THE EFFECTS OF DIFFERENT WATER QUALITY PARAMETERS ON PRAWN (*MACROBRACHIUM ROSENBERGII*) YIELD, PHYTOPLANKTON ABUNDANCE AND PHYTOPLANKTON DIVERSITY AT NEW ZEALAND PRAWNS LIMITED, WAIRAKEI, NEW ZEALAND.

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1. ABSTRACT

Aquaculture is the fastest growing industry in the food sector and demand for aquaculture products is continuing to grow as many wild stocks from capture fisheries continue to decline. It is imperative that water quality in an aquaculture system is closely controlled in order to maintain the health of the species under culture and maximize production. New Zealand Prawns Limited (NZPL) is an aquaculture facility in Wairakei, New Zealand that cultures the freshwater prawn *Macrobrachium rosenbergii*. Dramatically reduced yields of prawns have been observed in ponds following periodic blooms of benthic algae. In this study, water quality variables were measured in grow out ponds at 9-11 day intervals. I measured temperature, phytoplankton abundance, phytoplankton diversity, turbidity, and concentrations of ammonia, nitrate, orthophosphate, dissolved oxygen and chlorophyll *a*. This data was combined with information on pond depth and prawn yield in order to investigate what variables influence the abundance and diversity of phytoplankton, benthic algal blooms and prawn yield. The difficulty of combining scientific endeavour with commercial enterprises resulted in only a small data set being available for analysis but it appears that benthic algal blooms at NZPL may be due to excessive light penetration to the benthos due to shallow pond depths, and reduced shading of the benthos when phytoplankton abundance is low. Low phytoplankton abundance may possibly be a result of low orthophosphate. There was insufficient data to determine what impacts, if any, the variables investigated have on prawn yield or how water quality variables change with time. Future studies and experiments are recommended in order to increase knowledge of farming *M. rosenbergii*; a valuable crustacean that has been shown to have a lower social and environmental impact than many other more common aquaculture species.

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2. INTRODUCTION

2.1.1 Aquaculture

The Food and Agriculture Organization of the United Nations (FAO) defines aquaculture as "the farming of aquatic organisms in inland and coastal areas, involving intervention in the rearing process to enhance production and the individual or corporate ownership of the stock being cultivated" (FAO 2007a). Naylor et al. (2000) state that two key criteria separate aquaculture from wild capture fisheries: 1) ownership of stock, and 2) intervention in the lifecycle or husbandry of the cultured species. It involves the culture of both animals (such as fish, molluscs, crustaceans etc) and plants (such as algae) and occurs in both freshwater and saline water, the latter often being referred to as 'mariculture' to distinguish it from freshwater aquaculture.

A variety of forms of aquaculture exist, distinguished from one another by their intensity (output of product per unit of area) although it may not always be clear what category a farm may fall into. The FAO (2007a) uses the following criteria:

- *Extensive* - Production system characterized by (i) a low degree of control (e.g. of environment, nutrition, predators, competitors, disease agents); (ii) low initial costs, low-level technology, and low production efficiency (yielding no more than 500 kg/ha/yr); (iii) high dependence on local climate and water quality; use of natural waterbodies (e.g. lagoons, bays, embayments) and of natural often unspecified food organisms.

- *Semi-intensive* - Systems of culture characterized by a production of 2 to 20 tonnes/ha/yr, which are dependent largely on natural food, which is augmented by fertilization or complemented by use of supplementary feed, stocking with hatchery-reared fry, regular use of fertilisers, some water exchange or aeration, often pumped or gravity supplied water, and normally in improved ponds, some enclosures, or simple cage systems.

- *Intensive* - System of culture characterized by (i) a production of up to 200 tonnes/ha/yr; (ii) a high degree of control; (iii) high initial costs, high-level technology, and high production efficiency; (iv) tendency towards increased independence of local climate and water quality; (v) use of man-made culture systems.

Aquaculture is the fastest growing industry within the food sector (Kutty 2005). In 1950, total world aquaculture totalled less than one million metric tonnes. By 2004, total world aquaculture production had increased to 59.4 million metric tonnes valued at $US 70.3 billion (FAO 2007b). The aquaculture industry continues to diversify (develop new products) and intensify (increase output per unit area). With the increasing human population and decline of wild capture fisheries, the FAO estimates that global aquaculture production will need to reach 80 million metric tonnes by 2050 (FAO 2007b).

This is not without its problems however, as aquaculture can cause severe social, economic and ecological problems. Examples of these problems are discussed in the next section, with particular reference to Decapod aquaculture.

Management of water quality is also a central concern in aquaculture and can be a complicated task involving many different variables that are often difficult to control. In order to include the focal aquaculture species of this study (the giant river prawn *Macrobrachium rosenbergii*) water quality and its common management strategies will be discussed later in the chapter (see section 2.3: '*Water quality in aquaculture'*).

2.1.2 Decapod aquaculture

Most farmed decapods are shrimps or prawns (usually referred to as shrimps) although some crabs are also commercially cultured, such as the mud crab *Scylla serrata* (Ruscoe et al. 2004). The commercial-scale farming of shrimps began in the 1970s. There are a wide variety of shrimps under culture around the world but the majority of them are marine shrimp of the family Penaeidae. In 2003, the estimated farmed production of shrimps totalled 2.7 million metric tonnes worth almost $US 10.6 billion (FAO 2007d).

Like many forms of aquaculture, the culture of shrimps has received much criticism for its detrimental environmental impacts such as habitat modification and pollution. In particular, the destruction of mangrove forests to make way for shrimp farms has been cited as one of the worst effects. Since the 1980s, it has been estimated that around 35% of the world's mangrove forests have been lost and of these losses, 52% has been attributed to clearance for mariculture, with shrimp culture contributing to 38% alone (Valiela et al. 2001). Mangroves provide important ecosystem services such as nursery grounds for the juvenile stages of a variety of marine life such as a number of *Barracuda* species, mullet, bream, flathead and (wild) Penaeid prawns (Little et al. 1988, Laegdsgaard and Johnson 1995, Primavera 1997a), many of which

are of economic importance in wild capture fisheries. Mangroves have also been found to reduce coastal erosion (Mazda et al. 2002) and their soils have been shown to remove heavy metals and nutrients from water such as copper and phosphorous (Tam and Wong 1999), reducing the amount that reaches estuaries and open ocean.

Aquaculture has been championed by many as the saviour of wild capture fisheries, supposedly relieving pressure on stocks by providing an alternative source of fish. The reverse is often true as carnivorous species under culture require large amounts of wild-caught fish for feed (Naylor et al. 2000), increasing or at best sustaining the pressure on wild fisheries. Further stress is placed on wild fisheries through the ecological impacts that aquaculture can have, such as the introduction of exotic species and pathogens, collection of wild seed stock, nutrient enrichment, habitat modification and alteration of food web dynamics and shrimp aquaculture is no exception to this (Naylor et al. 2000).

Disease has also had a significantly detrimental impact on shrimp farming, particularly in intensive systems where stock may be overcrowded. In particular the white spot syndrome virus (WSSV) has caused massive problems in shrimp farms around the world (Wang et al. 1997, Rajendran et al. 1999, Kutty 2005), costing jobs, millions of dollars and could potentially infect wild populations of crustaceans (Naylor et al. 2000).

Shrimp culture, can also have serious socio-economic impacts. Traditional livelihoods such as rice farming are displaced or rice farmers are forced to sell their land when it becomes polluted by adjacent shrimp farms (Primavera 1997b). In India,

the costs of shrimp farming have been found to outweigh the benefits, and in particular is associated with increasing unemployment (Primavera 1997b). The productivity of shrimp ponds decreases at a rate of 3%-8% per production cycle due to deterioration in pond sediment and soil quality and poor pond management (Dierberg and Kiattisimkul 1996). After a period of around seven years, ponds cease to be profitable and are abandoned. This sees farmers switch to new sites, leaving useless salinated land in their wake. In Thailand alone it is estimated that between 4,500 and 16,000 hectares of shrimp ponds have been abandoned, the majority of which continue to lay idle (Dierberg and Kiattisimkul 1996). Rice farming cannot be resumed following farm closure due to salinization of soil.

2.1.3 Freshwater Decapod aquaculture

There are three species of freshwater prawns cultured around the world, all of which belong to the genus *Macrobrachium*. The feasibility of large-scale commercial culture of the monsoon river prawn (*Macrobrachium malcolmsonii*) is currently underway in India (New 2005). The Oriental river prawn *Macrobrachium nipponense* is farmed in China where approximately 120,000 tonnes was produced in 2001, rapidly catching up to the giant river prawn *M. rosenbergii* (approximately 130,000 tonnes produced in China in 2001). At current rates of expansion, global production of all *Macrobrachium* species combined is expected to be between 700,000 and 1.4 million metric tonnes by 2010 (New 2005). Currently, *M. rosenbergii* is by far, the biggest overall contributor to freshwater prawn culture and is farmed in many countries around the world.

The complete life cycle of *M. rosenbergii* was closed (all life history stages observed and understood) in 1962 when the first laboratory-hatched larvae were successfully reared through to adulthood (Ling 1977). While *M. rosenbergii* had for some time been captured from the wild and grown to marketable size (~25g+) in captivity, it was proper understanding of the life cycle that allowed true culture systems to develop. The Anuenue Fisheries Research Centre in Hawaii pioneered technology for mass-rearing of *M. rosenbergii* larvae in 1965 (Fujimura and Okamoto 1972). This was followed by a series of successful growout experiments and the development of a number of commercial farms in the USA during the 1970s. At around the same time, Thailand and Taiwan both began developing what would become significant industries for both countries (Chen 1976).

By 1987 global production of farmed *M. rosenbergii* was estimated to be around 27,000 metric tonnes per annum (New 1990). Global production increased to 213,861mt by 2001 as more countries (particularly China) began to culture the species (New 2005). While the vast majority of *M. rosenbergii* is farmed in Asia, significant producers also include Africa, Israel, and Central and South America. Production also occurs in countries that at first seem unlikely candidates for a tropical species. The temperate country of New Zealand has just one *M. rosenbergii* farm, New Zealand Prawns Ltd (NZPL). NZPL uses heat exchange with geothermal water to warm fresh water to the optimum culture temperature of 28°C. A joint venture has seen this technology being used to culture *M. rosenbergii* in Iceland (New Zealand Prawns Ltd, personal communication). Russia has also recently begun to culture the species using heated effluent from electrical power generation (New 2005).

The freshwater farming of *M. rosenbergii* has a number of advantages over marine farming. The territorial nature of the males (see section 2.2 *Biology of Macrobrachium rosenbergii*) requires that culture is less intensive than is typically seen in marine shrimp farms. This means that pollution of land and adjacent waterways through nutrient enrichment is lower and the incidence and severity of disease among the prawns is reduced (Kutty 2005). There is also good potential for integration with other aquaculture species such as carps and tilapia (Kutty 2005). With the growout phase occurring entirely in freshwater, land does not become salinated and can be used for other purposes such as growing crops following a farm's closure. Freshwater culture also means that farming can take place much farther inland than marine species, allowing for a far greater number of potential farm sites. Farms can also be situated in close proximity to large, lucrative inland urban markets and deliver fresh or even live product in short time-frames and at lower transport costs (Tidwell et al. 2005).

2.1.4 Aquaculture in New Zealand

Aquaculture is a significant industry in New Zealand. In 2005, total aquaculture production was approximately 105,301 metric tonnes worth over $US 204 million (FAO 2007c). The New Zealand Seafood Industry Council (Seafic) reports that the industry in NZ as a whole is aiming to be worth $NZ 1 billion by the year 2025 (Seafic 2007). Green-lip mussels (*Perna canaliculus*) are by far the biggest contributor to the aquaculture industry in NZ (Gall et al. 2000, Markowitz et al. 2004), followed by king salmon and pacific oysters (Seafic 2007). The paua (abalone) industry for both meat and pearls is a growing industry and kingfish, eel, turbot, rock

lobster, seahorses and some species of seaweed and sponges are also showing potential (Seafic 2007).

Aquaculture facilities span the length of the country, from Stewart Island in the far south to Kaitaia and beyond in the far north. The vast majority though are located in the Marlborough Sounds, the centre for mussel farming. Of 520 mussel farms operating in New Zealand in 2000, 455 were located in the Marlborough Sounds (Gall et al. 2000).

Like the rest of the world, aquaculture in New Zealand can have detrimental environmental and socio-economic impacts. Mussel farming has been found to significantly lower phytoplankton abundance in Beatrix Bay, Marlborough Sounds (Ogilvie et al. 2000). Chlorophyll *a* is the primary photosynthetic pigment in plants and algae, frequently used as an index of phytoplankton biomass (Desortova 1981, Canfield et al. 1985, Voros and Padisak 1991). In their 1997/1998 study, Ogilvie et al. measured chlorophyll *a* levels both within and outside four mussel farms and found that chlorophyll *a* was significantly lower inside farms compared to outside, attributing this to filter feeding by mussels. This poses potential problems for higher trophic levels in the ecosystem and the sustainability of the mussel farming industry if it continues to intensify and farm sites increase in number.

Farms may also compete for space with marine mammals such as the dusky dolphin. Over the course of five consecutive winters Markowitz et al. (2004) observed the occurrence, distribution, abundance, and behaviour of dusky dolphins in the Marlborough Sounds. In particular they focussed on Admiralty Bay, where dolphins were observed to spend significantly less time inside mussel farm boundaries

compared with outside farm boundaries. It is thought that the suspended lines on which mussels are grown obstruct foraging and that the floats used to keep lines suspended may impair dolphin echo-location, essential for dolphins to find food. This could pose a serious problem for dusky dolphins, who spend much of the winter in Admiralty Bay, and whose observed distribution within the bay overlaps significantly with areas where many more mussel farm sites are being proposed (Markowitz et al. 2004).

Salmon farming in New Zealand can also have detrimental effects on the ecosystem. The sedimentation rate directly under salmon cages in the Marlborough Sounds has been found to be very high, and its physical and chemical characteristics (particularly for nitrogen and phosphorous) very different to those of sediments nearby (Kaspar et al. 1988). Similar effects on the benthos have been found for mussel farming in Keneperu Sound where sediment beneath a mussel farm was found to contain twice as much ammonium as a comparable reference site with no mussel farming (Kaspar et al. 1985). Infauna of the mussel farm sediment consisted only of polychaete worms whereas the reference site consisted of polychaete worms, brittle stars, bivalve molluscs and crustaceans.

The mussel farming industry alone employs approximately 2,500 people (NZMFA 2007), most of this in the Marlborough Sounds. Other important human activities take place in the Marlborough Sounds as well, such as tourism, commercial fishing and the area sees much boat traffic (Markowitz et al. 2004) including the Interislander ferries that link road and rail traffic between the North and South Islands of the country. New Zealand's mussel farming industry illustrates that much

like the rest of the world, aquaculture in New Zealand can have important environmental and socio-economic consequences.

2.2.1 Biology of Macrobrachium rosenbergii

Macrobrachium rosenbergii (de Man, 1879) are Decapod crustaceans of the family Palaemonidae. They are not strictly 'prawns' such as the more familiar tiger prawn (*Penaeus monodon*, family Penaeidae) but the term "prawn" is still used informally. The species is found throughout much of Southeast Asia and the tropical regions of northern Australia (New 2002).

The species is found in lakes, rivers, swamps and irrigation canals with a preference for turbid conditions. They are capable of climbing waterfalls and can also traverse land where there is plenty of moist vegetation (New 1990). The adult stage of the life cycle is spent entirely in freshwater although they are euryhaline (tolerant of a wide variety of salinities). Armstrong et al. (1981) found that *M. rosenbergii* transferred from freshwater to water of 24 psu (practical salinity units) without acclimation showed no signs of stress or decrease in activity. At least one aquaculture farm, New Zealand Prawns Ltd, keeps egg-bearing females in full strength seawater so that larvae immediately find themselves in the required conditions on hatching (see below).

Macrobrachium rosenbergii are sexually dimorphic. Males are larger than females and have much larger second periopods (the prominent clawed arms, see Appendix 1, Diagram 1), and larger cephalothoraxes (the fused structure comprised of the head and thorax, Appendix 1, Diagram 1). For males, society is highly structured with

three distinct morphotypes being recognized, each with differing growth rates and behaviour (Kuris et al. 1987). All three morphotypes are sexually active but with different success and mating strategies.

The first of the male morphotypes is the small or ‘SM’ male (Appendix 1, Photo 1, lower specimen). As the name might suggest they are the smallest of the three and make up around 50% of the male population (Ra'anan and Sagi 1985). They are characterized by short, relatively un-pigmented claws and are subordinate to the other morphotypes. They also exhibit “sneak” reproductive behaviour, quickly copulating with females when opportunity permits (Ra'anan and Sagi 1985). The age at which they become capable of sexual reproduction varies, but usually occurs at a carapace length of just 10mm (Kuris et al. 1987).

The second male morphotype is the orange-claw or OC male (Appendix 1, Photo 2) comprising around 40% of the male population (Ra'anan and Sagi 1985), and is characterized (unsurprisingly) by having orange claws. The transition from SM to OC male is gradual, with an intermediate form, the “weak OC” being recognisable (Kuris et al. 1987). They are dominant over small males but subordinate to blue-claw males (the third morphotype, see below) and hence have poor mating success.

The largest of the three castes are the dominant blue-claw or ‘BC’ males (Appendix 1, Photo 3), making up the remaining 10% of the male population (Ra'anan and Sagi 1985). They are characterized by their large body size and especially large second periopods (see diagram 1, Appendix 1) that are deep-blue in colour. BC males are

dominant over both SM and OC males, are territorial, and gather harems of soft-shelled females in preparation for mating (Kuris et al. 1987).

