phosphatases in the plant cells and associated bacteria and sufficient organic phosphates in the surrounding water medium, can satisfy phosphorous requirements (Admiraal, 1977) for the photosynthetic phytoplankton populations. Other minor but sometimes limiting nutrients are silicate, trace metals such as iron and manganese and vitamins (Eppley, 1977).

According to McCarthy and Goldman (1979), phytoplankton populations need only be exposed to intermittent pulses of nitrogen in order to aquire their daily ration. Such pulses can be in the form of waste discharges (municipal, urban, industrial and agricultural) and or bacterial decomposition of detritus and dissolved organic matter.

In stratified waters, the surface layers are generally nutrient depleted in comparison to middle and bottom layers (Holmes et.al, 1967; Eppley et.al, 1968; Eppley & Harrison, 1975). This is due to the higher photosynthetic rates by phytoplankton populations in surface waters due to a higher surface irradiance level as compared to lower depths of the estuary. Photoinhibition at greater depths results in the decrease in primary productivity due the lack of sufficient sunlight to support biomass production and physiological and morphological functions. However, low dissolved inorganic concentrations do not necessarily mean that phytoplankton growth is under strong nutrient control, since the pools may be rapidly renewed through remineralization of nitrogen and phosphorus by heterotrophs as well as through mixing mechanisms (Harrison & Gilbert, 1983).

When sufficient light is present, the availability of biolimiting nutrients such as nitrogen and phosphorus often limit primary productivity within estuaries. The determination of the limiting nutrient in estuaries is accomplished by comparing the ratio of nitrogen to phosphorous within the system (Howarth, 1988), within constraints of low concentrations.

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NITROGEN

It is generally agreed that primary production in estuarine environments is dependent on nutrient inputs and light supply (Fichez et.al, 1992; Collos, 1986; Sciandra & Amara, 1994). Studies have indicated that in an estuarine environment, nitrogen is the biolimiting nutrient for phytoplankton production (Ryther & Dunstan, 1972; Thayer, 1974; Boynton et.al, 1982). Consequently, uptake kinetic rates of nitrogenous compounds by phytoplankton species within estuaries have long been an interest to physiologists and ecologists (McCarthy, 1981; Cembella et.al, 1984). In an estuarine environment, the uptake of nitrogenous compounds by phytoplankton is controlled by the availability of the nutrient (MacIsaac & Dugdale, 1969; Probyn, 1985) and to the photosynthetic flux density (Priscu, 1984). In turbid waters, the photosynthetic flux density is generally low as compared to clear waters. As a consequence, besides fluctuations in daily light exposure, turbidity plays a major role in determining uptake rates of nitrogenous compounds by phytoplankton species (Ryther & Dunstan, 1972; Thayer, 1974; Boynton et.al, 1982).

Dinitrogen present in the atmosphere has a low chemical reactivity and is not directly available to photosynthetic organisms such as phytoplankton except for the nitrogen fixing cyanobacteria. Consequently, the lack of available nitrogen is often a major limiting factor for phytoplankton productivity (O'Neill, 1990; Brock, 1979). It is well known that most marine phytoplankton communities rely heavily on reduced nitrogen sources such as ammonium and urea which are regenerated from organic compounds within the photic layer (Sorensson & Sahlsten, 1987). The nitrogen for new production would be nitrate and nitrogen gas whilst regenerated forms would be ammonium and urea (Larsson & Hagstrom, 1982).In order for phytoplankton to utilize available nitrogen pools for biomass productivity, nitrogen from the atmosphere needs to be converted into forms that can be readily utilized.

The three principle stages of the nitrogen cycle are : (1) ammonification: Much of the nitrogen found in waters is a result of the decomposition of organic materials and is in the forms of proteins, amino acids, nucleic acids and nucleotides. These compounds are rapidly deaminated into ammonium by the action of bacteria and fungi. Microorganisms such as phytoplankton use the proteins and amino acids to build their own needed proteins and release the excess in the form of ammonia or ammonium ion. This process is termed ammonification.

(2) Nitrification: The oxidation of ammonia by a number of bacterial species is an energy yielding process. The energy released is used by these bacteria as their primary energy source allowing them to fix carbon dioxide into cellular carbon. This process is termed nitrification. During this stage, ammonia is oxidized to nitrite which in turn is oxidized to nitrate. (3) Denitrification : Is the conversion of nitrates back into nitrogen gas via reductive processes.

Estuaries are referred to as sinks for dissolved inorganic nitrogen and phosphorous and act as filters for downstream coastal systems (Fisher et.al, 1988a). The dissolved inorganic nitrogenous compounds are utilized by the phytoplankton and incorporatrd into particulate fractions. This can eventuate into population explosions of phytoplankton communities if the influx of nutrients are at a dangerously high level. Phytoplankton species assimilate different forms of inorganic nitrogen such as ammonia, nitrate and nitrite (Sciandra & Amara, 1994). Nitrite is known to be toxic to phytoplankton if present in high concentrations (Laanbroek et.al, 1985). For this reason, phytoplankton prefer to utilize ammonia and nitrate for biomass productivity and metabolism. Concentrations of ammonia, nitrate and nitrite tend to fluctuate on an annual basis within estuaries. This is due to seasonal patterns associated with biological activity which are modified by rainfall and flow (Christian et.al, 1991). If present in sufficiently high concentrations, nitrate is often rapidly depleted (Fisher et.al, 1988b). Ammonia is then the most utilized compound for phytoplankton productivity. Sediment denitrification is an important pathway for the loss of fixed, available nitrogen from estuaries (Nowicki, 1994).

Loss of nitrogen through sediment denitrification, adsorption, and sedimentation can contribute to the nitrogen limitation in estuaries (Ryther & Dunstan, 1971; Howarth, 1988) where nitrogen fixation is relatively unimportant.

Denitrification rates within estuaries appear to be stimulated by increases in external nutrient loading (Seitzinger &Nixon, 1985; Seitzinger, 1988).

If such is the case, then the process of denitrification on a sufficiently high rate can cause nitrogen limitation within the estuary itself (Crawford & Purdie, 1992). This process can itself make nitrogen the biolimiting nutrient in estuaries. Generally, estuaries have a higher nutrient load at the end of winter due to decrease in photosynthetic rates. This means that in early spring to summer, phytoplankton blooms are primarly light dependent (Lancelot & Mathot, 1987). With an adequate supply of nitrogenous compounds present in the water column, phytoplankton communities can undergo a population explosion if physical, chemical and biological parameters are at their optimum.

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PHOSPHORUS

Like nitrogen, phosphorus is also considered to be a common biolimiting nutrient to phytoplankton communities (Sorokin, 1985; Collos, 1986; Christian et.al, 1991; Aksnes & Egge, 1991). Phytoplankton growth rates and the total productivity is determined by phosphorus flux rates as well as concentrations present (Dugdale & Goering, 1967). Therefore it is not adequate to just rely on dissolved inorganic nutrient concentrations in the estuary but also take into account flow rates of the nutrient itself. For instance, inorganic phosphorus concentrations of 0 .2 to 0 .5 ug/L at surface layers are usually in regions of upwelling and not in oligotrophic waters where the rates of primary production can 😓 2 to 3 orders of magnitude lower (Rigler, 1956). Therefore, estimation of nutrient flow rates can/provide valuable information into the relationship between nutrient and primary productivity.

In almost all cases, phosphorus is present as orthophosphate in estuarine waters (Mallin & Pearl & Rudek,1991). The orthophosphate is bound to insoluble inorganic compounds or as a component of organic molecules.

Phosphorus is an essential constituent of ATP, ADP, AMP, DNA and RNA. This means that phosphorus is an essential nutrient and can be often a biolimiting nutrient in aquatic systems. The low solubility of the inorganic compound limits its availability as a nutrient (Fourgurean & Noves & Aieman, 1992) and is therefore found in sufficent high concentrations in sediment beds, bound to other compounds. The major causes of increased dissolved phosphorus loadings in estuaries is due to sewage disposal and fertilizer inputs in the form of super phosphates. Phosphorus loadings entering estuaries are rapidly immobilized by inorganic compound formations and as stated previously are not readily available for phytoplankton uptake. Sediment analysis for phosphorous concentrations are routinely undertaken, and tend to be higher than overlying waters (Seitzinger, 1991).

PHYSIO-CHEMICAL PARAMETERS

The vertical migration of phytoplankton populations in marine and freshwaters is regulated by light intensities, temperature, salinity gradients, dissolved oxygen concentrations and turbidity (Fichez et.al, 1992; Anderson & Stolzenbach, 1985). It is well understood that phytoplankton populations in estuaries are subjected to a fluctuating nutrient regime. Due to the build up of nutrient concentrations over the winter period, phytoplankton blooms generally develop in early spring or summer (Ryther & Dunstan, 1972; Boynton et.al, 1982). Phytoplankton blooms during such periods can be said to be light dependent.

Turbid estuaries are often referred to as being unproductive ecosystems and that primary production tends to be less than the clearer waters like the ocean (Cadee & Hegeman, 1974; Milliman & Boyle, 1975).

The greater the turbidity of the water body, the lower the penetration depth of sunlight. This in itself is a limiting factor in phytoplankton productivity. The critical depth (maximum light penetration depth) in estuaries regulates phytoplankton productivity and is dependent on both mixing processes and light penetration.

According to Wofsy (1983), Cole & Cloern (1984), productivity is possible in turbid waters such as in estuaries with phytoplankton species such as cyanobacteria migrating vertically through a fluctuating light regime, photosynthesising in the light and only respiring in the dark. A majority of Australian estuaries are turbid. Due to this, light is more likely to be a biolimiting factor for primary productivity. If the phytoplankton communities are maintained within the water columns via suspended sediments, turbidity tends to allow time for bloom development (Postma, 1980; Mclusky, 1989). This means that if turbidity is decreased in Australian estuaries, phytoplankton populations are exposed to higher levels of irradiance which can lead to explosions in population numbers.

The sediments of estuaries are an important source of recycled nutrients such as nitrogen and phosphorous for phytoplankton production. According to Meyer, 1979 & Newbold, 1987, increases in pH values can make these nutrients bio-available for phytoplankton populations. This resuspension and mobilization of limiting nutrients can lead to phytoplankton population explosions. A study of the Potomac estuary in America, found that pH played a major role in the release of phosphorous from the estuarine sediment bed. A quantitative study undertaken here suggested that high pH values mobilized phosphorus from the sediment bed which was at an adequate concentration to initiate and propogate bloom development (Clayton & King, 1994). Thermal stratification of estuaries allows the distribution of phytoplankton species vertically. Vertical migration within an estuary by phytoplankton has a number of advantages. The most common advantage is that of access to nutrients located below the depleted surface waters (Holmes et.al, 1967; Eppley et.al, 1968). Thermal stratification of shallow water estuaries can therefore be said to allow for the vertical and horizontal movement of phytoplankton populations (on thermal currents, surface currents, salinity gradients and tidal influxes) in response to environmental fluctuations.

Clearly it can be seen that a number of contributing parameters are responsible for the development of phytoplankton blooms. An interaction of the chemical, physical and biological parameters mentioned above, governs the population explosions and or stabililty of phytoplankton species in estuarine environments.

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TOXIC PHYTOPLANKTON BLOOMS

Over the years, the relative intensities, distribution and frequency of harmful algal blooms in Australian aquatic systems has increased (Hallegraeff, 1992). However, it must be clearly stated that not all algal blooms are toxic. The following section will be sub-divided into two categories; toxic and non toxic algal blooms.

a) Toxic algal blooms: The accumulation of surface microalgae in coastal systems are collectively known as "red tides", since they give the water a red appearance. Red tides are generally associated with dinoflagellate species, however, cyanobacteria species as well as raphidophyte species may also contribute to this biological phenomenon (Olsgard, 1993; Nielson, 1993; Rao et.al,1993).

Approximately 30 out of the 1500 identified species of Dinoflagellates are known to produce toxic compounds. The consumption of these toxic compounds by filter feeders such as mussels, scallops and oysters, initiates the biomagnification process of the toxin up the food chain. The consumption of contaminated sea food by humans can cause severe gastrointestinal and neurological illnesses. The unarmoured toxic dinoflagellate " Gymnodinium catenatum", was responsible for contaminating the shellfish industry of Tasmania in 1986, 1987 and 1988 (Clayton & King, 1994). "Pyrodinium bahamense" is a toxic dinoflagellate that causes fatal cases of paralytic shellfish poisoning (PSP). Blooms of " Dinophysis fortii" have been known to cause diarrhetic shellfish poisoning (DSP). Ciguatera fish poisoning is caused by toxic dinoflagellates such as "Gambierdiscus toxicus", "Ostreopsis siamensis" and "Prococentrum lima" (Olsgard, 1993; Nielson, 1993; Clayton & King, 1994).

The contamination of shellfish products with algal toxins has also been recorded for blooms of diatom species such as "Rhizosolenia chunii" (Lukatelich & McComb, 1986). The contamination of water resources and the poisoning of cattle and wildlife by "Nodularia spumigena" and "Anabaena circinalis" is a present problem being faced by the Murray-Darling river in New South Wales and in the Peel Harvey Estuary in Western Australia (Hallegraeff, 1992).

The above mentioned toxic species of phytoplankton are capable of causing severe economic, environmental, recreational and health related problems. Toxins excreted by the above mentioned species that are responsible for DSP and PSP can result in death.

Therefore the immediate arrest of phytoplankton blooms on a world wild basis is of primary importance, due to the health, economic, aesthetic and recreational implications involved.

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NON-TOXIC PHYTOPLANKTON BLOOMS

Certain species of dinoflagellates have been known to cause water discolourisations. The dinoflagellate "Scrippsiella trochoidea" has been known to cause red-brown sea water discolourisation (Hallegraeff, 1992). Other dinoflagellates such as "Gymnodinium sanguineum" and "Gonyaulax polygramma" have also been known to cause water discolourisations. Prymnesiophyte and dinoflagellate species have also been known to damage fish gills either by increased sea water viscosity due to secretions of algal mucilages or through the production of substances which affect cell permeability (Hallegraeff, 1992).

The majority of non-toxic phytoplankton species when develop into bloom proportions can cause water discolourisations. The bloom that developed in the Maribyrnong estuary in 1992, involved the discolorization of the estuary due to the population explosion of the photosynthetic phytoflagellate "Fibrocapsica japonica" caused by the influx of nutrients via a sewer collapse (Melbourne Water, 1994).

These non toxic phytoplankton species are a threat to the recreational industry but no evidence is yet to be cited on any harmful effects on humans or marine life (Hawser et.al, 1991).

LIMITATIONS OF LITERATURE CITED

The vast majority of studies undertaken on a global scale in respect to algal blooms, has been primarily investigated overseas, especially in the Northern hemisphere. The extent of research undertaken in Australian waters to date has been done mostly in New South Wales and Western Australia. Bloom developments in these two states have been on a much more regular occurrence as compared to Victorian waters (Hallegraeff, 1992; Clayton and King, 1994). The Peel-Harvey estuary in Western Australia and the Murray-Darling river system in New South Wales have been subjected to regular algal blooms. The literature available for algal blooms within the Australian sub-continent, cites these two states regularly. Other regions of algal bloom investigations as cited by Hallegraeff 1984; 1987 & 1992 have been as follows;

1) West lakes in South Australia;

2) Port Phillip Bay in Victoria;

- 3) Gippsland lakes in Victoria;
- 4) Lake Macquarie in New South Wales;

5) Lake Illawara and Batemans Bay in New South Wales;

6) Moreton Bay in Queensland;

7) Hawksbury and Parramatta rivers in New South Wales.