Growth rates for both males and females vary, involving genetic and environmental factors, as well as the social factors outlined above. In males, OCs has the highest somatic growth rates of all three morphotypes. Eventually OC males will grow large enough to overthrow a territory's BC male, take over his harem and transform into a BC male at a single metamorphic moult (Kuris et al. 1987) at which point growth ceases (Ra'anan et al. 1991). This is an ongoing process, known as the "leapfrog" phenomenon (Ra'anan et al. 1991) and sees BC males becoming increasingly larger with each successive generation.

Unlike the male population there is only one morphotype for females (Appendix 1, Photo 4) and no territoriality. Growth effectively ceases at sexual maturation. The length of time taken to reach sexual maturity for females is variable, but observations of a captive population by Ra'anan et al. (1991) ranged from 6-20 weeks with weights ranging from 12-32g.

Mating occurs between recently moulted, ovigerous (egg-bearing) females and hard-shelled males. External fertilization takes place when the male deposits a gelatinous sperm-packet (spermatophore) around the gonopores between the walking legs of the female. Fertilized eggs are transferred to a ventral brood chamber beneath the abdomen and are periodically ventilated through beating of the pleopods (paired structures under the abdomen used primarily for swimming, (see Diagram 1,

Appendix 1). Eggs will hatch in 18-23 days under the optimum temperature of 28°C (New 1990).

Unlike adults, larvae in the early stages of development require brackish water to survive and will die if they don't find themselves in such conditions within five days of hatching (Ling 1977). Typically, newly hatched larvae are washed down freshwater streams and rivers until they reach the required brackish conditions of estuaries. There are generally 11 recognized larval stages before metamorphosis into post larvae, although Gomez Diaz and Kasahara (1987) have reported another six instars, bringing the total to 17. As they develop into post-larvae, they move further upstream away from estuaries into progressively less saline water, over a period of around three to six weeks (New and Singholka 1985). At adulthood, they live exclusively in fresh water.

2.3 Water quality in aquaculture

Water quality is important in natural environments, particularly for the health of the organisms that live within it. It can be described as "physical, chemical and biological factors that influence the beneficial use of water" (Aquaculture 1999). Water quality parameters are often closely linked to and dependent on one another. Of particular concern in water quality is eutrophication, described by Nixon (1995) as "the process of increased organic enrichment of an ecosystem, generally through increased nutrient inputs". These "inputs" Nixon refers to are most often nitrogen and phosphorous, important limiting nutrients for photosynthetic organisms (Lobban and Harrison 1994). Human activities such as sewage discharge and run-off from agriculture and aquaculture are common sources of these nutrients (Anderson et al.

2002). In particular, harmful algal blooms linked to eutrophication are of concern to resource managers. Harmful algal blooms can have a variety of detrimental effects including oxygen depletion when blooms crash, alteration of habitat through shading of the benthos, contamination of drinking water and mass mortality of fish and shellfish. The latter has the potential to cause human illness and even death when contaminated water or fish and shellfish are consumed (Anderson et al. 2002).

Despite their artificiality, aquaculture environments such as prawn ponds share many similarities with natural ecosystems. As in natural environments, good quality water is essential in aquaculture in order to maintain the health, optimal growth and survival of the cultured species, prevent eutrophication and maximize value for the farmer. Many water quality variables are often closely linked to one another, and maintaining appropriate levels of each in aquaculture is often a 'balancing act'. This may require the farmer to compromise on the ideal level of one variable in order to avoid the detrimental level of another. For example, the level of feed that may allow for optimal nutrition may not necessarily be the best option if the system is not able to adequately deal with the level of ammonia excreted by stock. Management in aquaculture often involves trade-offs, and optimal targets may shift, for instance due to the biomass of stock, day length or temperature etc. Common water quality parameters of interest to farm managers are discussed in the next section, with special reference to *M. rosenbergii* where possible.

2.3.1 Phytoplankton & chlorophyll a

Phytoplankton is essential in maintaining good water quality. Phytoplankton affects oxygen levels, nutrient concentrations, light levels, and zooplankton biomass (Chien

1992). Farm managers often deliberately fertilize aquaculture ponds to stimulate phytoplankton blooms. These blooms shade stock, prevent growth of benthic algae (by shading the benthos), oxygenate water, reduce toxic ammonia levels and provide a food source for zooplankton which in turn can provide a food source for higher trophic levels that may be eaten by stock (Burford 1997).

Chlorophyll *a* is present in all photosynthetic organisms including algae. Its measurement as an index of water quality (Papista et al. 2002) and phytoplankton biomass (Desortova 1981, Canfield et al. 1985, Voros and Padisak 1991) is widely accepted. Generally, higher chlorophyll *a* concentrations translate into higher individual cell counts and biomass of phytoplankton, though not always, as not all algal cells produce equal amounts of chlorophyll *a* (Felip and Catalan 2000). For this reason it is also important to identify and count phytoplankton cells in water samples.

The identification of algal species and cell number is also important as some species can produce toxins that are harmful to animals and humans. For example, the deaths of many dogs, cattle, sheep and horses have been reported in Australia and Scotland after ingestion of a number of neurotoxin-producing cyanobacteria and benthic *Oscillatoria* species in lakes and rivers (Codd et al. 1992, Steffensen et al. 1999). Some species of cyanobacteria and dinoflagellates produce Paralytic Shellfish Poisons or PSPs, which have been widely documented to affect humans, sometimes lethally (Rodrigue et al. 1990, Anderson et al. 2002). PSP events have also been associated with the products of aquaculture such as bivalve molluscs (Paez-Osuna et al. 1998). Certain species of cyanobacteria have also been found to affect the flavour of some cultured species (Tucker 2000). The bottom line for farm managers is the

value of their product. With certain algal species being able to affect the safety and flavour of aquaculture products, management of phytoplankton is essential.

If phytoplankton levels are too high farm managers may reduce the level of feeding to reduce nutrient inputs and/or flush ponds with clean fresh water. If phytoplankton levels are not sufficient they may deliberately fertilize ponds to encourage growth (Burford 1997).

2.3.2 Nutrients: nitrate, ammonia & orthophosphate

Nutrient levels are an important consideration in farm management. Adequate nutrient levels will allow for the right structure and biomass of phytoplankton (Alonso-Rodriguez and Paez-Osuna 2003). In terms of aquaculture this means phytoplankton species composition and abundance that allows for maximum production, health and ultimately value of the cultured species. Both species composition and biomass of phytoplankton communities can be influenced by nutrient concentrations (Smith 1982, Hecky and Kilham 1988) which in turn can influence other dynamics in aquaculture ponds.

Nitrogen is an essential nutrient for the growth of phytoplankton, and is an important component of metabolic compounds such as amino acids (Lobban and Harrison 1994). Nearly all phytoplankton will utilize nitrate (Burford and Pearson 1998). Inorganic nitrate (NO_3^- must be converted to nitrite (NO_2^-) by the enzyme nitrate reductase, and then converted further to ammonium by nitrite reductase (Lobban and Harrison 1994). This process is known as denitrification. The end-product of ammonium can then be directly incorporated into amino acids and proteins. The

enzymes involved in this process are produced by algae and the conversions occur within algal cells, although some bacteria found in water are also capable of denitrification (Betlach and Tiedje 1981). Some bacteria can also convert nitrogen back from ammonia into nitrite and then nitrate (Durborow et al. 1997).

Ammonia enters aquatic systems mainly through excretion by living organisms, especially by the species under culture, and through the decay of organic material including dead organisms and uneaten feed. Given the abundance of sources, nitrogen is not expected to be limiting to phytoplankton growth in aquaculture, particularly in intensive systems.

Aside from being a nutrient source for phytoplankton, ammonia is of interest to farm managers because it can be highly toxic to aquatic animals (Chin and Chen 1987, Noor-Hamid et al. 1994, Ostrensky and Wasielesky 1995, Naqvi et al. 2007) and has been found to affect prawn growth and cause mortality (Wickins 1976, Armstrong et al. 1978, Mallasen and Valenti 2005). Problems can occur at concentrations as low as 0.5 parts per million, or ppm (Naqvi et al. 2007). In juveniles of the crab *Callinectes sapidus* it has been demonstrated that ammonia accumulates in the body, altering growth and causing death (Kormanik and Cameron 1981). Mallasen and Valenti (2005) offer a similar explanation for altered larval development and growth in *M. rosenbergii*.

In crustaceans, ion exchange with the environment occurs mainly through the gills (Henry and Wheatly 1992). Mallasen and Valenti (2005) found that in *M. rosenbergii*, larvae in later stages of development were more sensitive to ammonia

than those in the earlier stages, attributing this to having more developed gills with a larger surface area. In a controlled experiment Naqvi et al. (2007) found that for late juveniles (4.13-4.49g) of *M. rosenbergii*, mortality increased and growth decreased significantly as ammonia levels increased. They also found significantly reduced feeding activity in ammonia levels as low as 0.5 ppm. As well as stunting growth through poor nutrition, reduced feeding activity results in further increases in ammonia and decreased oxygen as feed goes uneaten and pollutes the water during bacterial decomposition of the material.

Phosphorous is generally the least abundant of essential (and therefore limiting) nutrients required by photosynthetic organisms in freshwater systems, including phytoplankton (Schindler 1977). For the majority of algae, it is most readily available in the form of the orthophosphate ion, PO_4^{-3} (Lobban and Harrison 1994, Correll 1998). Phosphorous is an important component of nucleic acids, proteins, phospholipids and ATP (Correll 1998), the latter being essential for the transport of energy in cells for metabolism (Lobban and Harrison 1994).

Nutrient enrichment may cause algal blooms which can cause water to become hypoxic (low in oxygen) or even anoxic (no oxygen) (Anderson et al. 2002), particularly at night. As in the day time, oxygen continues to be consumed at night by all the organisms in the pond, including algae which are now consuming more oxygen than they produce in the day time while photosynthesizing. This creates what is known as a deficit in the oxygen budget (Brunson et al. 1994) where the demand for oxygen of all the organisms in the water exceeds the production of oxygen.

A number of management strategies exist to control ammonia and other nutrient levels (Aquaculture 1999). These include:

- Stopping or reducing feeding (this reduces nutrient input).
- Flushing ponds with fresh water (to dilute the nutrient concentrations).
- Reduce stocking density (reduces the level of feed needed and reduces the level of nitrogenous excretion by the cultured species).
- Reduce pH level of the pond (reduces the conversion of non-toxic ammonium into toxic ammonia ions).

2.3.3 Temperature

Being ectothermic, *M. rosenbergii* obtain their heat from the water in which they live. Temperature affects the chemical and biological processes of ectothermic organisms in the water which in turn affects many other important variables such as oxygen consumption, feeding rates and growth. Depending on the type of farm, temperature may not be controlled at all, or it may be heated by a number of methods such as solar, electrical and geothermal energy.

New (1990) reports temperatures below 14°C and above 35°C as lethal for *M. rosenbergii*, with 29-31°C being optimal. Niu et al. (2003) reported 33°C as significantly increasing feeding rates and being optimal for growth. However, in their experiment water had to be artificially saturated with oxygen. This was to compensate for the increase in oxygen consumption by prawns due to the increased feeding activity (and hence decreased dissolved oxygen) that resulted from the high temperature. Rearing prawns at this temperature in a commercial situation would

require a highly effective method of aeration which would increase operating costs, offsetting benefits from higher growth rates.

2.3.4 Turbidity

Turbidity (clarity of water) in aquaculture systems is also an important water quality variable. Turbidity is affected by zooplankton and phytoplankton densities in the water column and also suspended particulate matter such as silt, faecal matter and uneaten feed.

Turbidity affects the level of light penetration in the water column which has influential effects on photosynthesis and hence algal growth. Highly turbid ponds have shallow light penetration which lowers the temperature as well as photosynthetic activity. Highly turbid ponds often have decreased amounts of algae growing on the bottom of ponds. In ponds with low turbidity, we see the opposite effect (Aquaculture 1999). Evidence for light limiting phytoplankton growth has been found in both commercial penaeid prawn ponds (Burford 1997) and in *M. rosenbergii* ponds (Costa-Pierce et al. 1984).

When ponds are too turbid, farmers may flush ponds with fresh, clean water to reduce nutrient levels to discourage phytoplankton growth. Flushing can also dilute the amount of particulate matter to reduce turbidity. If not sufficiently turbid they may add fertilizer to stimulate phytoplankton blooms.

2.3.5 Dissolved oxygen

Dissolved oxygen is recognized as one of the most important water quality parameters for aquatic organisms, affecting a variety of physiological processes. Low oxygen, or hypoxia, has been found to inhibit moulting and growth and cause mortality in Penaeid prawns (Clark 1986, Allan and Maguire 1991). In freshwater habitats, dissolved oxygen levels can fluctuate markedly, particularly on the bottom of growout ponds where prawns spend most of their time (Cheng et al. 2003).

Hypoxia (low oxygen) in aquaculture ponds is due in particular to the respiration of all present organisms, water temperature and the decomposition of faecal matter and uneaten feed. Farm managers try to control dissolved oxygen levels by controlling stocking densities of the cultured species, feeding level, paddle-wheel aeration, temperature control, and by managing algal species composition and abundance.

In aquaculture, high dissolved oxygen is vital (Costa-Pierce et al. 1984). In *M. rosenbergii*, survival rate is most closely linked to dissolved oxygen than any other water quality parameter (New 1990). Dissolved oxygen levels down to 1 ppm can be tolerated (Avault 1987) but stress is visible when levels drop below around 4 ppm. Farm managers generally aim to keep levels at 6-8 ppm (New 1990).

2.4 New Zealand Prawns Limited

New Zealand Prawns Ltd (NZPL) is a commercial freshwater prawn farm located just north of Taupo in Wairakei, New Zealand. It was established in 1987 to investigate the feasibility of culturing *M. rosenbergii* in a temperate country using

geothermal water to heat fresh water from the Waikato River to the species' optimal temperature of 28°C.

The current population of *M. rosenbergii* at NZPL was founded in 1987 by 20 males and 5 females imported live from Malaysia. A further 25 males and 30,000 post-larvae were imported from the same source one year later (Wear 1996). Importing the foreign species into New Zealand was helped by the fact that they would be unable to establish themselves in the wild and compete with native species if they ever escaped the confines of the farm (Wear 1991). This is because the tropical species would be unable to tolerate the cold winter temperatures of New Zealand; the warming of freshwater with geothermal water within the prawn farm is the key to their survival.

NZPL now consists of indoor brood-stock holding tanks, hatchery facilities and nursery tanks for culturing larvae and nursing post-larvae, and 19 outdoor growout ponds with depths ranging from 0.8 to 1.2 metres (see Diagram 1, below). Good quality freshwater (well oxygenated, low in nutrients) from the Waikato River is brought up to the required temperature using a plate heat exchange device (similar in principal to a car radiator) with waste geothermal water from a neighbouring geothermal power plant run by Contact Energy Ltd. Geothermal water cannot be used directly because the heavy metal content is far too toxic for aquaculture.

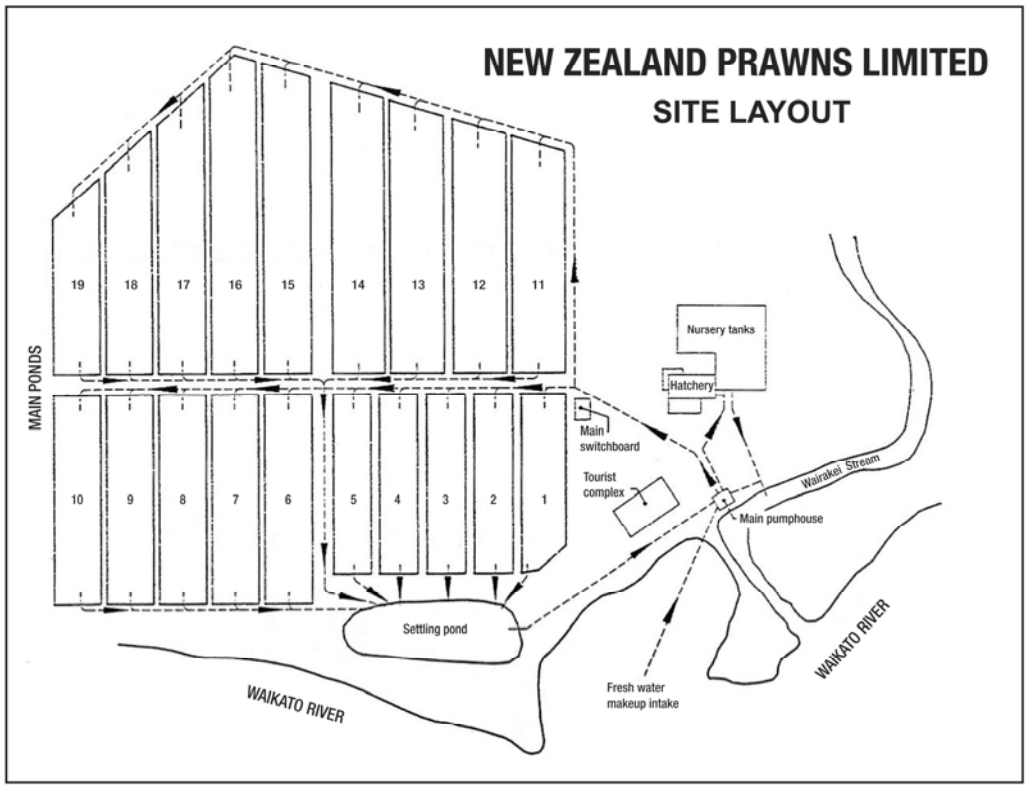


Diagram 1 – site layout, New Zealand Prawns Ltd

Post-larval prawns of between 0.5 and 5.0 grams are stocked from nursery tanks into empty growout ponds. Harvest occurs around three months later if a sub-sample of prawns shows that the mean weight of individuals is marketable (25g+). When a pond is ready for harvesting, water is completely drained and prawns are collected by hand from the bottom once the pond is empty.