The Maribyrnong river estuary in Victoria has had previous occurrences of blooms developments (Melbourne Water, 1994). However, Morumentation of the bloom has been neglected and or minimal. An intensive physio-chemical study of the estuary was first undertaken by Melbourne Water in 1992 due to a sewer collapse which led to a population explosion of the photosynthetic phytoflagellate "Fibrocapsica japonica". Prior to this research, sightings and occurrences in the river estuary has resulted in very limited attention being given by the Victorian Government and its associated environmental organizations.

As a consequence of the limited volume of literature available for Victorian water ways, this sampling program will provide valuable information and data for the Maribyrnong river estuary in Victoria. As a result of such research, a management strategy for the control of algal blooms can be provided. The physio-chemical readings and nutrient analyses of the estuary, will provide a valuable insight into the dynamics and seasonal fluctuations of the estuary and determine the limiting parameter/s for bloom development.

STATEMENT OF AIMS

1) Construct a nutrient and physio-chemical profile of the estuary from surface to bottom through the summer-autumn months of 1994;

2) Monitor the estuary for possible outbreaks of algal blooms and outline dynamics of bloom development;

3) Determine the contributing factors which lead to the development of an algal bloom and especially identify "problem nutrients";

4) To propose and implement a management strategy in the control of algal blooms in the Maribyrnong river estuary.

RESEARCH PLAN

(1) Routine monitoring of river:

(a) Physio-chemical readings were undertaken on weekly or fortnightly intervals. Samples were always taken at high tide in the middle of the day. Readings were taken at three depths per site in the middle of the river from a boat. The parameters measured were pH, conductivity, dissolved oxygen and water temperature.

(b) Nutrient analyses were conducted at three depths per site in duplicate. Water samples were taken from each depth using a vertical Van Dorn water sampler. The nutrients that were measured were: Total Phosphorus, Ammonia, Nitrate + Nitrite, Orthophosphate and Total Oxidized Nitrogen.

(2) INCUBATION EXPERIMENTS:

(a) Time course for productivity studies at one depth;

(b) Productivity studies at surface, middle and bottom layers using light and dark bottles to measure photosynthetic and respiration rates respectively;

(c) Amendment incubation experiments were conducted on both nitrate and ammonia as the nutrient source at one concentration at one depth using light and dark bottles;

(d) Kinetic experiments using nitrate and ammonia over a 5-8 concentration range at one depth using light and dark bottles in order to measure community productivity.

HYPOTHESIS

The following hypothesis were drawn about the Maribyrnong river estuary;

 The Maribyrnong river estuary will be a stratified estuary;
Nutrient concentrations will increase with depth from surface to bottom;

3) An algal bloom will most probably take place during the summer months from March to June due to higher irradiance levels and warmer water conditions;

4) Nutrient concentrations will peak in the winter months from July to August due to decrease in irradiance levels thus affecting overall rate of photosynthesis and nutrient assimilation rates by phytoplankton;

5) Nutrient concentrations will be higher at sites 3 and 4 as compared to sites 1 and 2 due to the presence of stormwater drain and overflow sewer pipe constructed by Melbourne Water between site 2 and site 3 ;

6) Community productivity will be greatest at the surface layers in all four sampling stations as compared to middle and bottom layers;

7) Nitrogen will be the biolimiting nutrient in the Maribyrnong river estuary.

METHODS

DESCRIPTION OF MARIBYRNONG RIVER.

The Maribyrnong river has its source about 70 kms northwest of Melbourne and discharges its volume into the Yarra river about 3 kms from the mouth of Port Phillip Bay. Major tributaries such as Deep creek, Emu creek, and Jacksons creek discharge their loads into the river. The estuarine region of the river extends approximately 12 kms from the confluence with the Yarra to the Canning street bridge in the suburb of Maribyrnong.

The river basin is used primarily for agriculture whilst the remaining areas of forest are within the Macedon and Cobaw ranges. In its lower regions, the river is used for intensive market gardening whilst the last 15 kms comprises urban areas. Urban and industrial development constitutes approximately 16 kms of the estuary. The last 0.5 kms is dredged and is therefore much deeper than other regions.

The average depth of the estuarine region of the river is approximately 3 meters deep but the dredged section is 10 meters deep.

Being a tidal estuary, its physio-chemical parameters fluctuate on a daily basis. Spatial and temporal variations in physio-chemical parameters are influenced by the mean influx of saline water from Port Phillip Bay during high tides. Retention times within the estuarine region of the river is approximately seven days.



Hobsons Bay

Fig. 1. Map of Yarra and Maribyrnong estuaries, showing location of sampling stations and of water-level recorders and average annual freshwater inflows (\rightarrow). Sampling stations 3 and 6 were manned on 1–3 June, and 9 on 3 June only; 1, 3–8 and 10 on 12–13 July, and 2 on 12 July and 9 on 13 July only; and 3, 6 and 7 on 24–25 August.

The Yarra and Maribyrnong Rivers represent by far the greatest source of freshwater input to Port Phillip Bay and drain 56% (or 5460 km²) of the total catchment area (Anon. 1973). The main river inflows to the Yarra estuary are summarized in Fig. 1. The Yarra at Dight's Falls contributes an estimated annual average flow of $6 \cdot 6 \times 10^8$ m³, which is almost three-quarters of the total annual inflow to Hobson's Bay (Anon. 1973). The Maribyrnong is the other main contributor $(1 \cdot 8 \times 10^8 \text{ m}^3 \text{ or } \sim 20\%)$, with additional small contributions from Gardiners Creek $(3 \cdot 4 \times 10^7 \text{ m}^3, \sim 4\%)$ and Moonee Ponds Creek $(2 \cdot 1 \times 10^7 \text{ m}^3, \sim 2\%)$. There are no estimates available for the storm-water inflows discharging directly to the estuary.

The flow patterns in both the Yarra and Maribyrnong Rivers are quite seasonal, with small flows generally recorded in summer and autumn and higher flows in winter and spring. This is shown in Fig. 2 which contains the mean daily flows recorded in the Yarra River at Banksia Street during 1976 and 1977, and in the Maribyrnong River at Brimbank (near Keilor) during 1977; the three survey periods are also marked on this figure.

DESCRIPTION OF SITE AREA.

The 1992 sewer collapse at Plantation Street, Maribyrnong resulted in the development of an algal bloom of the photosynthetic phytoflagellate "Fibrocapsica japonica". Due to this reason, the four sites chosen for the Honours research program were situated along this stretch of the estuarine section of the river.

Four sampling stations along Chifley drive in the suburb of Maribyrnong were chosen to conduct physio-chemical and nutrient analyses. The total distance of study sites was approximately 3.5 kms. The four sampling station locations were as follows;

STATION 1: Anglers Tavern Bridge on Maribyrnong road;

STATION 2: Emergency relief structure;

STATION 3: Major storm water drain;

STATION 4: Bridge walkover.

Station 1 was closest to Port Phillip Bay, whilst station 4 was the furtherest.

** The attached maps indicate sampling station locations. Physio-chemical readings were conducted on weekly or fortnightly intervals at high tide in the middle of the day from a boat.



MATERIALS AND METHODS FOR ROUTINE MONITORING OF RIVER Physio-chemical readings for pH, conductivity (mS/cm), temperature (degrees Celcius) and dissolved oxygen concentrations (ppm) were conducted at high tide in the middle of the day from a boat. In Situ field readings were undertaken from March 1994 upto October 1994. The 90 FL Microprocessor Field Analyzer which was supplied by Melbourne Water was used to conduct physio-chemical readings at three depths per site from the middle of the river.

Calibration of the field instrument for all four parameters was done after each run to maintain accuracy in readings. The instrument was calibrated according to the 90 FL Microprocessor Field Analyzer users manual.

The dissolved oxygen probe was transported in a moist, plastic capsule in order to avoid damage to the enclosed membrane. The probes were allowed to settle at each depth for a certain fixed period of time prior to taking readings. This allowed for stabilization of the probes at that particular depth.

MATERIALS AND METHODS FOR NUTRIENT ANALYSES

Water samples were taken in duplicate at three depths per site using a vertical Van Dorn water sampler. The Van Dorn water sampler is an open cylinder of known capacity that is let down into the water and is automatically closed at both ends by a metal "messenger" which slides down the cable. The enclosed water is under pressure so that water from other layers cannot enter during its return journey to the surface. Water samples from the Van Dorn water sampler were immediately transferred to clean, opague polypropelene bottles for

transportation back to the laboratory for analyses. The water samples were kept out of direct sunlight in order to avoid photo-degradation. The samples were filled to the top in order to reduce the rate of oxidation of the water sample (Wetzel & Likens, 1993).

The samples were transported back to the laboratory on ice and refrigerated at -14 degrees celcius as recommended by Valcarrel & Luque de Castro, (1988).

The Aquatec 5400 flow injection analyzer was used to measure concentrations of phosphorus, nitrogen, ammonia,

orthophosphate , nitrate and nitrite.

Nutrient determinations in water samples were in accordance to standard recommended methods by Aquatec 1990.

A Cadmium reductor was used during the determination of nitrate + nitrite as well as for total oxidized nitrogen.

Prior to nutrient analyses, all samples were filtered through a 0.45 um membrane to remove any suspended solid particles. Nutrient samples for nitrogen and phosphorus concentrations had to undergo a variation in sample preparation which was as follows;

SAMPLE PREPARATION FOR NITROGEN AND PHOSPHOROUS

15 ml of the sample was pipetted into a centrifuge tube;
3 ml of Potassium Persulphate was added to sample;
3) Heated for 30 minutes in an autoclave (digested);
4) Cooled and analyzed.

The reproducibility of the Aquatec 5400 flow injection analyzer is usually better than 1% r.s.d. (Aquatec Technical Specifications, 1990).
MATERIALS AND METHODOLOGY FOR INCUBATION EXPERIMENTS

The incubation bottles were made out of pyrex glass bottles and had a total volume of 1 litre. The dark bottles were made by covering them with a double layer of water resistant electricians tape and aluminium foil. This method was as recommended by Wetzel & Likens, 1990 for the exclusion of light in dark bottles.

The methodology and materials for each of the four incubation experiments followed by Wetzel & Likens (1990) are summarized as follows;

(a) TIME COURSE FOR PRODUCTIVITY STUDIES

In order to determine the time course required to run the incubation experiments, the light and dark bottles were suspended in duplicate at site 4 in the estuary (bridge walkover). A total of 24 bottles were used in this experiment. Surface water samples were then added to the bottles for this experiment. Dissolved oxygen concentrations were taken at time 0 prior to immersion of the bottles into the estuary. Dissolved oxygen concentrations in the remaining duplicate light and dark bottles were taken at hourly intervals using the 90 FL Microprocessor Field Analyzer. This experiment formed the basis for the incubation experiments, since it provided a time course to run future

nutrient incubation experiments in situ.

(b) PRODUCTIVITY AT THREE DEPTHS

Surface, middle and bottom productivity studies were undertaken on two occasions in much the same way. This experiment provided a means of comparing the community productivity at three depths using changes in dissolved oxygen as a measure of productivity (Strickland, 1960; Saunders et.al, 1962; Parsons et.al, 1984). The duplicate light and dark bottles were suspended from site 4 (bridge walkover) using strings. The productivity bottles at each depth contained water from that depth respectively using the vertical Van Dorn water sampler.

(c) NUTRIENT AMENDMENT EXPERIMENTS

Amendment incubations for nitrate (sodium nitrate) and ammonia (ammonium chloride) were conducted at the surface layer of the estuary with a concentration of 10 mg N/L in each of the duplicate light and dark bottles.

Surface water samples were used for this exercise because surface productivity with nutrient additions were being measured.

Changes in dissolved oxygen concentrations were recorded at the end of the four hour incubation period in the controls as well as for the nutrient "enriched" bottles using the 90 FL Microprocessor Field Analyzer.

Changes in Dissolved Oxygen concentrations (p.p.m) were used as a measure of Gross Photosynthesis expressed as mg Carbon/Litre/Hour. (d) KINETIC EXPERIMENTS USING SODIUM NITRATE AND AMMONIUM CHLORIDE OVER A 5 - 8 CONCENTRATION RANGE

The kinetic experiments were done on both nitrate and ammonia. The duplicate light and dark bottles recieved a range of nutrient concentrations for ammonia (ammonium chloride) and nitrate (sodium nitrate) respectively.

0 mg N/L (control), 2 mg N/l, 4 mg N/L, 6 mg N/L, 8 mg N/L and 10 mg N/L of either ammonium chloride or sodium nitrate were added to the duplicate light and dark bottles. The filled bottles were stored under a dark cloth (light proof) until all bottles were ready for incubation (Wetzel & Likens, 1990). The bottles were suspended at the surface layer at sight 4. Dissolved oxygen concentrations for each nutrient concentration range were recorded at the end of the four hour incubation period using the 90 FL Microprocessor Field Analyzer.

RESULTS

INCIDENCE OF ALGAL BLOOMS DURING STUDY PERIOD

The 1994 sampling of the Maribyrnong estuary over the summer-autumn months did not indicate the outbreak of an algal bloom. Monitoring changes in Dissolved Oxygen concentrations (p.p.m) as a measure of Gross Photosynthesis expressed as mg Carbon/Litre/Hour might not have been as efficient in determining primary productivity as chlorophyll a and or fluorometric analyses.

Measuring oxygen changes in the light and dark bottles measures community metabolism. The respiration measured not only measures phytoplankton repiration but also zooplankton and bacterioplankton respiration. Therefore the changes in Dissolved Oxygen concentrations as an indicator of bloom development has its limitations.

RESULTS FOR THE ROUTINE MONITORING OF THE RIVER

1) The following tables provide the Mean pH readings at three depths for the four sites over the study period.

Table 1: Mean pH readings at three depths for Site 1 over study period.

DEPTH				
SURFACE	MIDDLE	BOTTOM		
7.73	8.05	8.21		
7.71	8.12	8.70		
7.42	8.17	8.90		
7.88	7.99	8.06		
8.10	7.61	8.00		
7.79	7.43	8.13		
	E SURFACE 7.73 7.71 7.42 7.88 8.10 7.79	DEPTH SURFACE MIDDLE 7.73 8.05 7.71 8.12 7.42 8.17 7.88 7.99 8.10 7.61 7.79 7.43		

TABLE 2: Mean pH readings at three depths for site 2 over study period.

DEPTH

	SURFACE	MIDDLE	BOTTOM	
DATE			. d	
24/3/94	7.91	7.90	8.10	
6/4/94	7.85	8.20	7.80	
20/4/94	7.42	8.61	8.65	
1/6/94	7.98	7.99	8.08	
27/6/94	8.13	8.54	8.65	
12/7/94	8.37	9.27	9.87	
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TABLE 3 : Mean pH readings at three depths for site 3 over study period.