In order to help keep ponds oxygenated and warm, water is constantly flowing into and out of the ponds. Complete turnover of water in each pond takes an average of three days in summer, and one and a half days in winter. Turnover is higher in winter because the colder ambient air temperature cools pond water more rapidly than in summer. Water flowing out of ponds (including water drained at harvest) ends up in a 'settling pond' rather than being expelled into the Waikato River. Water from the

settling pond is pumped back to the heat exchanger where it is brought back up to the required temperature. This ranges from 39°C in the peak of summer to 58°C in the peak of winter. These differences in water heating temperatures are again due to differences in ambient air temperatures between seasons. Water is then recycled back into the growout ponds.

While water first entering grow out ponds is hot enough to be lethal to prawns, it cools rapidly to the optimum temperature of 28°C, and prawns do not inhabit the ends of ponds where water enters. Fresh water is taken from the Waikato River when water levels in the growout ponds get low. This is due mainly to evaporative loss which on a daily basis farm managers estimate to be around 1% of total farm water volume in summer and 2% in winter. Once again, this is due to the seasonal differences in ambient air temperature.

2.5 Algal blooms in freshwater prawn culture

Algal blooms have had detrimental impacts in the culture of *M. rosenbergii*. Green et al. (1977) reported anoxic conditions following an algal bloom as being responsible for a mass kill (45%) of pond-reared *M. rosenbergii* in an experiment on an outdoor culture pond at the University of Malaya. The algal bloom was believed to have been caused by over-fertilization of the pond with feed, exacerbated by heavy rain causing suspension of organic material which in turn stimulated bacterial growth. The combination of algal and bacterial respiration at night caused the pond to become anoxic, causing the kill. On the morning the kill was discovered, dissolved oxygen was 0.5 ppm, lower than the 1 ppm reported by Avault (1987) as being the lower limit *M. rosenbergii* can tolerate.

Blooms of benthic algae in spring and summer have been observed in growout ponds at NZPL. Farm managers have noticed that ponds in which these blooms occur often have dramatically reduced yield. Qualitative notes included with the production data dating back as far as 2000 show that on many occasions benthic algal blooms have coincided with very low yields, often less than 2 marketable tonnes per hectare (MTPH) and as low as 1 MTPH, while the overall mean for all ponds is 4.14 MTPH (calculated from data from January 1st 2000 to 28th March 2007). The lowest recorded harvest is 0.8 MTPH on April 8th 2004 but there are no notes included to suggest why the harvest was so poor. It is thought that benthic algae impair the ability of prawns to forage for food, which is of great concern in the farming of *M. rosenbergii*. Allan and Maguire (1994) found that in model ponds used to culture the Penaeid prawn *Penaeus monodon*, individual prawn growth (weight gain), biomass gain and food conversion efficiency were significantly lower ($p < 0.05$) in ponds where filamentous benthic algae was stimulated to bloom compared to ponds where phytoplankton was stimulated to bloom.

While pelleted feed is used at NZPL, Δ-carbon studies on prawns have shown they depend mainly on naturally occurring food regardless of whether pelleted feed is available or not (Schroeder 1983). In an experiment by Tidwell et al. (1997) *M. rosenbergii* were grown in ponds receiving three different treatments for four months. Prawns in treatment one were fed a complete diet of pelleted feed containing 32% protein. In treatment two prawns were not fed but the ponds in which they were grown were organically fertilized with distillers dried grains with solubles at a rate determined to be equal in nitrogen content with treatment one. Prawns in the third treatment were unfed and the ponds received no fertilizer. They found that prawns in treatment three were significantly smaller than those in treatments one and two, but

prawns in treatments one and two were not significantly different from one another in size, indicating that natural productivity could be at least as important as supplementary feeding. Green et al. (1977) also report that most food obtained by prawns is from naturally occurring sources.

In an experiment by Correia et al. (2002), *M. rosenbergii* grown for 63 days had significantly greater growth (more than double) in older ponds than in newer ones. Older ponds displayed higher levels of natural productivity, greater macroinvertebrate diversity and had 200 times more zoobenthos than newer ponds. The greater diversity and abundance of macroinvertebrates in older ponds was also reflected in the stomach contents of prawns grown in older ponds when compared to prawns grown in newer ponds. With the importance of naturally occurring food having been well demonstrated for *M. rosenbergii*, impairment in their ability to forage is of great concern.

The omnivorous diet of *M. rosenbergii* includes aquatic insects and larvae, algae, nuts, seeds, grains, fruits, molluscs, crustaceans, fish flesh and offal (New 1990). Insects, insect larvae, algae, crustaceans and fish have all been observed in growout ponds at NZPL by farm staff and myself. It is quite possible that fruits, grains and seeds dispersed by wind could be present at NZPL too. Any impairment in prawn foraging ability at NZPL is therefore of great concern.

Filamentous algae have also been found in the gill cavities of some prawns at harvest by farm staff, suggesting that algae may affect prawns directly through choking. Harvests after algal blooms appear to show a reduction in the *number* of individual

prawns as well as overall biomass. This suggests that blooms might cause mortality directly, as well as cause stunted growth through impaired foraging.

This study aims to investigate what variables related to water quality differ between ponds at NZPL, the effect these variables have on abundance and species composition of phytoplankton, and the effects they may have on the production of marketable prawns. Funding was gratefully received from a Technology Industry Fellowship provided by the Foundation for Research, Science & Technology (FRST).

3. MATERIALS AND METHODS

3.1 Field collection

Water samples were taken at 9-11 day intervals between 8th November 2006 and 28th March 2007 (for the complete sampling schedule and raw data see the attached compact disc). All sampling in the field was done at the same time each day (approximately 10:00am-noon). Water samples were taken from all 19 growout ponds. Ponds 10 and 19 were later randomly selected to be dropped from sampling due to budget constraints. Pond 12 was later dropped from the study as it did not get harvested during the sampling period. Samples were also taken from the farm's settling pond, as well as the Waikato River to enable monitoring of any changes that may occur in the farm's water source during the study.

500ml of pond water was taken from the centre of each pond, within 50cm of the bottom (the area of the ponds actually inhabited by prawns). Half of each water sample was fixed in Lugol's iodine solution (Montagnes et al. 1994) for identification and quantification of phytoplankton. The other half was frozen at -20°C for nutrient and chlorophyll *a* analysis.

Water temperature was measured using a Check-Temp 100-model (Aircon Ennis Ltd, Noughaville, Ireland) and dissolved oxygen was measured at the same time as water collection using an Insight IG 3100 dissolved oxygen meter.

Pond turbidity was measured using standard procedures with a 20cm diameter secchi disk (Koenings and Edmundson 1991). A secchi disk is a weighted disk divided into quarters. Two of the quarters are black and the other two are white to provide

contrast. The secchi disk was suspended by a cord marked at every centimetre and lowered into the water column. The disk was lowered until it could no longer be seen from the surface and this depth was recorded to the nearest centimetre using the markings on the cord.

3.2 Chlorophyll a *analysis*

Chlorophyll *a* was measured as an index of phytoplankton biomass. Measurement of chlorophyll *a* does not give a direct measure of phytoplankton biomass nor is there a standard formula to convert chlorophyll *a* readings into biomass, but several studies that have measured both have found significant positive correlations between the two (Desortova 1981, Canfield et al. 1985, Voros and Padisak 1991).

25ml sub-samples of water were taken from each 500ml sample and filtered through 47mm Whatman GF/F (catalogue number 1825 047) glass microfibre filters using a Millipore vacuum filter apparatus (model XX1004700) in a dimly-lit laboratory. Filters were wrapped in aluminium foil and kept frozen at -20°C in total darkness until chlorophyll *a* was ready for methanol extraction.

Chlorophyll *a* was extracted from filters by soaking them in 10 ml of analytical grade methanol (99.9%) for 24 hours in total darkness. While in the past acetone has been commonly used as an extraction solvent, methanol has been found to extract chlorophyll *a* faster and more thoroughly from phytoplankton than acetone, and does not interfere with fluorometric analysis (Holm-Hansen and Riemann 1978) which this study used. A Turner Designs Fluorometer (Model # 10-AU-005, Sunnyvale, California, USA) was used to take an initial reading from a 3ml sub-sample of the

methanol extract. Five drops of 1M hydrochloric acid was added to the 3ml sub-sample and another reading taken to correct for phaeophytins as per the fluorometer manufacturer's instructions. The difference between the two readings gives the actual measure of chlorophyll *a* (parts per million, ppm).

When chlorophyll *a* degrades (for instance, due to the death of an algal cell) waste products known as phaeophytins can remain in the water. Phaeophytins fluoresce at a similar but slightly shorter wavelength in the red light spectrum than chlorophyll *a* (Lorenzen 1967) and contribute to the reading given by the fluorometer. Phaeophytins must therefore be accounted for in fluorometric analysis to avoid obtaining falsely high chlorophyll *a* readings (Richards and Thompson 1952). Adding acid to the methanol extract converts the chlorophyll *a* molecules it contains into phaeophytins and the fluorescence of the extract is reduced (due to the shorter wavelength at which phaeophytins fluoresce). Thus we can calculate the concentration of chlorophyll *a* in the methanol extract by taking the difference in fluorometer readings from before and after the addition of acid.

All steps in the analysis of chlorophyll *a* were carried out in a dimly lit laboratory and all samples were stored in total darkness between steps in the process. This was to prevent degradation of chlorophyll *a* into phaeophytins by sunlight and fluorescent light, as recommended in the Turner Designs fluorometer instruction manual, which would have resulted in falsely low readings of chlorophyll *a*.

3.3 Nutrient analysis

Orthophosphate and ammonia levels were measured using an Aqua Analyzer (Orbeco-Hellige, model 952, Farmingdale, New York, USA). The catalogue numbers for the orthophosphate and ammonia test kits were 957-77 and 957-43 respectively. Nitrate was measured using an Analyst (Orbeco-Hellige, model 975MP, Farmingdale, New York, USA). The catalogue number for the nitrate test kits was 975-07.

The Aqua-Analyzer 952 measures nutrient levels by colorimetric analysis. Procedures vary depending on the nutrient being analyzed but typically a 'blank' sample is poured into a vial. This is sample water that has not had any chemical reagents added to it. The vial is placed in the Aqua-Analyzer and light is passed through it. The machine measures the amount of light transmitted (as a percentage) through the blank vial and the machine is then calibrated so that the transmittance reads 100%. The sample then has various chemical reagent(s) added to it and left for a specified time (the reagents used and the time varies depending on the nutrient being measured) during which it will change colour. The darkness of this colour will depend on the concentration of the chemical in the sample being analyzed. After the specified time the vial is placed back in the Aqua-Analyzer which will give a different reading to the blank due to the change in colour after the addition of reagents. The percentage of light transmitted through the sample is looked up in tables in the manual which gives a corresponding concentration of the nutrient in parts per million.

The Analyst 975MP also measures nutrients colorimetrically but differs from the Aqua-Analyzer in that it gives a direct reading of the nutrient concentration in parts per million, with no need to consult tables. The use of blanks and chemical reagents is still required. Exact details of the test procedures are available in the manuals of these machines.

While phytoplankton can assimilate and use a variety of forms of nitrogen (e.g. nitrate, nitrite, ammonia) the budget and available equipment prevented all forms of nitrogen from being measured. Given that pond water is recycled at NZPL, and prawns are fed a high protein diet and farmed semi-intensively, nitrogen is not expected to be a limiting factor for algae. Ammonia was measured because of its potential toxicity to prawns (Naqvi et al. 2007) and because it is the most preferred form of nitrogen for phytoplankton (Hargreaves 1998). Nitrate was measured because it is also utilized, although it requires enzymatic reduction to ammonia within phytoplankton cells before it can be incorporated into amino acids. This makes it a less energy-efficient alternative to ammonia (Hargreaves 1998).

3.4 Phytoplankton identification & quantification

Phytoplankton identification was carried out using an inverted microscope and sedimentation chamber using the method described by Utermohl (1958). Samples fixed by Lugol's solution were well shaken to evenly distribute algal cells that may have settled to the bottom of sample jars. Without shaking the samples would have been biased towards those algal cells that sink slowly and stay in the water column near the top of the jar. After shaking, 0.5ml sub-sample of water was placed into

each sedimentation chamber and allowed to settle for 24 hours. Where possible, phytoplankton were identified down to genus level. Where not possible, they were grouped into either "pennate diatoms" or "coccoid unicells" as the case may be. Texts used to identify phytoplankton were Moore (2000) and Wehr & Sheath (2003). One other alga was noticed in multiple ponds on two sampling dates and could not be identified from texts. This alga was referred to in all ponds on both dates as "*Eyes sp.*" See Appendix 2 for photographs of phytoplankton identified in this study.

Different algal taxa were identified and counted under each field of view (FOV) under the microscope within each sedimentation chamber. Counting stopped when enough FOVs within each chamber had been viewed to reach 200 or more algal cells total (N.B. *all* cells within the FOV in which the 200th cell was counted were also counted and included). The number of FOVs required to reach 200 cells or more was also noted so that the mean number of algal cells per FOV could be calculated (no. algal cells counted/no. of FOVs).

The volume of water within each FOV was also calculated so that total cell counts could be standardized to cells/ml using the following equation:

Average no. of cells per ml = average no. of cells per FOV × no. of FOVs per ml

The Shannon Index of Diversity (Shannon and Weaver 1949) was then calculated for each pond for each sampling date using mathematical functions available in Microsoft Excel 2003. The Shannon Index of Diversity (*H'*) is commonly used in biological monitoring programmes and to measure species richness or diversity

(Spellerberg and Fedor 2003) and can be applied to both terrestrial and aquatic ecosystems, and plants and animals (Baker 1992, Abbot et al. 2006, Smith and Lester 2006) and is the most commonly used diversity index for phytoplankton (Figueredo and Giani 2001). The index is given by the equation:

$$H' = -\sum_{i=1}^{s} (P_i \ln[P_i])$$

where P is the proportion of individuals in the *i*th species, s is the total number of species and ln is the natural logarithm.

The Shannon Index of Diversity is being used in this study to investigate 1) if the species diversity of the phytoplankton community in ponds at NZPL may affect prawn yield, and 2) if the species diversity is influenced by the water quality parameters measured. If it turns out that phytoplankton species diversity does in fact influence prawn yield, it would then be useful to know what influences species diversity (and therefore prawn yield).

3.5 Statistical analyses

All statistical analyses were carried out using SPSS 14.0 for Windows.

Measurements of average depth for each pond were obtained from NZPL, as was prawn yield at harvest. Yield was standardized to MTPH (Marketable Tonnes Per Hectare) to allow for the differences in pond size. Production data from January 1st 2000 was also compiled in order to assess if there were any historical differences in prawn yield between ponds.

One-way Analysis of Variance (ANOVA) is a statistical technique used to determine whether there are any differences in mean between groups or treatments for a given variable of interest (Quinn and Keough 2003). Differences in mean values between ponds were analysed for each variable measured using one-way ANOVA. When ANOVA assumptions of normality and homoscedacity were violated, natural log transformations of data were made. If the ANOVA assumption of normality was still violated after transformation, Kruskal-Wallis tests were used in place of ANOVA to compare medians between ponds (Quinn and Keough 2003). If after transformation the assumption of equality of variance was still violated, Brown-Forsythe tests were used to compare means (Quinn and Keough 2003). Unfortunately, both the Kruskal-Wallis and Brown-Forsythe tests have less power than ANOVA to detect departures from the null hypothesis (in this case that there are differences in means of measured variables between ponds) where such departures exist. Therefore one-way ANOVA was used over Kruskal-Wallis and Brown-Forsythe tests wherever possible.

Three different step-wise multiple linear regressions were run to investigate which variables were most influential on prawn yield, the number algal cells per ml and phytoplankton diversity. This method uses one or more explanatory or predictor variables to explain or predict a response variable (Moore and McCabe 2003). An initial multiple linear regression is performed, after which the explanatory variable with the least statistical significance is removed from the analysis. The procedure is repeated and the explanatory variable with the least significance is again removed. This continues until only statistically significant explanatory variable(s) remain. If there are none left, then none of the explanatory variables are satisfactory predictors of the response variable.

All measured water quality variables (dissolved oxygen, temperature, chlorophyll *a*, ammonia, orthophosphate, nitrate, turbidity, phytoplankton cells per ml and Shannon Index of Diversity) were used as explanatory variables as well as mean pond depth, with prawn yield in Marketable Tonnes Per Hectare (MTPH) as the response variable. Only production data from within the sampling period was used in this analysis.

For the multiple regression on prawn yield for the sampling period, the mean value of each predictor variable for each grow out period was used. For example, while the overall mean ammonia concentration in Pond 4 for the entire study was 0.0163 ppm, the mean ammonia concentration was 0.0217 ppm between stocking of Pond 4 on 22/11/06 until its harvest on 27/2/07, so the latter value was used in the regression. This was done for each predictor variable for each pond. Where information on water quality variables for an entire grow out period was incomplete, the information available was used.

A second multiple regression was done to see what variables measured were the most influential on phytoplankton species richness. The water quality parameters chlorophyll *a*, dissolved oxygen, temperature, turbidity, nitrate, ammonia and orthophosphate were used as the explanatory variables with the Shannon Index of Diversity as the response variable.

A third and final multiple regression was carried out in order to see what water quality parameters were most influential on phytoplankton biomass. Chlorophyll *a* (the index of phytoplankton biomass for this study) was used as the response variable with dissolved oxygen, temperature, turbidity, nitrate, ammonia, orthophosphate, turbidity, and the Shannon Index of Diversity as explanatory variables.

To test for changes over time in the different variables, repeated measures ANOVA (Quinn and Keough 2003) was used. In repeated measures ANOVA, it is the difference or change in response variable(s) over time that we are investigating, rather than differences in mean between groups or treatments for variable(s) of interest as in regular ANOVA. For example, we may use regular ANOVA to test whether or not there is a significant difference between ponds for the mean number of phytoplankton cells per ml of water at NZPL, and we may use repeated measures ANOVA to see if there is a change over *time* in the number of phytoplankton cells per ml of water.