	-		
	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	7.72	7.84	7.95
6/4/94	7.65	7.57	7.83
20/4/94	7.48	7.65	7.74
1/6/94	7.66	7.69	7.84
27/6/94	8.13	7.98	8.12
12/7/94	8.27	8.29	9.01

TABLE 4 : Mean pH readings at three depths for site 4 over study period.

DEPTH

	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	7.85	7.84	7.90
6/4/94	7.73	7.85	7.91
20/4/94	7.49	7.84	7.97
1/6/94	7.90	7.89	7.95
27/6/94	7.88	7.62	7.73
12/7/94	7.83	7.87	8.08

DEPTH



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2) The following tables provide the Mean dissolved oxygen readings in p.p.m. for the four sites over the study period.

Table 1 : Mean dissolved oxygen concentrations in p.p.m at three depths for site 1 over study period.

	DEPTH			
	SURFACE	MIDDLE	BOTTOM	
DATE				
24/3/94	10.47	8.35	8.67	
6/4/94	10.51	9.42	8.34	
20/4/94	10.39	9.48	8.64	
1/6/94	10.50	8.46	8.53	
27/6/94	9.56	7.50	9.08	
12/7/94	10.36	8.23	7.94	

Table 2: Mean dissolved oxygen concentrations in p.p.m. at three depths for site 2 over study period.

			DEPTH	
	SURFACE		MIDDLE	BOTTOM
DATE		20		
24/3/94	8.25		7.19	7.20
6/4/94	8.21		7.25	7.24
20/4/94	9.98		8.46	8.28
1/6/94	8.30	22	7.18	7.14
27/6/94	10.52		6.93	12.40
12/7/94	10.75		8.36	7.10

Table 3: Mean dissolved oxygen concentrations in p.p.m. at three depths for site 3 over study period.

DEPTH

	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	12.15	10.96	10.55
6/4/94	12.20	10.99	10.70
20/4/94	11.84	11.58	11.49
1/6/94	12.40	10.88	10.62
27/6/94	9.97	6.75	5.65
12/7/94	9.81	6.67	5.83

Table 4: Mean dissolved oxygen concentrations in p.p.m. at three depths for site 4 over study peeriod.

DEPTH

	SURFACE	MIDDLE		BOTTOM
DATE				
24/3/94	11.60	9.15		8.95
6/4/94	11.50	9.25		8.80
20/4/94	11.85	11.41	2	10.46
1/6/94	11.55	8.93	N.	8.75
27/6/94	9.30	5.75		4.75
12/7/94	9.27	6.83		4.83







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3) The following tables provide the Mean Conductivuty readings in mS/cm at three depths for the four sites over the study period.

Table 1: Mean conductivity readings (mS/cm) at three depths for site 1 over study period.

	DEPTH		
	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	18.21	46.40	47.40
6/4/94	18.40	47.50	49.10
20/4/94	16.80	46.80	48.10
1/6/94	17.27	44.60	46.70
27/6/94	10.95	30.90	43.97
12/7/94	18.43	32.80	46.31

Table 2: Mean conductivity readings (mS/cm) at three depths for site 2 over study period.

	DEPTH			
	SURFACE	MIDDLE	BOTTOM	
DATE		4. 10	336. K.S *	
24/3/94	27.10	48.10	46.50	
6/4/94	26.50	45.80	48.25	
20/4/94	19.75	48.40	49.20	
1/6/94	24.60	44.90	45.80	
27/6/94	8.31	45.40	46.70	
12/7/94	7.92	46.31	47.93	

Table 3: Mean conductivity readings (mS/cm) at three depths for site 3 over study period.

DEPTH

	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	17.10	44.20	47.40
6/4/94	18.20	41.30	48.50
20/4/94	17.85	48.61	49.60
1/6/94	15.58	41.80	45.30
27/6/94	9.93	44.90	44.80
12/7/94	9.75	46.83	48.30

Table 4: Mean conductivity readings (mS/cm) at three depths for site 4 over study period.

	DEPTH			
	SURFACE	MIDDLE	BOTTOM	
DATE				
24/3/94	12.50	46.40	47.20	
6/4/94	13.70	49.50	49.40	
20/4/94	15.60	46.40	48.61	
1/6/94	11.86	44.50	45.50	
27/6/94	8.52	42.00	42.40	
12/7/94	8.67	43.91	47.30	



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4) The following tables provide the mean temperature reading (Degrees Celcius) at three depths for the four sites over the study period.

Table 1: Mean temperature readings (degrees Celcius) at three depths for site 1 over study period.

DEPTH

	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	10.50	10.90	10.90
6/4/94	10.62	10.70	10.71
20/4/94	10.12	10.02	10.06
1/6/94	10.40	10.80	9.90
27/6/94	8.50	9.20	8.80
12/7/94	10.50	9.41	8.85

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Table 2: Mean temperature readings (degrees Celcius) at three depths for site 2 over study period.

	DEPTH		
	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	9.70	10.60	11.00
6/4/94	9.85	10.50	10.70
20/4/94	9.46	9.98	9.61
1/6/94	9.90	10.80	10.90
27/6/94	9.20	9.40	9.80
12/7/94	9.41	9.53	9.84

Table 3: Mean temperature readings (degrees Celcius) at three depths for site 3 over study period.

	DEPTH		
	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	10.50	10.80	10.90
6/4/94	10.70	11.10	10.40
20/4/94	10.50	10.70	10.78
1/6/94	10.40	10.70	10.90
27/6/94	9.30	9.90	9.90
12/7/94	9.47	9.81	9.89

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Table 4: Mean temperature readings (degrees Celcius) at three depths for site 4 over study period.

DEPTH

	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	10.20	10.95	11.00
6/4/94	10.40	10.85	11.20
20/4/94	10.12	10.06	10.91
1/6/94	10.10	10.90	11.00
27/6/94	8.40	9.50	9.90
12/7/94	8.61	9.81	9.89









MONTHS

(B) RESULTS FOR NUTRIENT CONCENTRATIONS FOR THE FOUR SITES OVER STUDY PERIOD.

The following tables will provide the Mean concentrations for Nitrate + Nitrite (ug/L), Total Phosphorus (mg/L), Orthophosphate (ug/L), and Total Oxidized Nitrogen (mg/L) at three depths for the for the four sites over study period.

Table 1: Mean Nitrate + Nitrite concentrations (ug/L) at three depths for site 1 over study period.

	_		
	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	249.30	263.30	266.25
6/4/94	269.00	263.55	245.30
20/4/94	265.10	264.15	247.65
1/6/94	286.35	74.17	76.14
27/6/94	279.00	85.39	75.41
12/7/94	290.30	83.28	80.95

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DEPTH

Table 2 : Mean Nitrate + Nitrite concentrations (ug/L) at three depths for site 2 over study period.

	DEPTH		
	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	249.30	368.80	353.45
6/4/94	418.70	379.45	360.25
20/4/94	398.30	390.70	349.80
1/6/94	325.15	80.80	70.95
27/6/94	347.85	309.50	218.15
12/7/94	361.50	316.15	263.75

Table 3: Mean Nitrite + Nitrate concentrations (ug/L) at three depths for site 3 over study period.

	DEPTH		
	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	395.55	376.50	319.45
6/4/94	392.90	363.50	332.65
20/4/94	392.60	347.95	324.60
1/6/94	278.10	84.00	75.50
27/6/94	325.15	317.85	178.85
12/7/94	348.87	319.20	170.75

Table 4: Mean Nitrate + Nitrite concentrations (ug/L) at three depths for site 4 over study period.

	DEPTH		
	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	258.45	244.30	239.65
6/4/94	246.95	241.75	239.30
20/4/94	249.90	249.40	242.60
1/6/94	359.80	82.60	75.50
27/6/94	267.20	105.40	75.47
12/7/94	273.80	124.60	65.53

Table 5: Mean Ammonia concentrations (mg/L) at three depths for site 1 over study period.

湖	DEPTH		
DATE	SURFACE	MIDDLE	BOTTOM
24/3/94	.6890	.6472	.6181
6/4/94	.6440	.6102	.6468
20/4/94	.6160	.6720	.6590
1/6/94	.6705	.5920	.5695
27/6/94	.6660	.4070	.6090
12/7/94	.6200	.5010	.6355

Table 6: Mean Ammonia concentrations (mg/L) at three depths for site 2 over study period.

DEPTH		
SURFACE	MIDDLE	BOTTOM
.7907	.7178	.6928
.8833	.6960	.6640
.7770	.6590	.6520
.7890	.7280	.7230
.7390	.5570	.6700
.7050	.5810	.5360
	SURFACE .7907 .8833 .7770 .7890 .7390 .7050	DEPTH SURFACE MIDDLE .7907 .7178 .8833 .6960 .7770 .6590 .7890 .7280 .7390 .5570 .7050 .5810

Table 7: Mean Ammonia concentrations (mg/L) at three depths for site 3 over study period.

	DEPTH		
	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	.6507	.6252	.5886
6/4/94	.6405	.6338	.5885
20/4/94	.6240	.6190	.6030
1/6/94	.8290	.7580	.6870
27/6/94	.8830	.8260	.8190
12/7/94	.9090	.7870	.7770

Table 8: Mean Ammonia (mg/L) concentrations at three depths for site 4 over study period.

	DEPTH		
	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	.6222	.5889	.5923
6/4/94	.6336	.5915	.5761
20/4/94	.5970	.5613	.5720
1/6/94	.5890	.5190	.4975
27/6/94	.6990	.5020	.3780
12/7/94	.6260	.4610	.3370

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Table 9: Mean Orthophosphate concentrations (ug/L) at three depths for site 1 over study period.

	DEPTH
SURFACE	MIDDLE

BOTTOM

24/3/94	165.45	154.10	156.90
6/4/94	178.20	143.25	161.30
20/4/94	233.75	145.50	148.85
1/6/94	174.70	133.95	118.80
27/6/94	173.90	142.54	120.71
12/7/94	160.57	135.31	128.27

DATE

Table 10: Mean Orthophosphate contentrations (ug/L) at three depths for site 2 over study period.

		DEPTH		
	SURFACE	MIDDLE	BOTTOM	
DATE	32			
24/3/94	190.40	166.05	166.95	
6/4/94	193.95	164.05	169.85	
20/4/94	183.80	162.25	160.70	
1/6/94	186.60	182.75	158.55	
27/6/94	203.10	150.50	123.20	
12/7/94	185.40	150.25	132.45	
Table 11: Mean Orthophosphate concentrations (ug/L) at three depths for site 3 over study period.

DEPTH

	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	171.90	166.75	133.45
6/4/94	171.15	143.00	142.35
20/4/94	165.10	153.25	150.45
1/6/94	161.05	147.75	133.85
27/6/94	182.05	194.60	188.65
12/7/94	193.75	174.20	184.25

Table 12: Mean Orthophosphate concentrations (ug/L) at three depths for site 4 over study period.

		DEPTH	
	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	131.05	129.65	129.55
6/4/94	128.80	135.35	138.95
20/4/94	134.05	133.85	134.80
1/6/94	167.90	96.50	78.30
27/6/94	163.80	96.00	87.65
12/7/94	157.40	113.75	73.76

Table 13: Mean Total Phosphorus concentrations (mg/L) at three depths for site 1 over study period.

	DEPTH		
	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	.1729	.1301	.1543
6/4/94	.1721	.1345	.1708
20/4/94	.1808	.1262	.1608
1/6/94	.1697	.1170	.1030
27/6/94	.1850	.2500	.2130
12/7/94	.1600	.1500	.1470

Table 14: Mean Total Phosphorus concentrations (mg/L) at three depths for site 2 over study period.

	DEPTH		
	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	.2731	.3214	.3525
6/4/94	.2741	.2345	.7510
20/4/94	.2637	.2789	.7060
1/6/94	.2550	.2050	.1920
27/6/94	.3560	.3210	.1930
12/7/94	.4100	.3900	.2700

Table 15: Mean Total Phosphorus concentrations (mg/L) at three depths for site 3 over study period.

	DEPTH		
	SURFACE	MIDDLE	BOTTOM
DATE		¥	
24/3/94	.4821	.2931	.2012
6/4/94	.4410	.2860	.2240
20/4/94	.4390	.2974	.2147
1/6/94	.3970	.2950	.1550
27/6/94	.4950	.4350	.4210
12/7/94	.4670	.4230	.4010

Table 16: Mean Total Phosphorus concentrations (mg/L) at three depths for site 4 over study period.

	DEPTH		
	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	.1321	.1830	.1244
6/4/94	.2341	.2135	.1524
20/4/94	.1187	.3528	.1382
1/6/94	.5870	.4350	.3950
27/6/94	.3170	.3950	.3980
12/7/94	.4210	.3870	.3670

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Table 17: Mean Total Oxidized Nitrogen concentrations (mg/L) at three depths for site 1 over study period.

	DEPTH		
	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	6.123	6.145	5.961
6/4/94	7.132	4.831	5.829
20/4/94	6.060	6.188	5.980
1/6/94	6.498	5.620	5.685
27/6/94	7.473	7.473	5.230
12/7/94	6.430	6.430	5.590

Table 18: Mean total Oxidized Nitrogen concentrations (mg/L) at three depths for site 2 over study period.

	DEPTH			
	SURFACE		MIDDLE	BOTTOM
DATE				
24/3/94	6.120	0]	6.145	5.961
6/4/94	5.385		6.280	6.168
20/4/94	5.195		5.813	5.996
1/6/94	5.803		5.391	4.870
27/6/94	6.283	n X	6.140	7.115
12/7/94	6.060	•345	5.700	5.980

Table 19: Mean Total Oxidized Nitrogen concentrations (mg/L) at three depths for site 3 over study period.

DEPTH

	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	4.815	5.168	5.221
6/4/94	4.856	5.456	5.302
20/4/94	4.970	5.383	5.217
1/6/94	5.830	5.489	3.230
27/6/94	6.090	5.810	5.360
12/7/94	5.875	5.640	5.225

Table 20: Mean Total Dissolved Nitrogen concentrations (mg/L) at three depths for site 4 over study period.

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	DEPTH		
	SURFACE	MIDDLE	BOTTOM
DATE			
24/3/94	5.799	5.720	6.178
6/4/94	6.183	6.338	6.192
20/4/94	5.763	5.798	6.262
1/6/94	6.429	5.920	5.310
27/6/94	6.160	5.630	5.070
12/7/94	6.315	5.770	5.205

NUTRIENT INCUBATION EXPERIMENTS

A) TIME COURSE FOR PRODUCTIVITY STUDIES

Date: 20/7/94

Time: 11.45 a.m.

Tide: High

Site: Bridge Walkover

Depth: Surface

Initial Conductivity: 12.21 mS/cm

Initial pH: 8.11

Initial Dissolved Oxygen: 8.68 p.p.m.

Initial Temperature: 9.0 Degrees Celcius.