4. RESULTS

This study sought to investigate what water quality variables impact prawn yield, and what water quality variables impact phytoplankton diversity and abundance at New Zealand Prawns Ltd, how these variables change over time, and whether they differ between ponds. For investigating impacts on prawn yield, phytoplankton diversity and abundance were themselves included in analyses as water quality variables of potential influence. Water samples were taken at 9-11 day intervals between November 8th 2006 and March 28th 2007. The following water quality parameters were measured from water samples:

- Phytoplankton abundance and diversity
- Chlorophyll *a* concentration
- Ammonia
- Nitrate
- Orthophosphate
- Secchi disk depth (turbidity)
- Temperature
- Dissolved oxygen

The water quality variables measured were compared between ponds to investigate any differences. Yield of marketable prawns was also recorded during the sampling period and used as the response variable in a multiple regression to investigate what (if any) impacts recorded water quality variables may have on prawn production. A further two multiple regressions were also carried out with chlorophyll *a* and the Shannon Index of Diversity as response variables. Repeated measures ANOVA

could not be used to investigate changes over time in different variables due to gaps in the data.

4.1 Differences between ponds

Few differences were found between ponds for the water quality variables sampled and yield of marketable prawns. Table 3 summarises the outcomes of tests for differences in water quality variables between ponds.

***Phytoplankton abundance & diversity, and chlorophyll* a**

One way ANOVA showed a significant difference between ponds for the mean number of phytoplankton cells per pond ($p = 0.003$, natural log transformed data). Post-hoc pair-wise comparisons (Tukey tests) found Ponds 2, 3 and 4 all had significantly higher numbers of algal cells per ml than Pond 18 ($p = 0.031$, 0.027 and 0.043 respectively). A barplot of the mean number of phytoplankton cells per ml for each pond is shown in Figure 1.

The highest number of cells per ml was 2.99×10^5 in Pond 3 on 17th January 2007 and lowest in Pond 17 on 8th December 2006 at 1.58×10^4 cells per ml. The overall mean number of cells per ml for the entire sampling period was 6.95×10^4. Coccoid unicellular green algae were by far the dominant group, on average accounting for 69.9% of total cell counts. A complete list of all phytoplankton taxa and mean proportion of each taxa for the sampling period is shown here in Table 1.

Table 1: Complete list of phytoplankton taxa found at New Zealand Prawns Ltd, listed in descending order of abundance

Algal taxa	Mean proportion of total
Coccoid unicellular green algae	0.699
Coelastrum	0.093
Filamentous cyanobacteria	0.039
Scenedesmus	0.037
Anabaena	0.035
Merismopedia	0.027
Pennate diatoms	0.023
Dictyosphaerium	0.017
Pediastrum	0.014
"Eyes sp."	0.006
Staurastrum	0.004
Golenkinia	0.002
Oscillatoria	0.002
Thinner filamentous cyanobacteria	0.001
Euglena	0.001
Ceratium	0.001
Oocystis	7.46^{-5}
Lyngbya	4.33^{-5}
Spirogyra	2.05^{-5}

No significant difference in phytoplankton diversity was found between ponds (Kruskal-Wallis test, $p = 0.06$). The highest score for the Shannon Index of Diversity was 1.76, recorded in Pond 3 on 17th January 2007. The lowest diversity score was 0.42 in Pond 9 on 14th February 2007. Overall mean species diversity for all ponds was 1.02 for the entire sampling period. Mean species diversity for all ponds is shown in Figure 2.

Figure 1: Mean number of phytoplankton cells per ml for all sampled ponds, entire sampling period (error bars are standard deviations).

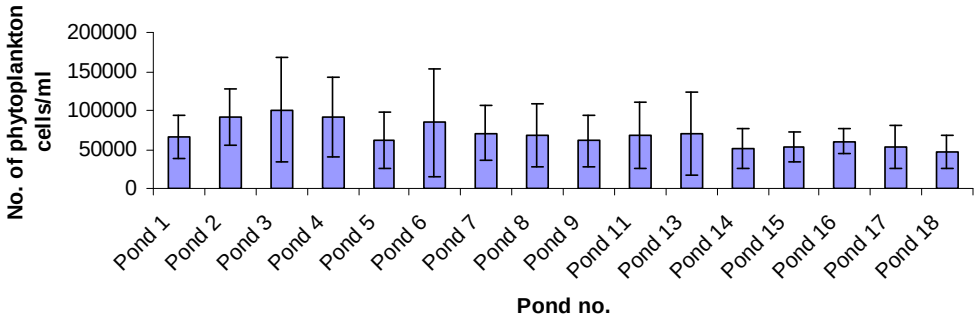


Figure 2: Mean phytoplankton diversity (*H'*) for all sampled ponds, entire sampling period (error bars are standard deviations).

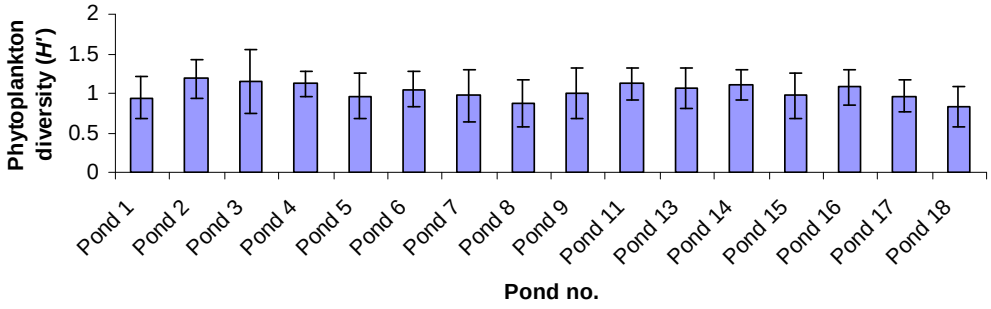
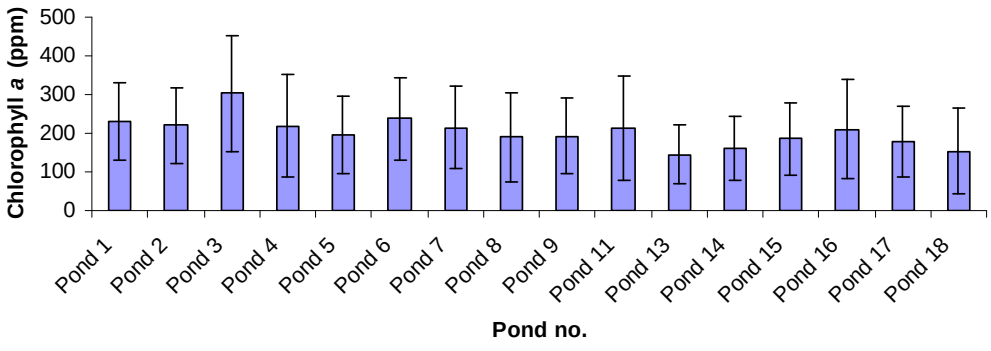
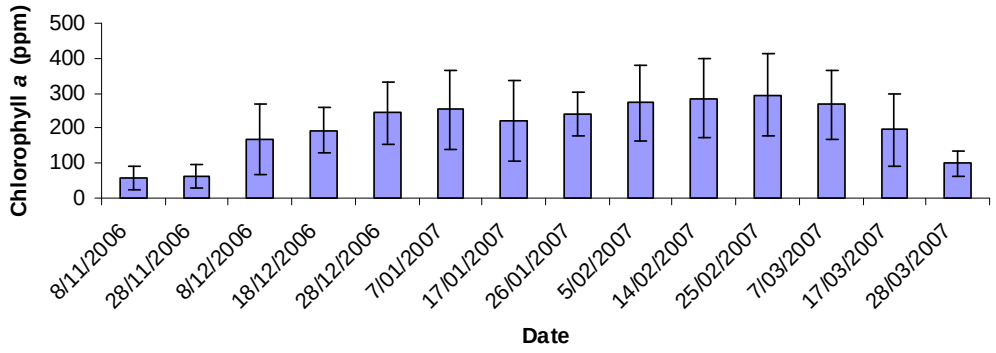


Figure 3: Mean chlorophyll *a* levels for all sampled ponds, entire sampling period (error bars are standard deviations).



No difference in chlorophyll *a* was found between ponds (Kruskal-Wallis test, p = 0.278). Chlorophyll *a* levels peaked at 571 parts per million (ppm) in Pond 3 on 25th February and were lowest in Pond 13 at 6.14 ppm on 8th November 2006. A lot of filamentous benthic algae, consisting mainly of *Oedogonium sp*. was observed in Pond 13 on this day and throughout much of the sampling period. Farm managers decided on manual removal of the benthic algae from this pond. Overall mean chlorophyll *a* was 204.5 ppm for the entire sampling period. Mean chlorophyll *a* levels for each pond during the sampling period are shown in Figure 3. Mean chlorophyll *a* levels by date are shown in Figure 4.

Figure 4: Mean chlorophyll *a* levels by date (error bars are standard deviations).



Nutrients

There were no significant differences between mean ammonia levels between ponds (Browne-Forsythe test, p = 0.172). The highest recorded ammonia level for the sampling period was 0.670 parts per million (ppm) in Pond 15 on 8th November, 2006. The lowest *detected* level of ammonia was 0.003 ppm recorded in most ponds on multiple occasions. All ponds had at least one occasion during the sampling period where ammonia levels were too low to be detected. The overall mean

ammonia level for the sampling period was 0.066 ppm. Mean ammonia levels for each pond are shown in Figure 5.

No significant differences between ponds were detected for mean nitrate levels (Browne-Forsythe test, $p = 0.571$). Pond 7 had the highest recorded nitrate level on 18th December 2006 with 0.22 ppm. The lowest recorded nitrate level was 0.01 ppm and was shared by all ponds except Pond 6 at least once in the sampling period. Nitrate was also too low to be detected on multiple occasions for several ponds. The overall mean nitrate level for the sampling period was 0.034 ppm. Mean nitrate levels for each pond are shown in Figure 6.

Mean orthophosphate did not differ significantly between ponds (Kruskal-Wallis test, $p = 0.218$). The highest recorded orthophosphate concentration was 0.71 ppm recorded in Pond 2 on 26th January 2007. Ponds 3, 9 & 16 all share the lowest recorded concentration of 0.05 ppm. Mean orthophosphate for the sampling period was 0.21 ppm. Mean orthophosphate levels for each pond are shown in Figure 7.

Figure 5: Mean ammonia levels for all sampled ponds, entire sampling period (error bars are standard deviations).

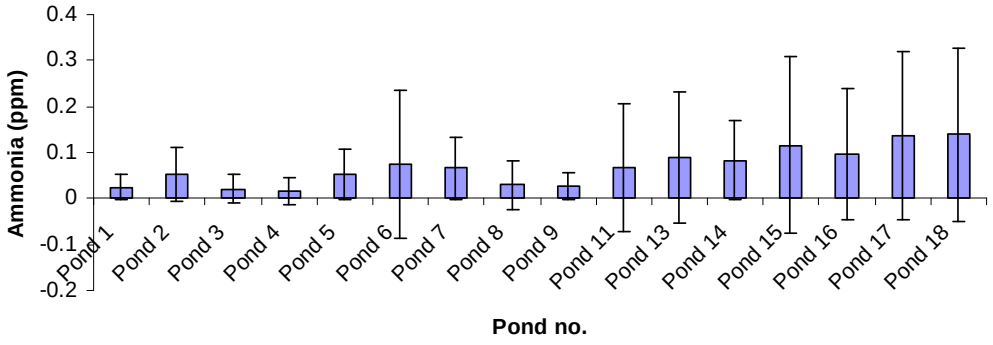


Figure 6: Mean nitrate levels for all sampled ponds, entire sampling period (error bars are standard deviations).

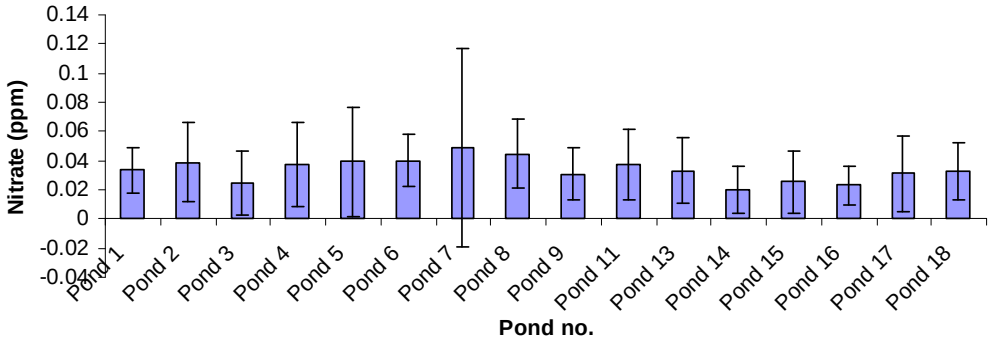
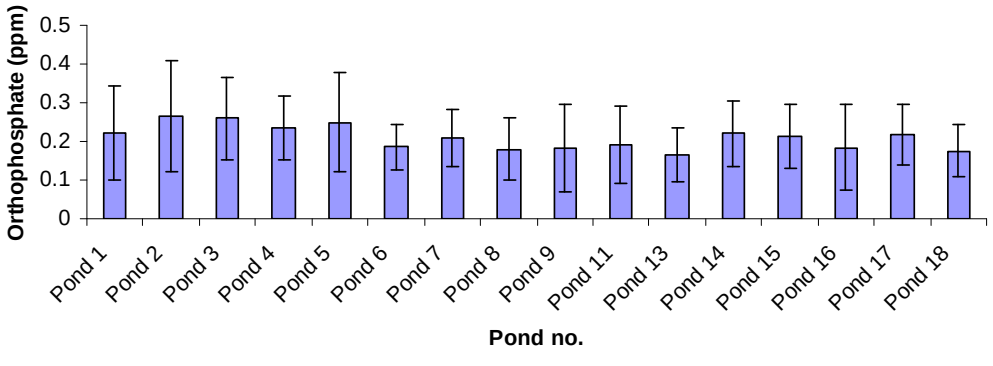


Figure 7: Mean orthosphosphate levels for all sampled ponds, entire sampling period (error bars are standard deviations).



Temperature

Mean temperature did not differ significantly between ponds (Kruskal-Wallis test, $p = 0.971$). The highest recorded was in Pond 3 at 30.2°C on 8th November 2006. The lowest temperature recorded was 25.5°C in Pond 4 on 28th November 2006. This abnormally low temperature occurred during maintenance by Contact Energy Ltd on their power plant (during which time the prawn farm had no geothermal water supply). The overall mean temperature for the sampling period was 27.7°C, close to the optimum temperature. Mean temperatures for each pond are shown in Figure 8.

Dissolved oxygen

There were no significant differences between ponds for mean dissolved oxygen (one-way ANOVA, $p = 0.720$). The highest dissolved oxygen level was in Pond 15 on 25th February 2007 with a concentration of 11.6 ppm. The lowest recorded level was in Pond 8, 4.5 ppm on 17th March 2007. Overall mean for the sampling period was 8.4 ppm, and the mean dissolved oxygen levels for each pond are shown in Figure 9.

Secchi depth (turbidity)

Mean secchi depth was significantly different between ponds (Kruskal-Wallis test, $p = 0.007$). Unfortunately no post-hoc pair-wise comparison tests exist for the Kruskal-Wallis test so we cannot determine which ponds were different in turbidity. The highest secchi depth (lowest turbidity) recorded during the sampling period was 124cm, shared by Ponds 3 & 18 on 8th November 2006 and 17th January 2007 respectively. As well as sharing the lowest turbidity recording, Pond 3 also had the highest turbidity recording with a secchi depth of 30cm on 25th February 2007. The

overall mean secchi depth for the sampling period was 59.47 cm, with a standard deviation of 18.31cm. Mean secchi depth for all ponds is shown in Figure 10.

Prawn yield

From the *historical* data, yield of marketable sized prawns was significantly different between ponds (one-way ANOVA, $p = 0.007$). A post-hoc Tukey test found Ponds 3 and 16 differ significantly from each other with mean yields of 4.86 and 2.83 marketable tonnes per hectare (MTPH) respectively. The highest recorded yield ever came from Pond 11 on 8th September 2004 with 7.1 MTPH. Pond 16 has the lowest recorded yield of 0.8 MTPH on 8th April 2004. Mean prawn yield for all ponds between 1 January 2000 and 28 March 2007 is shown in Figure 11. A comparison between Ponds 3 and 16 for all variables measured during the *sampling* period is shown in Table 2. No information is available for these variables for the period in which historical yield data has been compiled.

Table 2: Comparison between Ponds 3 & 16 for all variables, entire sampling period.