Changes in Dissolved Oxygen concentrations (p.p.m) as a measure of Gross Photosynthesis and expressed as mg Carbon/Litre/Hour have been calculated from the following formulae as an indicator of primary productivity within the estuarine waters;

Gross Photosynthesis (mg Carbon/Litre/Hour) formulae: [Oxygen Light Bottles - Oxygen Dark Bottles] (1000 x .375) /PQ x Hours (Wetzel & Likens, 1990).

Where :

Light Bottles = Oxygen in mg/L/unit volume/Time;

dark Bottles = Oxygen in mg/L / unit volume/Time;

1000 x .375 = Conversion from mass of oxygen to mass of carbon;

PQ = Photosynthetic quotient;

Hours = Length of incubation period.

Table 1: Productivity at surface layer over time period expressed in mg C/L/H.

TIME	MEAN LIGHT (p.p.m)	MEAN DARK (p.p.m)	PRODUCTIVITY
0	8.67	8.69	-1.25
1	8.71	8.62	5.63
2	8.89	8.42	29.38
3	8.95	8.26	43.13
4	9.02	8.17	53.13
5	9.07	8.13	58.75



B) PRODUCTIVITY AT SURFACE, MIDDLE AND BOTTOM LAYERS EXPRESSED IN mg CARBON/LITRE/HOUR OVER 4 HOUR INCUBATION PERIOD.

Date: 2/8/94

Time: 12.30 p.m

Tide: High

Site: Bridge Wallover (4)

Depth: Surface

Initial Conductivity: 13.10 mS/cm

Initial pH: 8.01

Initial Dissolved Oxygen: 9.83 p.p.m.

Initial Temperature: 8.40 Degrees Celcius.

1) SURFACE PRODUCTIVITY EXPRESSED IN mg CARBON/LITRE/HOUR.

TIME	MEAN LIGHT (p.p.m)	MEAN DARK (p.p.m)
1	9.49	9.05
2	9.41	9.40
3	9.80	9.18
4	9.89	8.60

Gross Photosynthesis at surface = 80.60 mg C/L/H.

2) MIDDLE PRODUCTIVITY EXPRESSED IN mg CARBON/LITRE/HOUR.

TIME	MEAN LIGHT (p.p.m)	MEAN DARK	(p.p.m)
1	9.48	9.04	2
2	8.77	9.06	
3	9.54	8.95	
4	9.53	8.89	

Gross Photosynthesis at middle = 40 mg C/L/H.

3) BOTTOM PRODUCTIVITY EXPRESSED IN mg CARBON/LITRE/HOUR.

TIME	MEAN LIGHT (p.p.m)	MEAN DARK (p.p.m)
1	9.81	9.75
2	9.69	9.54
3	9.49	8.89
4	9.20	8.94

Gross Photosynthesis at bottom = 16.25 mg C/L/H.

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SECOND PRODUCTIVITY EXPERIMENT AT SURFACE, MIDDLE AND BOTTOM LAYERS EXPRESSED IN mg CARBON/LITRE/HOUR OVER 4 HOUR INCUBATION PERIOD.

Date: 4/8/94

Time: 11.30 a.m.

Tide: High

Site: Bridge Walkover (4)

Depth: Surface

Initial Conductivity: 18.12 mS/cm

Initial pH: 8.02

Initial Dissolved Oxygen: 10 p.p.m.

Initial Temperature: 8.10 Degrees Celcius.

1) SURFACE PRODUCTIVITY EXPRESSED IN mg CARBON/LITRE/HOUR.

TIME	MEAN LIGHT (p.p.m)	MEAN DARK (p.p.m)
1	10.06	9.56
2	9.90	9.31
3	10.12	9.23
4	10.26	8.83

Gross Photosynthesis = 111.72 mg C/L/H.

2) MIDDLE PRODUCTIVITY EXPRESSED IN mg CARBON/LITRE/HOUR.

TIME	MEAN LIGHT (p.p.m)	MEAN	DARK (p.p.m)
1	9.67	8.57	
2	9.20	9.10	
3	9.71	8.67	
4	9.75	8.58	

Gross Photosynthesis = 91.41 mg/C/L/H.

3) BOTTOM PRODUCTIVITY EXPRESSED IN mg CARBON/LITRE/HOUR.

TIME	MEAN	LIGHT	(p.p.m)	MEAN	DARK	(p.p.m)
1	9.54			9.47		
2	9.33			9.16		
3	9.20			9.00		
4	9.08			8.96		

Gross Photosynthesis = 9.38 mg C/L/H.

C) NUTRIENT AMENDMENT EXPERIMENTS

Date: 16/9/94

Time: 1.00 p.m

Tide: High

Site: Bridge Walkover (4)

Depth: Surface

Initial Conductivity: 28.95 mS/cm

Initial pH: 7.70

Initial Dissolved Oxygen: 7.86 p.p.m.

Initial Temperature: 10.90.

Nutrients used:

a) Sodium Nitrate - 10 mg N/L

b) Ammonium Chloride - 10 mg N/L

Table 1: Gross Photosynthesis for light and dark bottles for Sodium Nitrate with a concentration of 10 mg N/L expressed in mg Carbon/Litre/Hour as an indicator of productivity over a 4 hour incubation period.

	MEAN LIGHT	(p.p.m)	MEAN DARK (p.p.m)	Productivity
0 mg N/L	8.45		7.65	62.50
10 mg N/L	8.57	38	7.59	76.57

Table 2: Gross Photosynthesis for light and dark bottles for Ammonium Chloride with a concentration of 10 mg N/L expressed in mg Carbon/Litre/Hour as an indicator of productivity over a 4 hour incubation period.

	MEAN LIGHT	(p.p.m)	MEAN DARK	(p.p.m)	PRODUCTIVITY
O mg N/L	8.19		7.81		29.29
10 mg N/L	8.69		7.59		85.94

Table 2: Gross Photosynthesis for light and dark bottles for Ammonium Chloride with a concentration range of 0 mg N/L to 10 mg N/L expressed in mg mg Carbon/Litre/Hour as an indicator of productivity over a four hour incubation period. CONCENTRATION PRODUCTIVITY MEAN MEAN (mg N/L) LIGHT(p.p.m) DARK (p.p.m) (mg C/L/H)0 7.50 7.30 15.63 46.09 2 7.71 7.12 7.14 6.94 15.63 4 55.47 6.31 6 7.02 6.35 46.09 6.94 8 6.46 5.95 39.84 10

D) KINETIC NUTRIENT EXPERIMENTS FOR SODIUM NITRATE AND AMMONIUM CHLORIDE.

Date: 21/9/94

Time: 11 a.m.

Tide: High

Site: Bridge Walkover (4)

Depth: Surface

Initial Conductivity: 29.90 mS/cm

Initial pH: 7.8

Initial Dissolved Oxygen: 7.00 p.p.m.

Initial Temperature: 10.70 Degrees Celcius

Table 1: Gross Photosynthesis for light and dark bottles for Sodium Nitrate with a concentration range of 0 mg N/L to 10 mg N/L expressed in mg C/Litre/Hour as an indicator of productivity over a four hour incubation period.

CONCENTRATION	MEAN	MEAN	PRODUCTIVITY
(mg N/L)	LIGHT(p.p.m)	DARK (p.p.m)	(mg C/L/H)
0	7.06	6.92	10.94
2	7.19	7.89	-54.69
4	7.62	7.53	7.03
6	6.48	6.03	35.16
8	6.13	5.99	10.95
10	6.23	6.13	7.81

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DISCUSSION

The sampling program for the Maribyrnong river estuary, which ran from March to October 1994, indicated that the estuary is Ammonium biolimited.