Variable	Pond no.	Mean	Std. Dev.	Range
DO2(mg/L)	3	8.24	1.76	5.6-11.1
	16	8.96	1.43	7-10.7
Temp°C	3	27.7	1.4	25.5-30.2
	16	27.81	1.22	25.5-28.8
Secchi(cm)	3	49.38	23.88	30-124
	16	56.67	11.99	21-77
Chlorophyll *a* (ppm)	3	303.48	155.52	19-571
	16	210.35	137.9	34-417
Ammonia (ppm)	3	0.02	0.03	0-0.1
	16	0.1	0.14	0-0.38
Orthophosphate (ppm)	3	0.26	0.11	0.05-0.48
	16	0.18	0.11	0.05-0.39
Nitrate (ppm)	3	0.02	0.02	0-0.08
	16	0.02	0.01	0.01-0.04
Cells per ml	3	1×10^5	6.7×10^4	3.1×10^4 - 2.99×10^5
	16	6.03×10^4	1.63×10^4	3.82×10^4- 8.53×10^4
Pond depth (mm)	3	1155	n/a	1000-1310
	16	1085	n/a	1070-1100
Shannon Index	3	1.15	0.4	0.65-1.76
	16	1.07	0.22	0.64-1.34
Yield (marketable tonnes per hectare)	3	5.12	n/a	5.12-5.12
	16	3.04	n/a	3.04-3.04

Due to the low number of harvests (1-2 harvesting events per pond) mean yield between ponds cannot be compared for the sampling period. The highest yield for the sampling period came from Pond 4 with 5.88 MTPH, harvested on 16th November 2006. The lowest yield came from Pond 7 with 2.86 MTPH, harvested on 19th December 2006. Pond 6 had the lowest *mean* yield of 2.9 MTPH and Pond 1 had the highest mean yield with 5.42 MTPH. Overall mean yield was 4.18 MTPH. The mean yield for each pond during the sampling period is shown in Figure 12. As most ponds were only harvested once during the sampling period most of these 'mean' values are actually values from single harvests (and hence error bars displaying standard deviations are not present for most ponds in Figure 12).

Figure 8: Mean temperature for all sampled ponds, entire sampling period (error bars are standard deviations).

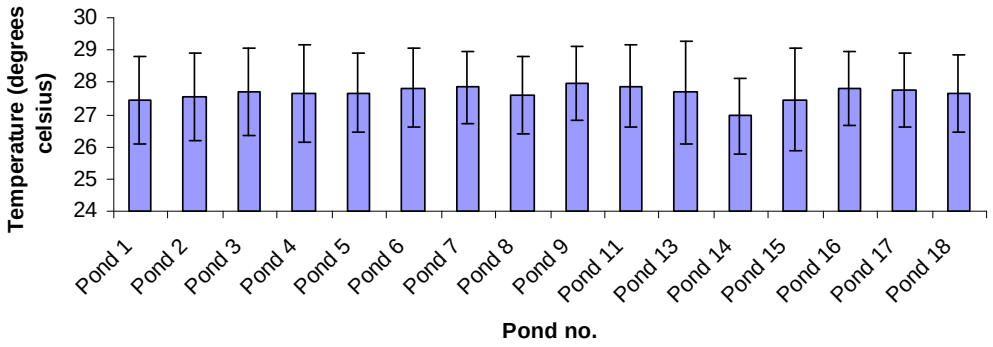


Figure 9: Mean dissolved oxygen for all sampled ponds, entire sampling period (error bars are standard deviations).

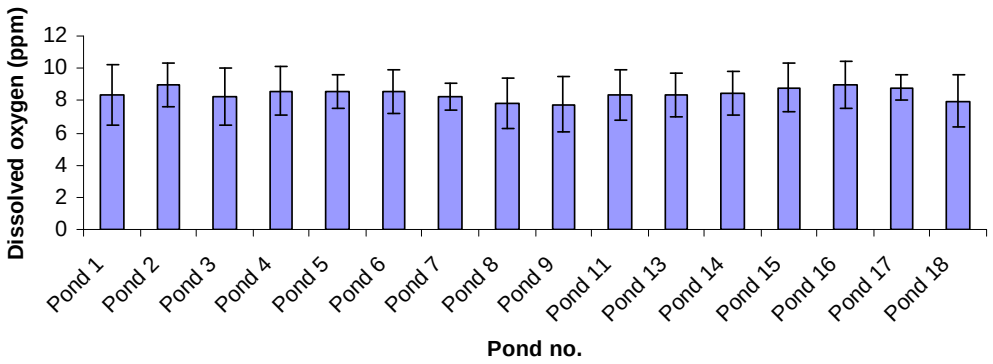


Figure 10: Mean secchi depth for all sampled ponds, entire sampling period (error bars are standard deviations).

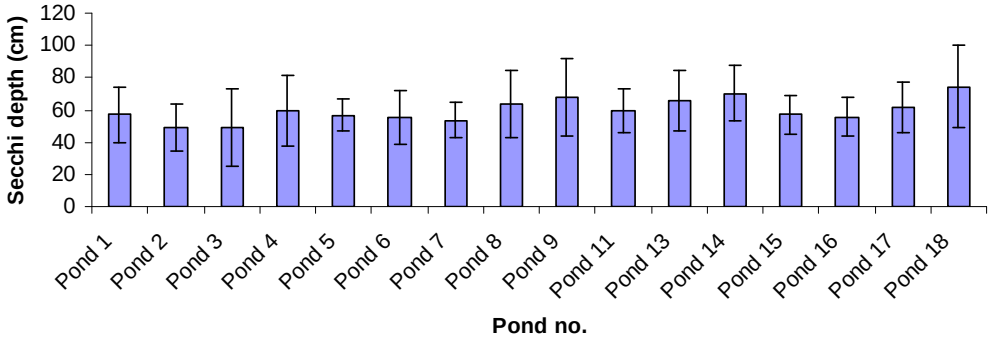


Figure 11: Mean prawn yield for all ponds between January 1st 2000 and March 28th 2007 (error bars are standard deviations).

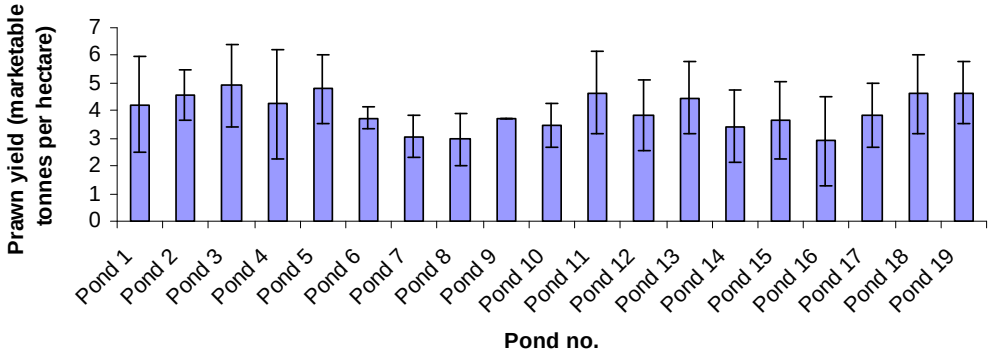


Figure 12: Mean prawn yield for all sampled ponds, entire sampling period (error bars are standard deviations).

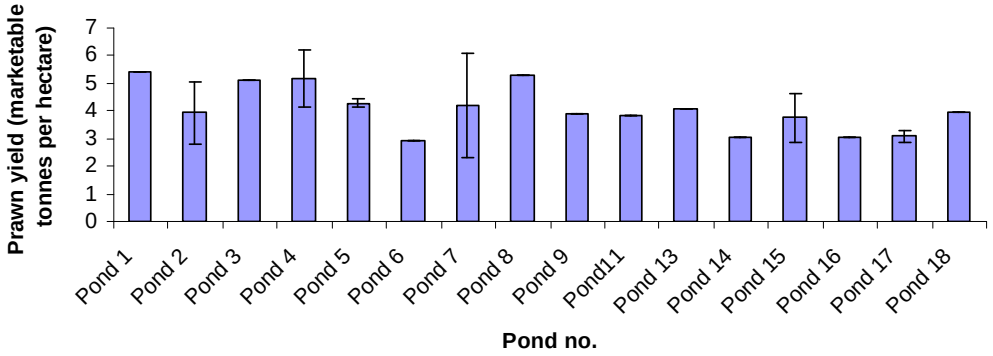


Table 3: Summary of outcomes of tests for differences between ponds for water quality variables.

Variable	Test used	*P*-value	Significant?
Historical prawn yield (MTPH)	ANOVA	0.007	Yes
Algal cells per ml	ANOVA	0.003	Yes
Secchi depth (cm)	Kruskal-Wallis	0.007	Yes
Chlorophyll *a* (ppm)	Kruskal-Wallis	0.287	No
Phytoplankton diversity (H')	Kruskal-Wallis	0.06	No
Temperature (°C)	Kruskal-Wallis	0.971	No
Ammonia (ppm)	Kruskal-Wallis	0.459	No
Nitrate (ppm)	Kruskal-Wallis	0.304	No
Orthophosphate (ppm)	Kruskal-Wallis	0.218	No
Dissolved oxygen (ppm)	ANOVA	0.72	No

4.2 Step-wise multiple linear regressions

Prawn yield

None of the water quality parameters sampled were found to be statistically significant predictors of yield as a response variable. We cannot make any valid future predictions of yield based on water quality parameters based on the data from this study.

Table 4: Summary of multiple regression of water quality variables on prawn yield

Predictor variable	*P*-value	Significant?
Ammonia	0.171	No
Nitrate	0.267	No
Orthophosphate	0.304	No
Chlorophyll a	0.478	No
Temperature	0.592	No
H'	0.606	No
Secchi depth	0.623	No
Phytoplankton cells per ml	0.634	No
Dissolved oxygen	0.972	No

Shannon Index of Diversity

Dissolved oxygen, orthophosphate and temperature were found to be significant predictors of phytoplankton diversity (p = 0.000, 0.003 & 0.007 respectively). Phytoplankton cells per ml, nitrate, ammonia, secchi depth and chlorophyll *a* were not significant predictors (p = 0.208, 0.381, 0.426, 0.533 & 0.845 respectively).

Table 5: Summary of multiple regression of water quality variables on Shannon Index of Diversity

Predictor variable	*P*-value	Significant?
Dissolved oxygen	0.000	Yes
Orthophosphate	0.003	Yes
Temperature	0.007	Yes
Phytoplankton cells per ml	0.208	No
Nitrate	0.381	No
Ammonia	0.426	No
Secchi depth	0.533	No
Chlorophyll a	0.845	No

Algal biomass (chlorophyll a)

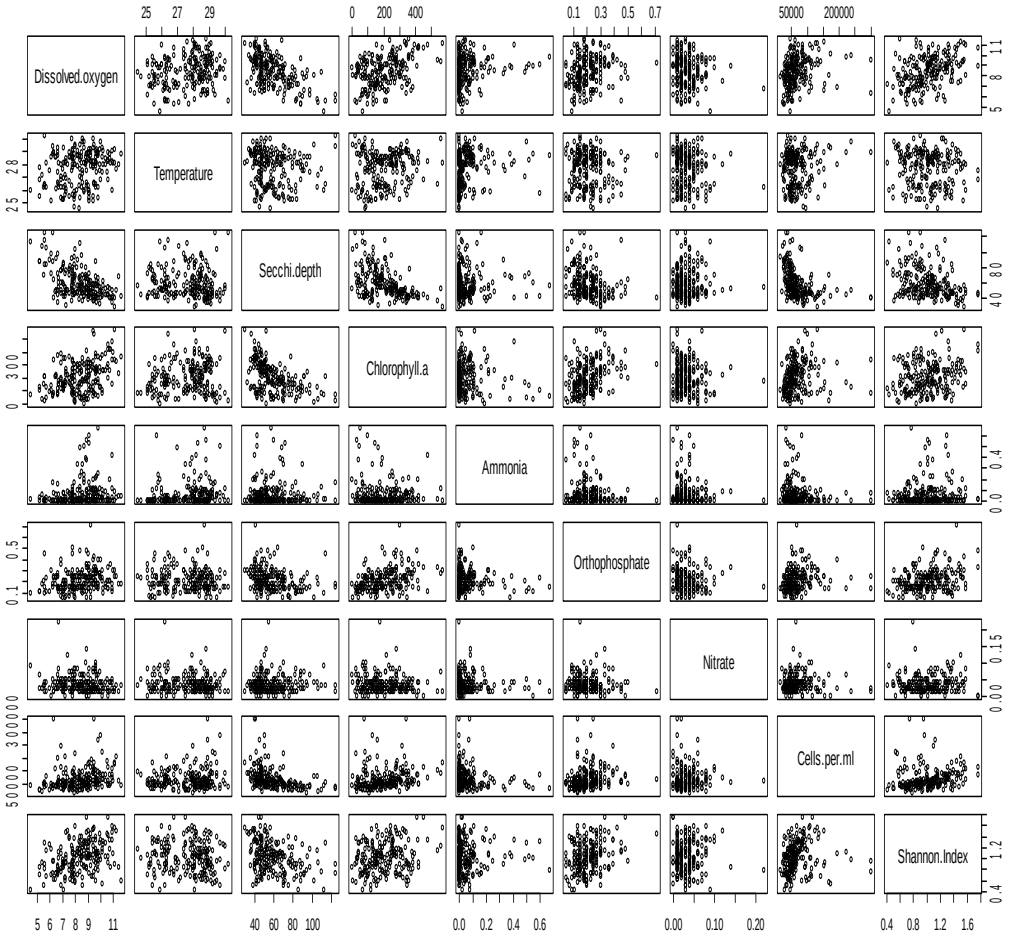
Secchi depth, orthophosphate, ammonia and dissolved oxygen were all found to be significant predictors of chlorophyll *a* (p = 0.000, 0.002, 0.011 & 0.011 respectively). Temperature, nitrate, phytoplankton cells per ml and the Shannon Index of Diversity were not found to be significant predictors of algal biomass (p = 0.054, 0.471, 0.513 & 0.845 respectively).

Table 6: Summary of multiple regression of water quality variables on chlorophyll a

Predictor variable	*P*-value	Significant?
Secchi depth	0.000	Yes
Orthophosphate	0.002	Yes
Ammonia	0.011	Yes
Dissolved oxygen	0.011	Yes
Temperature	0.054	No
Nitrate	0.471	No
Phytoplankton cells per ml	0.513	No
H'	0.845	No

Pairwise scatter plots of all water quality variables are shown in Figure 13.

Figure 13: Pairwise scatterplots of all variables sampled during the study period



4.3 Changes in variables over time

Unfortunately, repeated measures ANOVAs could not be performed for any of the variables measured. This is because there are too many missing data values for all variables measured, due to the number of sampling events where ponds were either being harvested, refilling or being brought back up to temperature before stocking. Graphs of all water quality variables for each pond by sampling date are shown in Figures 1-9 of Appendix 3.

5. DISCUSSION

Aquaculture is the fastest growing sector in the food industry (Kutty 2005). Increasing global human population, increasing demand for sea food and declining wild capture fisheries (FAO 2007b) means that aquaculture is an important industry. Therefore it is important that we expand our knowledge of aquaculture and improve techniques to maximize production while minimizing the detrimental social and environmental impacts it may incur. Due to semi-intensive stocking densities and its freshwater habit, the focal species of this study, *Macrobrachium rosenbergii* has been identified as having a light social and environmental footprint (Kutty 2005).

This study sought to investigate what variables related to water quality differ between ponds at New Zealand Prawns Ltd, the effect these variables have on the abundance and species composition of phytoplankton, the effects they may have on the production of marketable prawns, and how they vary over time. A lack of phytoplankton in the water column can lead to excess light reaching the bottom of ponds, resulting in the proliferation of benthic algae (Burford 1997, Aquaculture 1999). This process has been associated with reduced growth in cultured penaeid prawns (Allan and Maguire 1994) and is thought to be a significant cause of reduced prawn yield at NZPL.

Budget constraints and the difficulty of combining sampling with normal farm operations resulted in only a small data set being available for analysis and few statistically significant conclusions. Based on observation and literature searches on similar environments, it appears that blooms of benthic algae at NZPL probably occur mainly:

- In shallower ponds where more sunlight can reach the benthos allowing benthic algae to photosynthesize and proliferate.
- During summer months when there is increased intensity and duration of sunlight.
- When orthophosphate in pond water is low, reducing the amount of phytoplankton, which shade the benthos.

Eutrophication of waters by increased nutrient inputs is a serious problem in marine and freshwater, and natural and aquaculture habitats. Increased nutrients can stimulate phytoplankton blooms to detrimental levels which can be directly harmful through the toxins they may produce (Anderson et al. 2002) or cause anoxic conditions when blooms crash and decompose, which has the potential to kill animals living in these anoxic conditions (Burford 1997). This is of paramount concern to aquaculture farmers, hence knowledge of conditions that lead to eutrophication and any appropriate steps that can be taken to prevent or minimize it are of great value. Such knowledge may also have useful applications in the management of other settings such as lakes, rivers, estuaries and bays.

What constitutes eutrophication can be subjective and also depends in part on what the intended purpose of the water body is, and whether or not factors that influence eutrophication prevent that water body from serving its full potential (Correll 1998). At NZPL this full potential is maximum production of marketable sized prawns. Variable production at NZPL demonstrates that ponds are not consistently fulfilling their potential. If it is a lack of phytoplankton that causes yield-reducing benthic algae, I would suggest that conditions at NZPL are *not* eutrophic.

Unfortunately, a lack of algal blooms and a small number of harvests during the sampling period prevented any conclusion as to whether or not such blooms actually had a significant impact on prawn yield. Gaps in the data due to normal farm operations (harvesting, pond refilling and reheating) prevented rigorous statistical analyses of how variables changed over the study period.

Chlorophyll *a* concentration was used as an index of phytoplankton biomass and at NZPL is probably driven by ammonia and orthophosphate levels in the water. Of nitrate and ammonia, the latter is the more energy efficient form of nitrogen for algae to utilize (Hargreaves 1998) and is unlikely to be in short supply in a semi-intensive re-circulating aquaculture system. Phosphorous is often regarded as the most important limiting nutrient in freshwater systems (Schindler 1977) and has often been used to successfully predict phytoplankton abundance (Schindler 1978). Orthophosphate was also a significant predictor of phytoplankton diversity at NZPL. Changes in phytoplankton species composition with changes in orthophosphate are well documented (Anderson et al. 2002). Other variables found to be significant predictors of chlorophyll *a* and phytoplankton diversity are more likely to be their by-products than directly influential variables.

No water quality variables were found to be significant predictors of prawn yield. This could be due to the small sample size for prawn yield during the sampling period.