Surface productivity was highest in terms of mg C/L/H as an indicator of community productivity. This was due to the availability of sunlight in surface waters being greater than that reaching the turbid, lower depths of the water column. An increase in conductivity and pH readings for the middle and bottom layers indicated the penetration of a salt wedge at high tide. Concentrations of dissolved oxygen were highest at the surface as compared to bottom layers. Temperature readings were lowest at the surface as compared to bottom layers which could mean that the penetrating salt wedge at high tide is slightly warmer than the overlying fresh water. Nutrient analyses of the water samples at the four sites, indicated an increase in the concentrations of nutrients in the winter months of June to August. This was due to the drop in productivity and nutrient utilization due to decreased light availability and increased turbidity. Increased turbidity during the winter months was due to increased rainfall, which resulted in a higher volume of turbid water discharging into the Maribyrnong river estuary from land run off. Seasonal fluctuations in rainfall can be said to play a major role in determining the turbidity of the Maribyrnong river estuary which results in the drop in community productivity.

All four sites along Chifley drive, indicated a higher surface concentration of nutrients as compared to middle and bottom layers. Such a result could possibly be due to the surface waters being constituted primarily with nutrient rich fresh water as compared to the nutrient poor salt wedge penetrating the sampling region.

Nutrient concentrations were higher upstream of site 2 as compared to site 1 at all three depths. Sites 2, 3 and 4 had higher concentrations of Nitrate + Nitrite, Ammonia, Total Phosphorus, Total Oxidized Nitrogen and Orthophosphate as compared to site 1. This indicates that nutrient additions are taking place upstream of site 1 which is increasing the nutrient status of the water column.

The major storm water drain and the emergency relief structure at sites 2 and 3, are in some way adding to the overall increase in the nutrient status of the water body. This is indicated by site 1 having a lower nutrient status as compared to sites 2, 3 and 4 respectively.

Nitrate + Nitrite, Ammonium, Orthophosphate, Total Oxidized Nitrogen and Total Phosphorus in the Maribyrnong river estuary showed marked seasonality, though the pattern at each site was fairly different. Nitrate + Nitrite had a maximum in winter, presumably after the soils had become sufficiently wet to allow wash out of soil nitrate. The decline or lower concentration readings probably reflected biological uptake or denitrification, because ammonium levels increased in the winter months.

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Orthophosphate concentrations were fairly uniform in all four sites at all four depths. Concentrations peaked in the winter at site 2.

Total Phosphorus concentrations ranged from 0.117 mg/L to 0.709 mg/L. Bottom waters tended to have a higher Phosphorus content due to the mobility of Phosphorus from sediments at high pH readings caused by the penetration of the salt wedge at high tide. The winter months indicated a peak of Phosphorus concentrations in surface waters. Site 3 in general, had a greater Phosphorus count than any of the other sites. Productivity at the surface was the highest as compared to the bottom lying waters. Gross Photosynthesis was an indicator of community productivity and explained in terms of mg Carbon/Litre/Hour. Productivity readings ranged from 80.6 mg C/L/H in surface waters to 10 mg C/L/H in bottom waters. This drop in productivity from surface to bottom, indicates that light availability is a limiting factor in the Maribyrnong river estuary. The general pattern suggested that surface productivity was greater than middle or bottom productivity in the Maribyrnong river estuary. Nutrient amendment experiments at the surface layer using 10 mg N/L, indicated an increase in dissolved oxygen concentrations. Gross Photosynthesis at surface layers was expressed in mg C/L/H for Sodium Nitrate and Ammonium Chloride.

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Ammonium Chloride at a concentration of 10 mg N/L, had a higher productivity of 85.94 mg C/L/H as compared to the lower productivity of sodium Chloride which was 76.57 mg C/L/H. Ammonium Chloride as the source of Nitrogen for community metabolism, is the more biolimiting nutrient in the Maribyrnong river estuary as compared to Nitrate. The nutrient Kinetic experiments indicated once again that Ammonium Chloride as the source of Nitrogen for community metabolism, was biolimiting in the estuary. Productivity rates for pulses of Ammonia ranged from 15.63 mg C/L/H to 55.47 mg C/L/H. Pulses of Nirtate as the source of Nitrogen on the other hand, had productivity rates which ranged from 7.03 mg C/L/H to 35.16 mg C/L/H. Ammonium Chloride as the source of Nitrogen, biolimited the phytoplankton community in the Maribyrnong river estuary.

The Maribyrnong river estuary did not experience a Phytoplankton bloom during the study period. Sampling methods employed, suggested that light and turbidity are the two main biolimiting factors in the estuary with pulses of Ammonia increasing Gross Photosynthesis which was expressed as mg C/L/H.

The techniques employed during the course of study, can be regarded as being not as accurate as classical, present methods currently undertaken to determine Phytoplankton blooms. Chlorophyll a analyses and or Flourometric analyses would have provided more conclusive evidence on the development of a Phytoplankton bloom. Analytical problems encountered with the Aquatech 5400 flow injection analyzer were mainly for Phosphorus and Nitrogen concentration determinations. Standardizing the base line proved to be a problem, since the time taken to do this ran into hours. Other nutrient determinations using the flow injection analyzer were adequate, with reproducibility being fairly constant. The Aquatec flow injection analyzer had a reproducibility of 2 % r.s.d. Such a method provided fairly accurate results in the determination of nutrient concentrations in water samples.

Measuring changes in dissolved oxygen concentrations using the 90 FL Microprocessor Field Analyzer did have its limitations. The time required for physio-chemical readings to stabilize was fairly large. This had a constant affect on the battery life out in the field. A better method which could have been employed for measuring changes in dissolved oxygen concentrations in water samples would have been the Winkler method. However, due to the short period of time, the field analyzer provided adequate accuracy since the instrument was calibrated after each run as recommended by the manual. Phytoplankton identification and counts was undertaken on three occasions using Lugol's solution as a stain. However, on all three occasions, it was fairly difficult to differentiate between live and dead cells. As a result, a species profile of the estuary was not achieved. The sampling program of the Maribyrnong river estuary compared fairly well with other studies. Nutrient and physio-chemical analyses indicated the partial stratification of the estuary from surface to bottom. The three layers investigated had differing nutrient concentrations along with a variability in physio-chemical readings. Surface productivity was greatest due to the estuary being limited to light and turbidity. Seasonal fluctuations indicated that the winter months increased turbidity in the estuary which was reflected in a drop in community productivity during these months. Due to the samples being taken at high tide in the middle of the day, the method employed keeps the sampling regime at a constant.

One possible explanation why a bloom occurred upstream of the sewer collapse in 1992 towards the Canning street bridge area, could possibly be due to the flushing of the inorganic nutrients upstream of the sewer by the incoming tide. Tidal regimes in the Maribyrnong does play a role in diluting the nutrients present in suspension in the water column due to the increased volume of saline water recieved by the middle and bottom layers. On a management level it can be suggested that if the Maribyrnong river estuary had clear water, light and turbidity would no longer be biolimiting. Phytoplankton blooms would occur under such conditions due to the availablity of nutrients for phytoplankton metabolism. Maintaining present hydrological, physio-chemical and nutrient conditions would limit the development of a phytoplankton bloom in the Maribyrnong river estuary.

CONCLUSION

The Maribyrnong river estuary is Ammonium biolimited. Light and turbidity prevent the uptake of nutrients in the middle and bottom layers of the estuary. In order to prevent phytoplankton explosions occurring, the influx of Ammonium must be retarded. Since Ammonium is a constituent of sewage, the concentrations discharged into the estuary must be monitored closely.

Productivity at surface layers were higher in comparison to bottom and middle layers. This further suggested that light and turbidity play a major role in controlling phytoplankton population explosions in the Maribyrnong river estuary.

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