5.1 Differences in prawn yield between ponds

Significant differences were found between ponds for historical prawn yield, with Pond 3 having significantly higher mean yield than Pond 16. Table 2 in the results section summarises the differences in water quality variables between Ponds 3 and 16 for the sampling period. Although none of these variables were found to be significantly different between Ponds 3 and 16 it is interesting to note that Pond 3 has higher mean turbidity, chlorophyll *a*, phytoplankton diversity, phytoplankton cells per ml, pond depth and orthophosphate. It also had lower mean ammonia than Pond 16. Pond 3 also yielded more marketable prawns than Pond 16 during the sampling period (5.12 MTPH compared to 3.12 MTPH).

Unfortunately no information on water quality parameters is available for the period covered by historical yield. That there are differences in yield between ponds could be viewed as quite surprising. One would have hoped that in the past, all ponds were managed identically with consistent management practices across ponds. When management practices change, hopefully those changes are applied to all ponds, not just some. This raises the question of why there are differences between ponds. Unfortunately production data was not available from pre-2000. That production would be significantly higher now than when the prawn farm started 20 years ago would not be surprising as techniques, methods and knowledge improve with experience. Andrew Harrison has been managing biological aspects of NZPL since 2001. Since his appointment he has been able to raise production of prawns from one tonne per hectare to around 6-7 tonnes per hectare and has sought to make practices as consistent as possible (NZPL, personal communication).

But any improvements we'd assume were applied simultaneously to all ponds, which should result in proportionally equal improvements in yield between ponds. Not all ponds are the same size but yield was standardized to marketable tonnes per hectare (MTPH) to account for this.

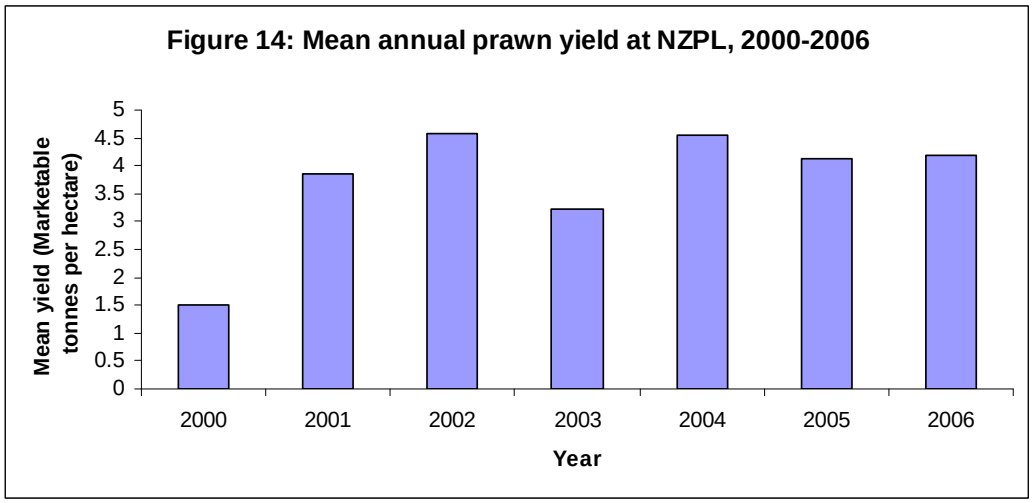


Figure 14 shows mean yield per harvest for each pond from 2000 to 2006. The year 2000 appears to have dramatically lower mean yield than the following years which appear fairly steady, with a possible decline in mean yield in 2003. With no information regarding water quality parameters for these years we can only speculate as to the causes of the apparent jump in production after the year 2000. As mentioned above, Andrew Harrison has been managing biological aspects of NZPL since 2001 and has since raised production of prawns from one tonne per hectare to around 6-7 tonnes per hectare and has always sought to make practices as consistent as possible (NZPL, personal communication). Perhaps having one person in charge consistently has translated into consistent and improved production. Prior to his appointment a number of different people had handled biological aspects of production and the future viability of the farm had often been in doubt, with the need for outside investment to keep it running (Wear 1996). The past environment of

uncertainty combined with frequently changing farm management practices was unlikely to make for a consistent and successful operation.

Mean prawn yield for each pond from January 1st 2000 to 28th March 2007 is shown in Figure 11 of the results section. Ponds 1-6 look like they may produce slightly higher yields than Ponds 7-19. Farm managers report anecdotally that the "bottom series" of ponds (Ponds1-10, see NZPL site lay-out, Appendix 4) are better producers than the "top series (Ponds 11-19), with Ponds 1-5 appearing to be the best and most consistent. Ponds 1-5 were in fact originally the only ponds at NZPL, built in 1987 while feasibility of the farm was being investigated. Farm managers report that the worst yields seem to come from ponds in the top series. The most obvious difference between the top and bottom series is that the top series are shallower on average than the bottom series (mean depth 954.4mm in the top series compared to 1098.5mm in the bottom series) and have been observed to suffer from benthic algal blooms far more often (NZPL, personal communication).

Unfortunately we cannot test statistically whether there were any differences in yield during the sampling period (November 8, 2006 – March 28, 2007) because the data set is far too small. Most ponds were only harvested once or at the most twice during this time. Ponds 1-5 at least appear to have produced higher yields than the rest of the ponds (see Figure 12, results section) during the sampling period. Ponds 3 and 16 yielded 5.12 and 3.02 MTPH respectively during the sampling period. While there may be a difference in yield of more than two tonnes between Ponds 3 and 16 we cannot say statistically whether the difference is significant as each pond was only harvested once during the sampling period.

Historical differences in yield between Ponds 3 & 16 *were* found to be statistically significant. Water is recycled among all ponds from the same source and ponds were found in nearly all cases to be the same with regards to the water quality parameters that were measured. This may suggest that something inherently different about the design of the ponds leads to the difference in yield. This could possibly be due to depth if say, ponds that were too shallow experience excessive benthic algal growth, impairing prawn foraging ability, which farm managers believe is the cause of reduced yield. A search of the literature failed to find any information on benthic algae causing impaired foraging ability in *M. rosenbergii* in either natural habitats or aquaculture situations. Allan and Maguire (1994) however conducted an experiment with the Penaeid prawn *Penaeus monodon* in fibreglass pools. They found that in pools where filamentous benthic was stimulated to grow individual prawn growth (weight gain), overall prawn biomass and food conversion efficiency were all significantly lower ($p < 0.05$) than in pools where phytoplankton blooms were stimulated. Differences in water quality arose between the two treatments due to different fertilization strategies needed to achieve the desired conditions, but these were not found to explain the differences in prawn growth observed. They believed the effect of the benthic algae was impairment of prawn's ability to forage for food, as well as entanglement of post-larvae. It is possible that benthic algae has the same effect on *M. rosenbergii* at NZPL.

Having too deep a pond can also be a problem if light starts to limit the growth of phytoplankton lower in the water column (Burford 1997) which shade the benthos and prevents the growth of benthic algae (see *'Cells per ml'* below). This could well

be the case here as it was Pond 18 which had the deepest average pond depth (and deepest average secchi depth) that was found to have significantly lower cells per ml than Ponds 2, 3 & 4, which have pond depths in the middle of the range seen at NZPL and secchi depths at the lower end of the range seen at NZPL. While there is no evidence to suggest that *M. rosenbergii* require phytoplankton directly, New (1990) reports that they have a preference for turbid conditions, and phytoplankton contribute to turbidity. As discussed in the introduction, the right abundance and composition of phytoplankton for a given system contributes positively to water quality in ways other than shading the benthos such as oxygenation of water, reduction of toxic ammonia and forming the basis of food webs that may ultimately benefit stock (Burford 1997).

Deeper ponds may also develop thermal stratification (Aquaculture 1999). While water flows through ponds at NZPL a stratified layer could potentially result in only the upper layer of water being turned over. This could result in the build up of nutrients on the bottom layer which in turn could be toxic to prawns if say, ammonia levels were too high and were not able to be flushed out. The out takes in ponds at NZPL remove water at the surface. If stratification is a problem this could potentially be solved by having another water out take beneath the surface, below the stratified layer to keep both layers moving and turning over. The speed of the out take could be adjusted to compensate for the increased rate of turn over from having two out takes per pond.

Water samples in this study were taken from close to the bottom of the ponds because prawns live within about 50cm of the bottom (the maximum height of the

artificial habitats used to increase habitable surface area in the ponds is approximately 50cm). Had the budget allowed, it would have been interesting to see if the water quality parameters sampled in this study differ with depth. Burford (1997) conducted a study on the phytoplankton dynamics in a Penaeid prawn farm on the Gold Coast of Australia and found that chlorophyll *a* levels were significantly lower at the surface of the ponds compared to the bottom despite a paddle-wheel mixing regime that resulted in all other water quality variables being evenly concentrated through the water column (including nutrients). Burford did not examine grazing by zooplankton in his study and offers different grazing rates at different depths as a possible explanation. Zooplankton was not examined in this study either and may help to explain why phytoplankton cell counts were significantly different between ponds (although this would then raise the question of why there are differential grazing rates by zooplankton in some ponds). Sampling at different depths would also be beneficial because while the prawns may all live within 50cm of the bottom, it is possible that prawns are affected by water quality dynamics occurring at other depths.

5.2 Chlorophyll a

Chlorophyll *a* levels in this study ranged from 6.14 to 571 ppm with a mean of 204.5 ppm (±116.5 ppm SD). These are similar values to those found in *M. rosenbergii* ponds by Costa-Pierce et al. (1984), which ranged from 2.3 to 693.7 ppm with a mean of 194.6 (±206.5 ppm SD). Both their results and mine show how wide ranging chlorophyll *a* levels can be in ponds. Burford (1997) found chlorophyll *a* levels in a Penaeid prawn farm can fluctuate markedly on even a *daily* time scale. Burford also reports that these fast-changing dynamics can be witnessed by farm

managers with the naked eye as ponds change colour during the course of a single day. Further studies may well require more frequent sampling than the 9-10 days used in this study in order to reflect how rapidly chlorophyll *a* levels can change. Burford (1997) recommends daily sampling of chlorophyll *a* with automated loggers but these were not available for this study.

That chlorophyll *a* did not differ significantly between ponds is perhaps surprising given that the number of algal cell per ml did. This could be a result of the Kruskal-Wallis test (required for chlorophyll *a*) having less power to detect differences than does ANOVA (used for cells per ml). Alternatively, the different cell counts may have produced the same level of chlorophyll *a* if cell sizes were larger, or if there had been a shift to species producing more chlorophyll *a* during times when cell counts were lower. Not all phytoplankton species produce equal amounts of chlorophyll *a* per unit of biomass, and even within a species the same biomass can produce different amounts of chlorophyll *a* depending on factors such as light intensity and temperature (Felip and Catalan 2000).

Chlorophyll *a* levels for each pond on each sampling date over the study period are shown in Appendix 3, Figure 3. While repeated measures ANOVA couldn't be performed for chlorophyll *a* (or any other variables) it appears that chlorophyll *a* levels built up, peaked around the middle and then started to taper off towards the end of the study period. A similar pattern is seen for mean chlorophyll *a* levels by sampling date (Figure 4, results section). Perhaps this is due to daylight hours increasing at the start of the study period (late spring), allowing phytoplankton to photosynthesise for longer each day and multiply more often. As daylight hours

decrease towards the end of the study period (early autumn), so do chlorophyll *a* levels. Burford (1997) found that in a commercial Penaeid prawn farm primary production was related to chlorophyll *a* concentration ($r^2 = 0.668$) with higher primary production being associated with higher light intensities. Both Winter et al. (1975) and Anderson and Sullivan (1986) concluded that in Puget Sound, USA, spring increases in phytoplankton were due mainly to increased solar irradiance, and thought that the decrease of phytoplankton in autumn was due to decreasing solar irradiance. Hence it would make sense that chlorophyll *a* at NZPL is lower in late spring, increases over summer and then starts to decrease in early autumn as light intensity and duration builds, peaks and then decreases.

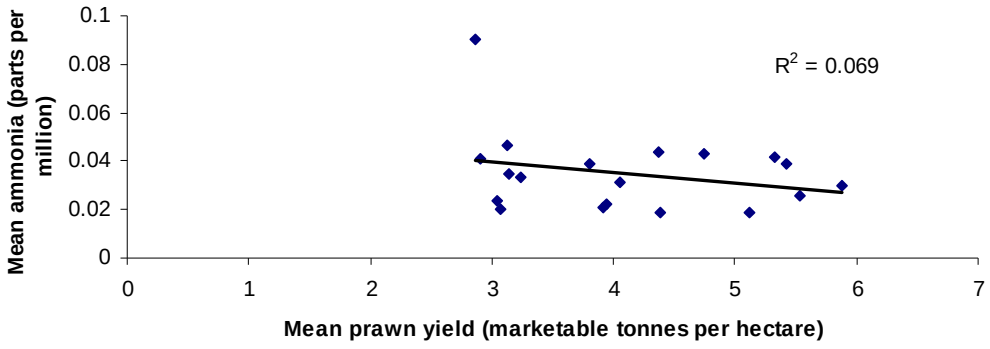
While no significant difference was found between ponds, it does also appear that there is more chlorophyll *a* overall in the historically more productive bottom series of ponds (1-9) than in the top series (11-18) (See Appendix 3, Figure 3)

5.3 Ammonia

During the study period, ammonia concentrations ranged from levels too low to detect up to 0.67 ppm with an overall mean of 0.066 ppm. This *mean* value is below the level of 0.5 reported by Naqvi et al. (2007) as being problematic for *M. rosenbergii*, and below the level of 0.1 ppm reported by Boyd (1998) as being the maximum acceptable level of ammonia for freshwater aquaculture in general. It is also lower than the mean of 0.3 ppm reported by Burford (1997) in a Penaeid prawn farm on the Gold Coast of Australia. However, on a number of occasions ammonia levels were higher than the 0.5 ppm reported by Naqvi et al. (2007) as being the beginning of problematic levels for *M. rosenbergii*. It is possible therefore that

ammonia had detrimental effects on prawn health and growth during the study. It is also interesting to note that Pond 3, which has historically been significantly more productive than Pond 16 had a mean ammonia concentration only one-fifth that of Pond 16 (0.02 ppm compared to 0.1 ppm). However, a scatter plot of mean ammonia for each growout period for each pond vs. each grow out period's yield shows only a very weak correlation ($r^2 = 0.069$) and is displayed in Figure 15.

Figure 15: Mean ammonia vs mean yield, entire sampling period.



Like the majority of water quality parameters sampled, ammonia was not found to differ between ponds, likely due to the homogenizing effect of the water recycling system in use at NZPL.

Ammonia concentrations for each pond on each sampling date of the study period are shown in Appendix 3, Figure 4. In all ponds, ammonia levels appear to be consistently very low, and below problematic levels. A number of ponds, however appear to have sudden spikes where ammonia levels increase dramatically and then drop just as suddenly. This is quite curious as it doesn't appear to coincide with any sudden changes in any other water quality parameters. The spikes in ammonia concentration appear to be more common in the top series of ponds which are also

shallower on average and are observed to experience more frequent and severe benthic algal blooms than the bottom series. Figure 5 (results section) also shows higher mean ammonia levels in the top series (Ponds 11-19) compared to the bottom series (Ponds 1-10), although no significant differences were found. If benthic algae is impairing foraging ability of prawns perhaps more of their high protein pelleted feed is going uneaten and is serving more to fertilize ponds than feed prawns, as protein-rich feed is a substantial contributor to ammonia levels in aquaculture ponds (Avnimelech 1999). This may explain the ammonia spikes, and it would be interesting in future to investigate if such spikes are associated with increased benthic algae.

5.4 Nitrate

Nitrate was not significantly different between ponds and appears to be consistently low over the sampling period for all ponds (Appendix 3, Figure 5) with a curious increase in Pond 7 on the third and fourth sampling dates (I can find no apparent reason for this). As ammonia is the preferred source of nitrogen for algae (Lobban and Harrison 1994) and is likely to be in fair supply due to the semi-intensive stocking density of prawns, and with phosphorous being the typical limiting nutrient in freshwater systems (Hecky and Kilham 1988), I am doubtful that nitrate has much influence at NZPL.

5.5 Orthophosphate

Boyd (1998) reports a range of 0.005 – 0.2 ppm as acceptable for orthophosphate in freshwater aquaculture ponds. At no time in the study period was orthophosphate below this level (minimum value 0.05 ppm). However the mean orthophosphate

level exceeded 0.2 ppm (0.21 ppm) and the highest recorded value of 0.71 ppm is more than three and a half times the recommended concentration. Phosphate is often cited as the major cause of eutrophication in freshwaters (Correll 1998, Anderson et al. 2002) and it may be a driving force behind phytoplankton abundance at NZPL (see '*Predicting chlorophyll* a *levels'* below). However, mean orthophosphate during the study only slightly exceeded Boyd's recommendation by 0.01ppm. Boyd's recommendation is also only a *general* guide for freshwater aquaculture, and not specific to *M. rosenbergii*.

Perhaps higher orthophosphate may even be good for the culture of *M. rosenbergii*. Elwood et al. (1981) conducted and experiment in which they enriched two stretches of a wooded stream in Tennessee with 60 and 450 μg of phosphate per litre for a period of 95 days. The result in both stretches was an increase in the amount of periphyton chlorophyll *a*, higher rates of leaf litter decomposition and increased secondary production in the form of populations of snails and leaf shredding macroinvertebrates. Peterson et al. (1985) enriched an Alaskan tundra river with 10 μg of phosphate per litre and also found an increase in periphyton and an increase in the mean size of aquatic insects. Given the demonstrated importance of secondary production (including snails, aquatic insects and other macroinvertebrates) to the nutrition of cultured *M. rosenbergii* (as discussed in the introduction) higher levels of orthophosphate than is recommended for aquaculture of other species may actually be beneficial to *M. rosenbergii* culture.

It would be interesting to study whether or not ponds with higher orthophosphate levels at NZPL also had higher levels of secondary production and/or higher yields

than ponds with lower orthophosphate levels. Although the differences were not found to be statistically significant, Pond 3 had a greater mean orthophosphate level than Pond 16 during the sampling period (0.26 ppm compared to 0.18 ppm) and greater yield (5.12 MTPH compared to 3.04 MTPH). Pond 3 has also had a higher mean yield than Pond 16 historically, a difference that *has* been found to be statistically significant.

It appears there was little change in orthophosphate over time in any ponds (Appendix 3, Figure 6). Phosphorous is retained in aquatic systems fairly efficiently by biological assimilation and the deposition of sediments and biota to bottom sediments (Correll 1998). In eutrophic systems when excessive algal blooms cause anoxia at night, bottom sediments often release excess orthophosphate into the water, which exacerbates the situation (Correll 1998). Dissolved oxygen levels were at satisfactory levels during the study period and certainly never anoxic (Appendix 3, Figure 8). I believe that the constant orthophosphate levels seen across ponds reflect the efficient retention of phosphorous typical of aquatic systems and the constant dissolved oxygen levels during the study period.

5.6 Phytoplankton cells per ml

Pond 18 had the lowest mean number of algal cells per ml for the sampling period. It is also the pond with the highest *recorded* secchi depth (124cm), highest *mean* secchi depth (74.2cm) and greatest mean *pond* depth (1185mm). Burford (1997) found that phytoplankton growth is light limited in Penaeid prawn ponds as did Costa-Pierce et al. (1984) in *M. rosenbergii* ponds. Being the deepest pond may result in not enough light penetrating to the lower depths of the water column in

Pond 18 for photosynthesis, resulting in lower numbers of phytoplankton at depth. Ponds 2, 3 and 4 were all found to have significantly greater mean numbers of algal cells per ml than Pond 18, and are all slightly shallower too at 1140, 1150 and 1075 respectively. Perhaps Ponds 2, 3 & 4 are at the right depth for optimal phytoplankton growth (sufficient light for photosynthesis penetrating to all depths of the water column) whereas Pond 18 is too deep. Water samples in this study were taken from near the bottom of ponds because prawns live within around 50cm of the bottom. As Pond 18 has the deepest mean depth, perhaps it was found to have significantly lower phytoplankton cells than Ponds 2, 3, and 4 (which are all shallower than Pond 18) because phytoplankton at the bottom of Pond 18 were limited by light and hence less abundant. Burford et al. (1997) suggest a shallower pond depth to counter light limitation in ponds but also note that this measure requires that phytoplankton blooms are maintained to prevent the growth of benthic algae (by shading the benthos which inhibits photosynthesis by, and proliferation of benthic algae, one of the reasons phytoplankton is desirable).

The growth of benthic algae has been a major problem at times at NZPL and was the impetus of this study. It is interesting to note that ponds in which benthic algae has been observed to be the worst are also the shallowest ponds (NZPL, personal communication). No benthic algal blooms were observed during the study period, but Pond 13 (one of the shallowest ponds at just 900mm deep,) was coming close until farm managers decided to manually remove the benthic algae before it got out of hand. It could well be of value to conduct experimental studies to ascertain optimal pond depths for *M. rosenbergii* culture.

Figure 1 of Appendix 3 shows that the number of phytoplankton cells per ml can vary somewhat and also appear to be higher in the bottom series compared to the top series. This is backed up by the ANOVA and Tukey tests, which found that Ponds 2, 3 & 4 (bottom series of ponds) all had significantly more phytoplankton cells per ml than Pond 18 (top series). There is however no clear trend over the sampling period (Appendix 3, Figure 1) and we do not see the same pattern of rise, peak and fall as we did for chlorophyll *a*. This may be due to shifts in the phytoplankton community to species that produce differing amounts of chlorophyll *a*, or phytoplankton being able to produce more chlorophyll *a* when daylight hours are longer.

5.7 Secchi depth (turbidity)

Secchi depth was one of the three variables found to differ between ponds. Turbidity and phytoplankton density can be closely related variables, often regarded as similar measures, although suspended particulate matter such as uneaten feed and faecal matter also contribute to turbidity. Further investigations will also need to be done in order to discover why secchi depth is different between ponds and which ponds actually differ. As a Kruskal-Wallis test was used to determine that there was a difference in secchi depth between ponds we are unable to do a post-hoc pair-wise comparison as we did with Tukey tests for cells per ml (analyzed by ANOVA).

While it may not be entirely surprising that secchi depth differs between ponds after finding that algal cell number does too, we cannot assume that ponds for which there are differences in secchi depth are the same as ponds for which there are differences in cell number. Further investigation is needed to determine this, and the relative

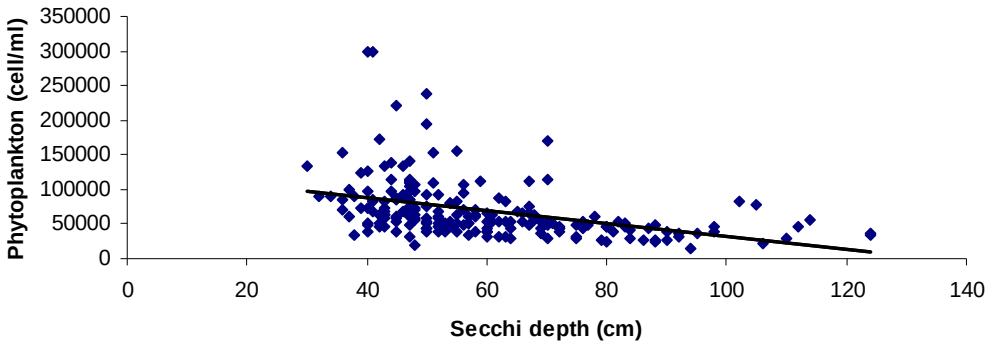
contributions of different factors to turbidity also need to be determined (as phytoplankton, silt, uneaten feed, faecal matter etc also contribute to turbidity).

However, it is worth noting that Ponds 2, 3, & 4 all have lower mean secchi depths (higher turbidity) than Pond 18 (see Figure 10, results section). In fact Pond 18, which had the deepest recorded secchi depth (lowest turbidity) for the study was also found to have statistically significantly less phytoplankton cells per ml than Pond 3, which also had the shallowest recorded secchi depth (highest turbidity) for the study. Secchi depths of between 30cm and 60cm are recommended for freshwater aquaculture (Aquaculture 1999). Mean secchi depths for Ponds 2, 3, and 4 all fall within this range (49, 49.4 and 59.4cm respectively) whereas the mean secchi depth of 74.2cm for Pond 18 exceeds this recommended range. New (1990) also reports that *M. rosenbergii* have a preference for turbid conditions. The overall mean secchi depth for the sampling period of 59.47cm, standard deviation ± 18.68 cm is similar to that found by Correia et al. (2002) of 53.92 ± 15.85cm SD and 57.06 ± 15.09cm SD for 1-3 month old and 8-10 month old *M. rosenbergii* ponds respectively.

It is possible that factors contributing to turbidity other than phytoplankton are more important. While secchi depth was found to be significantly different between ponds chlorophyll *a* was *not*. It may also be reasonable to assume that like chlorophyll *a,* phytoplankton *biomass* is also not significantly different between ponds, as strong positive correlations have been found between phytoplankton biomass and chlorophyll *a* concentration (Desortova 1981, Canfield et al. 1985, Voros and Padisak 1991). Future studies on phytoplankton biomass at NZPL may be helpful. Given this we may well find that the ponds for which secchi depth differ are not the

same as those for which cell number are different. However it is worth noting a strong negative correlation between the number of phytoplankton cells per ml and secchi depth (r = -0.41, 2d.p.). Fewer algal cells are associated with lower turbidity or deeper secchi depths (see Figure 16, below).

Figure 16: Secchi depth vs phytoplankton cells/ml, all ponds, entire sampling period.



Much like phytoplankton cells per ml, there is no clear trend over the sampling period for secchi depths (Appendix 3, Figure 9). Once again though there appear to be differences between the bottom series and top series of ponds, with the bottom series having lower secchi depths than the top series.

5.8 Temperature

New (1990) reports temperatures below 14°C and above 35°C as being lethal to *M. rosenbergii*. At no point in the study did temperatures become lethal. Temperature was not significantly different between ponds. At the start of the study period temperatures were lower than usual for NZPL due to a temporary cut off of the geothermal water supply for maintenance by Contact Energy Ltd of their geothermal power plant. This caused temperatures to fall below the optimal level of 29-31°C (New 1990), and would almost certainly have slowed growth. However the overall

mean of 27.7°C is close to the optimum temperature, and the lowest temperatures recorded during this study are an unusual event (Contact Energy Ltd carries out this maintenance only once a decade) and all ponds were equally affected. Temperatures also dropped below the optimum at other times during the study (e.g. sampling date 13, 17th March 2007, see Appendix 3, Figure 7) but again all ponds appeared to be affected so if there are current differences in yield between ponds temperature is unlikely to be the cause.

5.9 Dissolved oxygen

Dissolved oxygen was not significantly different between ponds and Figure 8 of Appendix 3 shows that across the sampling period levels were consistently at acceptable levels for the culture of *M. rosenbergii.* The overall mean of 8.4 ppm is well above the 4 ppm at which prawns start to get stressed (Avault 1987), although *slightly* above the 6-8 ppm New (1990) claims as being the usual target concentration in *M. rosenbergii* culture. There is nothing in the literature to suggest that the range of dissolved oxygen concentrations observed during the study period at NZPL would have been detrimental to prawns. When dissolved oxygen reaches 6 ppm or below farm mangers use paddle wheel aeration to increase oxygen levels (NZPL, personal communication). Dissolved oxygen does not appear to be problematic at NZPL.

5.10 Shannon Index of Diversity

The Shannon Index of Diversity (*H'*) for phytoplankton did not differ significantly between ponds. A search of the literature failed to find *H'* values of phytoplankton in *M. rosenbergii* culture to compare to the values observed in this study. Cremen et al.

(2007) in a study looking at phytoplankton in the culture of the tiger shrimp *Penaeus monodon* for two stocking density treatments of 10 and 15 post larvae per m^2 obtained mean *H'* values of 1.39 (range 0.6 to 2.23) and 1.56 (range 0.82 to 2.81) respectively. These stocking densities are comparable to NZPL's mean stocking density of 10.03 post larvae per m^2 per pond during the study period. The mean *H'* value of 1.02, and range of 0.42 to 1.76 observed at NZPL is lower than the values reported by Cremen et al. (2007) but this may be due to the rough grouping of some phytoplankton taxa in this study. This could be particularly true for the coccoid unicellular green algae, which are difficult to identify by light microscopy, especially after being fixed with Lugol's solution. During the study this group accounted for the vast majority of cells by number (69.9% on average) and is probably comprised of many different species. Unfortunately the resources were not available to identify this group down to a lower taxonomic level, had it been possible to identify unicellular green algae to level of genus, we may well have seen higher values of *H'*. No clear pattern in species diversity is seen over the sampling period (See Appendix 3, Figure 2).

5.11 Comparisons between Ponds 3 and 16

As stated earlier, Pond 3 had a significantly higher mean yield than Pond 16 historically. Here I discuss comparisons between these ponds with regards to the water quality parameters sampled during the study. Although no significant differences were found between Ponds 3 and 16 for these variables, and do not cover the period covered by historical yield, I believe it is still worth discussing as future investigations carried out over a longer time frame and with more frequent sampling may provide more significant results.

For mean dissolved oxygen, the difference between Ponds 3 and 16 is only 0.72 ppm. With means of 8.24 and 8.96 respectively both ponds' mean dissolved oxygen is well above the 4 ppm at which *M. rosenbergii* begin to get stressed (Avault 1987). The ranges of observed dissolved oxygen for both ponds are also within acceptable limits.

Mean temperature for Ponds 3 and 16 were 27.7 and 27.81°C respectively, the difference of 0.11°C is negligible. While these temperatures are slightly below the optimal 29-31°C reported by New (1990) this difference is also probably negligible. Both have similar ranges for temperature and are comfortably within non-lethal limits of 14-35°C and close to the optimal for *M. rosenbergii* (New 1990).

Nutrient levels are similar for both ponds. Pond 3 has a slightly higher mean orthophosphate level than Pond 16 (0.26 and 0.18 ppm respectively) and identical mean nitrate levels (0.02 ppm). Mean ammonia is five times higher in Pond 16 than in Pond 3 (0.1 ppm compared to 0.02 ppm) but neither of these values are reported as being problematic for *M. rosenbergii*, and the range seen in both ponds during the sampling period is below levels at which ammonia is toxic.

Both ponds have similar mean values for the Shannon Index of Diversity with 1.15 and 1.05 for Ponds 3 and 16 respectively. While these values are lower than that seen in other prawn culture systems such as was seen in *P. monodon* by Cremen et al. (2007), this could be due to the potential grouping of many different species into the 'coccoid unicellular green algae' group as discussed earlier. With such similar values

to one another anyway, it is not likely a good explanation for differences in yield between the ponds.

Although Pond 3 shared the highest recording for secchi depth (with Pond 18, 124cm) its overall mean turbidity was higher than Pond 16 with a mean secchi depth 7.3cm shallower. The lower turbidity of Pond 16 may also be exacerbated by having a shallower mean depth than Pond 3 (1085mm compared to 1155mm). Pond 3 also had a mean chlorophyll *a* concentration 44.3% higher than Pond 16 (303.48 ppm compared to 210.3 ppm), and Pond 3's mean number of phytoplankton cells per ml was 66.7% higher than Pond 16 (1×10^5 cells/ml compared to 6.03×10^4 cells/ml). These factors could all well contribute to more light reaching the bottom of Pond 16 than Pond 3, resulting in the proliferation of benthic algal blooms. Farm managers have observed that Pond 16 is one of the ponds to most frequently experience benthic algal blooms whereas Pond 3 seldom does. Most of the ponds in the top series (Ponds 11-19) are shallower than the bottom series (Ponds 1-10) and all have been observed to have benthic algal blooms more frequently than the bottom series. I suggest as a possible explanation that a combination of shallower pond depth and lower turbidity (the latter due mainly to lower phytoplankton numbers) causes the proliferation of benthic algae, which has contributed to significantly reduced yields in Pond 16 compared to Pond 3. As the data on which this possible explanation is based (apart from pond depth) has been obtained outside of the period covered by the historical yield data, we must however treat this explanation with caution.

5.12 Nutrient levels with regards to eutrophication

Based on comparisons with Pampulha Reservoir, a eutrophic tropical reservoir in Brazil studied by Figueredo & Giani (2001), I do not think that the water at NZPL is eutrophic. I have chosen to compare NZPL to a tropical reservoir because I believe that of the eutrophic water body types for which I could find nutrient concentration information in the literature, Pampulha is probably the most like NZPL. Although New Zealand is a temperate country, *Macrobrachium rosenbergii* is a tropical species and hence the conditions at NZPL are designed to mimic the tropical conditions in which the species has evolved and requires in order to flourish. Furthermore, the recycling system at NZPL means that the water and the nutrients it contains have a long residence time, similar to a reservoir (and unlike a river).

Figueredo & Giani (2001) took water samples at fortnightly intervals at Pampulha Reservoir for one year from February 1996 to January 1997. During this time the overall mean nitrate level was 0.65 ppm, nearly double that observed at NZPL (0.34 ppm). Ammonia at Pampulha ranged from 2 ppm to more than 6 ppm. Mean ammonia at NZPL was 0.071 ppm, just one-twenty-eighth the *minimum* observed at Pampulha. The maximum ammonia concentration of 0.67 ppm at NZPL is still only one-third of the minimum at Pampulha.

Orthophosphate is however higher at NZPL than it is at Pampulha. The maximum value of 0.07 ppm seen at Pampulha is only one-third of the *mean* value at NZPL (0.21 ppm) and one-tenth of NZPL's maximum orthophosphate concentration. Correll (1998) claims that for most lakes, streams, reservoirs and estuaries concentrations of *total* phosphorous (of which orthophosphate is only a fraction)

should not exceed 100µg/L, or 0.1 ppm. This is only half of NZPL's mean *ortho*phosphate concentration.

So if orthophosphate is so high at NZPL, and phosphates are the typical cause of eutrophication in freshwater systems, why does NZPL not appear to be a eutrophic system? First of all, Correll (1998) concedes that there is no clear, widely accepted consensus as to what is an 'acceptable' phosphorous level, despite his suggestion that it should not be more than 100µg/L. Second, the mean concentration of 0.21 ppm at NZPL is only just in excess of what Boyd (1998) recommends as the upper limit of orthophosphate in freshwater aquaculture. Third, the answer could lie in the water recycling and heat exchange system. As discussed in the introduction, water at NZPL reaches temperatures of between 39 and 58°C (depending on seasonal ambient air temperatures) when it is reheated in the geothermal heat exchanger before being pumped back into grow out ponds where it cools to 28°C. This probably sterilizes the water, the high temperature of the heat exchanger killing the phytoplankton so that while water and nutrients are continually recycled, phytoplankton is not. If this is the case it may be that eutrophication does not occur at NZPL because the phytoplankton population is being continually culled through heat exposure.

It could be useful to investigate the thermal tolerance of the different phytoplankton taxa at NZPL. Renaud et al. (2002) studied the effects of five different temperatures (25, 27, 30, 33 and 35°C) on the growth and nutritional quality on each of five different Australian tropical phytoplankton species. Only one, *Chaetoceros sp*. grew well at the highest temperature of 35°C while *Cryptomonas sp.* was killed outright.

All species grew best at 25-27°C. If the temperatures in the study by Renaud et al. (2002) had such a negative effect on the growth of *tropical* species of phytoplankton, it would seem unlikely that phytoplankton in New Zealand that have evolved in a *temperate* climate would fare any better when exposed to temperatures even higher than those used by Renaud et al. (2002). If phytoplankton are killed when water is reheated, then the temperature of the heat exchanger may need to be lowered, provided that this doesn't result in temperatures in the grow out ponds becoming too cold. If so, ponds in which phytoplankton levels are too low could be fertilized to help maintain satisfactory levels.

As in the natural world, phytoplankton can be important in aquaculture in forming the basis of food webs, ultimately providing nutrition for organisms at higher trophic levels (Burford 1997). Farmers may even stimulate blooms of phytoplankton for this very reason (Burford 1997). Therefore the nutritional value of phytoplankton will have implications for the nutrition of organisms feeding on phytoplankton, and therefore the nutrition of anything feeding on those organisms. Secondary production has been demonstrated as important in the culture of *M. rosenbergii* (Green et al. 1977, Schroeder 1983, New 1990, Tidwell et al. 1997, Correia et al. 2002). Renaud et al. (2002) also looked at the effects on the nutritional quality of the species in their study as they are sometimes used as feed for filter-feeding cultured bivalves. All species were found to have significantly reduced protein content at temperatures above 27°C and significantly lower energy content at the highest temperatures of 33 and 35°C. With respect to nutritional quality, *Chaetoceros sp.* was again the only species that did well at the highest temperature of 35°C. As *Cryptomonas sp.* was killed outright at 35°C chemical analyses of protein, lipid and carbohydrate was

impossible, as it was for the other three species because they were so few in number due to the high temperature. As with growth, all five species had the highest nutritional value at the lower temperatures of 25 and 27°C.

High growth rates of zooplankton have been related to high lipid and protein content in phytoplankton (Parsons et al. 1961). At NZPL, the heat exchanger reheats water to between 39 and 58°C depending on season. This is 4 to 24°C higher than the temperatures used in the experiment by Renaud et al. (2002). If phytoplankton at NZPL are affected by temperature in a similar way to the species studied by Renaud et al. (2002) then there could be potential consequences for the nutrition of prawns further up the food web if the high temperature they are subjected to are either killing them or reducing their nutritional content.

Phytoplankton is also more abundant at NZPL than in Pampulha (despite the possibility that phytoplankton are being killed by heat) but that doesn't necessarily mean that conditions at NZPL are eutrophic. Correll (1998) states that "From the human perspective it is desirable to prevent or minimize eutrophication of receiving waters for aesthetics and to maintain the productivity of animal species preferred for recreation and commercial fisheries". So eutrophication could be considered to be a relative term. If conditions identical to NZPL were found in a drinking water supply they probably would be considered eutrophic, but for the purposes of culturing *M. rosenbergii* they are not. If benthic algae proliferates due to inadequate shading of the benthos, phytoplankton levels at NZPL are actually not high enough.

5.13 Predicting chlorophyll a *levels (phytoplankton biomass)*

Neither ammonia nor nitrate were found to be significantly different between ponds. Ammonia was found to be a significant predictor of chlorophyll *a*. Nitrogen in the forms of both nitrate and ammonia are used by phytoplankton but ammonia is the preferred form as it can be directly assimilated and utilized. Phytoplankton must reduce nitrate to nitrite and then to ammonia before it can be utilized (Hargreaves 1998). As ammonia doesn't require reduction, it is the most metabolically efficient form of nitrogen, requiring the least amount of energy to meet nutritional requirements. Ammonia is unlikely to be in short supply in a system in which water is recycled, high protein feed is used and prawns are stocked semi-intensively. This may explain why ammonia but not nitrate was found to be a significant predictor of chlorophyll *a*. If ammonia had been in short supply, then perhaps nitrate would have been found to be a significant predictor of chlorophyll *a*, as phytoplankton would have been required to use nitrate more as a source of nitrogen.

Unsurprisingly, orthophosphate was also found to be a significant predictor of chlorophyll *a*. Orthophosphate is an essential limiting nutrient for phytoplankton, particularly in freshwater systems (Schindler 1977) and is required for a variety of metabolic functions (Lobban and Harrison 1994) as outlined in the introduction. Using data compiled from many studies from around the world, Schindler (1978) found a very strong relationship between mean annual total phosphorous concentration and mean annual chlorophyll *a* production. Soballe & Kimmel (1987) also analyzed data from 345 streams and 812 lakes and reservoirs. They found significant relationships between total phosphorous and algal cell abundance (closely related to chlorophyll *a* abundance), although they were different for each type of

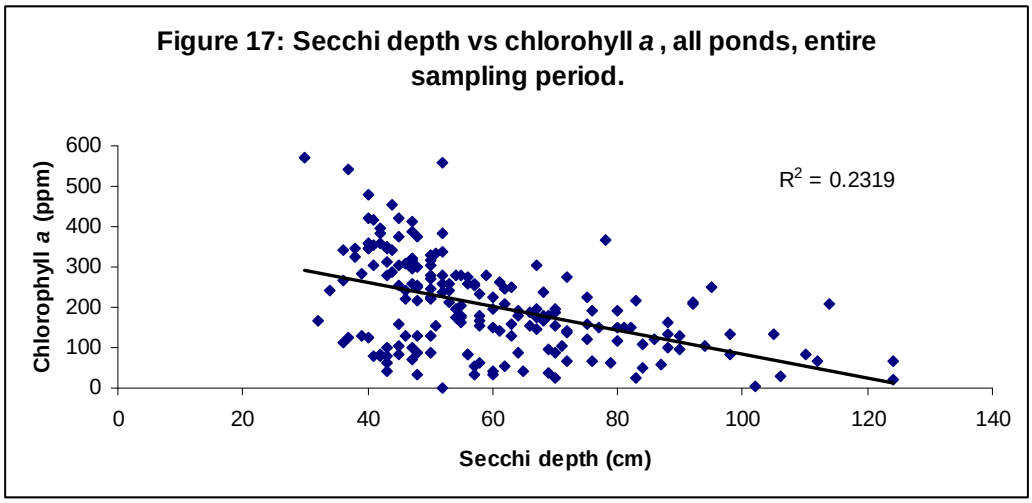
water body. They also found that statistical models showed lakes, followed by reservoirs as being the water body types most susceptible to additions of phosphorous due to long residence time. Orthophosphate had the least impact (though still important) on phytoplankton abundance in rivers. Although water is constantly moving at NZPL, due to the recycling system I would suggest that orthophosphate would have residence times more similar to lakes than rivers, and hence may also be very sensitive to any additions of phosphorous. Knowing the mean annual phosphorous levels of lakes has been found to allow managers to quite accurately predict the mean phytoplankton standing crop (Schindler 1978). NZPL could also possibly predict chlorophyll *a* levels by monitoring phosphorous levels. If the main source(s) of phosphorous in water at NZPL are identified they could potentially be controlled and increased or decreased depending on the state of phytoplankton levels at any given time. The Waikato River is unlikely to be a significant source of orthophosphate directly with a mean concentration of only 0.03 ppm for the study period compared to 0.21 for the prawn farm. However total phosphorous should be measured in the Waikato River (and prawn farm) as the various forms of phosphorous can be chemically and enzymatically transformed into orthophosphate and become available to phytoplankton (Correll 1998).

Oxygen was also found to be significant predictor of chlorophyll *a*. As oxygen is a by-product of photosynthesis this is not entirely surprising and it is probably more likely that dissolved oxygen levels in the water are a result of chlorophyll *a* levels, rather than the other way around. To investigate this possibility I ran another stepwise multiple regression with dissolved oxygen as the response variable and all the other water quality variables as predictor variables and found that chlorophyll *a*

was indeed a significant predictor of dissolved oxygen ($p = 0.003$) as well as secchi depth, Shannon Index of Diversity, temperature, and ammonia ($p = 0.000$, 0.000, 0.010, 0.002, & 0.003 respectively). Dissolved oxygen could however be a significant predictor as low dissolved oxygen can cause orthophosphate (an essential limiting nutrient, also found to be a significant predictor of chlorophyll *a* in this study) to precipitate out of bottom sediments (Correll 1998) and hence be more readily available to phytoplankton in the water column. As dissolved oxygen levels never reached anoxic levels during the study, this is unlikely to be the case here.

Secchi depth was also found to be a significant predictor of chlorophyll *a* levels. This is quite likely to be a significant predictor for much the same reason as dissolved oxygen. Chlorophyll *a* is an index of phytoplankton biomass and so we expect higher values of chlorophyll *a* to correspond to higher turbidity (lower secchi depth) as phytoplankton contribute substantially to turbidity. As was done above for dissolved oxygen, I ran another stepwise multiple regression with secchi depth as the response variable, and found that chlorophyll *a* was indeed a significant predictor of secchi depth (the only other being dissolved oxygen, $p = 0.000$ for both variables). It could be possible though, that if turbidity is caused by suspended particulate matter more than phytoplankton density, chlorophyll *a* levels are reduced because photosynthesis is inhibited by the lack of light. In such a situation secchi depth would not be a valid predictor of chlorophyll *a*. This is not likely however as a scatter plot of chlorophyll *a* against secchi depth (Figure 17) shows a negative correlation between the two variables with an R^2 value of 0.23. Deeper secchi depths (i.e. low turbidity) are associated with lower chlorophyll *a* levels because

phytoplankton biomass (which chlorophyll *a* is an index of) are an important contributor to turbidity.



5.14 Predicting phytoplankton diversity

Like they were for chlorophyll *a*, orthophosphate and dissolved oxygen were found to be significant predictors of phytoplankton diversity (*H'*). Temperature was also interestingly found to be a significant predictor of diversity. Ammonia, which was found to be a significant predictor of chlorophyll *a,* was not found to be a significant predictor of phytoplankton diversity.

As mentioned earlier in the discussion, this study may underestimate phytoplankton diversity due to the 'lumping' of species based on gross morphology into one taxonomic group that comprised the vast bulk of the different algal taxa by number (coccoid unicellular green algae, 69.9% of phytoplankton by number). If it had been possible to identify individuals in this group to lower taxonomic levels we may have seen both higher values of *H'* and more variation in *H'*. In this situation, ammonia may possibly have been a significant predictor of phytoplankton diversity as it was for chlorophyll *a*.

Given that orthophosphate is such an important limiting nutrient for phytoplankton its significance as a predictor of phytoplankton diversity is not a big surprise. Changes in phytoplankton species compositions with changes in nutrient regimes are well documented (Anderson et al. 2002). In freshwater systems, phosphorous is generally recognized as being the most important limiting nutrient for phytoplankton, so much so that often regulation of inputs into waterways from human activities (for example fertilizing agricultural land) has centred around limiting phosphorous use (Hecky and Kilham 1988, Correll 1998, Anderson et al. 2002). Algal blooms as a result of human-induced eutrophication tend to have low species diversity, and are in fact often nearly mono-specific (Hecky and Kilham 1988). If phytoplankton diversity is important for culturing *M. rosenbergii*, like most things in aquaculture, attaining the optimum level will be a trade-off with optimizing other key variables, including orthophosphate.

Temperature is necessarily tightly controlled at NZPL. The optimum temperature for farming *M. rosenbergii* is 29-31°C (New 1990). The mean temperature during the sampling period was 27.7°C (± 1.3°C SD) close to the optimum temperature. The range of recorded temperatures was narrow, 24.5 to 30.2°C. Temperatures in the mid-twenties were recorded in all ponds on November 28th 2006 following a shut down of the farms geothermal hot water supply. This was due to ten-yearly maintenance by Contact Energy Ltd of their geothermal power plant who supply the geothermal water NZPL uses. Had this not occurred during the sampling period, we would expect the temperature range to have been even narrower. With temperature being so controlled and varying little, it comes as some surprise that it was found to

be a significant predictor of phytoplankton diversity. Perhaps if even wider variations in temperature were seen we'd have seen even wider variations in phytoplankton diversity. Experiments investigating phytoplankton diversity at different temperatures would be necessary for this. Given that phytoplankton diversity was not found to be a significant predictor of yield, and that the farm's main objective is to make money, such an experiment actually taking place is not likely in the foreseeable future.

Appropriate levels of nitrogen must also be maintained in order to have the right balance of phytoplankton in the ponds. Ratios of nutrients are important for phytoplankton species composition, in particular the ratio of total nitrogen to phosphorous, or N:P ratio (Smith 1983). While nitrogen in the form of ammonia may be toxic to prawns, the removal of too much nitrogen from the system may lower the N:P ratio to the point where species of nitrogen-fixing cyanobacteria become favoured (< 29N:1P by weight) and form blooms (Schindler 1977, Smith 1983). This could be especially problematic if the bloom-forming species are of types that affect the flavour and odour of the product (and therefore value) such as cyanobacteria in the order Hormogonales (Tucker 2000). During this study *Anabaena spp.* were observed on a number of occasions to be more abundant in ponds during the early stages of grow out than they were in the later stages. This might possibly be due to lower prawn biomass in early stages of grow out excreting less ammonia into ponds. If ammonia is lower at earlier stages of grow out, this may reduce competitive pressure on *Anabaena spp.* which can fix their own nitrogen, and are at other times less abundant due to being inferior phosphorous competitors compared with other phytoplankton (Smith 1983).

Unfortunately total nitrogen (all forms of nitrogen) could not be measured for this study. However, it seems unlikely that the N:P ratio would have dropped below 29N:1P due to the use of high-protein feed, semi-intensive stocking, and water recycling system used at NZPL. Also, cyanobacteria were on no occasion the *dominant* phytoplankton group in any pond on any sampling event. Further, no problems with flavour or odour have been reported by customers at the prawn farm's on-site restaurant where nearly all of the product from the farm is sold and eaten. Nitrogen levels should however continue to be monitored to ensure that it does not get high enough to significantly impact prawn health, or low enough where undesirable cyanobacteria species may have an advantage over other phytoplankton species.

5.15 Comparison of water quality parameters between Pond 13 and the rest of NZPL

Of all ponds, Pond 13 came the closest to having a benthic algal bloom during the study. Manual removal of the algae took place before a full blown bloom actually occurred but I believe it is still worthwhile to compare Pond 13 to the rest of the farm as a whole. Table 7 shows the mean values of various parameters sampled during the study for Pond 13 in comparison to the mean values for farm as a whole.

Table 7: Comparison of water quality parameters of Pond 13 with overall farm means

Water quality parameter	Pond 13 mean	Farm mean	Difference
Dissolved oxygen (ppm)	8.35	8.4	0.05
Temperature (°C)	27.7	27.7	0
Secchi depth (cm)	65.77	59.47	-6.3
Chlorophyll *a* (ppm)	144.46	204.5	60.04
Phytoplankton cells/ml	6.98×10^4	6.95×10^4	$-.03 \times 10^4$
Ammonia (ppm)	0.09	0.066	-0.03
Orthophosphate (ppm)	0.17	0.211	0.04
Nitrate (ppm)	0.03	0.034	0.004
Shannon Index of Diversity (*H'*)	1.05	1.022	-0.028
Pond depth (mm)	900	1030	130
Yield (MTPH)	4.05	4.18	0.13

Pond 13 has a mean depth 130mm shallower than the farm mean, and slightly lower yield. While Pond 13 had a slightly higher mean number of phytoplankton cells per ml, it had 29% less chlorophyll *a* (the phytoplankton biomass index for this study) than the farm as a whole. Interestingly, there are also differences for dissolved oxygen, secchi depth, ammonia, and orthophosphate. These four water quality parameters were found to be significant predictors of chlorophyll *a* which as discussed earlier plays an important role in shading the benthos and preventing the growth of benthic algae. When compared to the overall farm mean, Pond 13 was lower in dissolved oxygen, turbidity (higher secchi depth), and orthophosphate, all of which were positively correlated with chlorophyll *a*. Dissolved oxygen and turbidity are most likely by-products of chlorophyll *a* (phytoplankton contributing to turbidity, and producing oxygen through photosynthesis). As the most important limiting nutrient for phytoplankton in freshwater systems however, orthophosphate levels probably contribute directly to chlorophyll *a* levels and lower orthophosphate in Pond 13 may directly result in its lower chlorophyll *a* concentration.

Although Pond 13 was not found to have significantly different mean values for any water quality parameters to any other pond the results are nonetheless interesting. I believe it is quite possible that a combination of shallow pond depth and less shading of the benthos by phytoplankton at the very least exacerbates the proliferation of benthic algae and further study in this area would be valuable.

5.16 Difficulties in this study

Combining controlled science with practical commercial enterprises is a difficult undertaking. Altering management practices to suit scientific endeavours will often be a gamble and could result in loss of profit. This study was no exception, and had to be a purely observational one in order not to interfere with the commercial imperatives of the business.

Often during sampling events one or more ponds were either in a state of being harvested, empty, refilling or being brought back up to temperature prior to stocking. This resulted in a lot of missing data and meant that SPSS could not perform repeated measures ANOVAs for any of the variables measured. Because of this we cannot make statistically validated claims about how variables changed over the study period.

Another major problem with this study was that no two ponds are at the same stage of the grow out cycle at any one time at NZPL. There is usually one pond harvested each week, occasionally two. After harvest, a pond is usually refilled, reheated and re-stocked with juveniles from the hatchery within a few days, and will be ready for harvest again by the time the other 18 ponds have been harvested. This creates

difficulties when comparing ponds: while all ponds receive identical water due to the recycling system used at NZPL, the water may not necessarily affect prawns in the same way due to differences in prawn age between ponds.

This may be particularly true where ammonia is concerned. Mallasen and Valenti (2005) found that larvae of *M. rosenbergii* increase in their sensitivity to ammonia as they develop, attributing this to increased gill surface area. Perhaps this sensitivity continues to increase as prawns continue to grow (assuming that gill surface area continues to increase with growth). This could well be a worthwhile area of future study in *M. rosenbergii* culture. With respect to NZPL, what may be a problematic ammonia concentration in one pond may not be in another because of differences in gill surface area associated with prawn age between ponds. Naqvi et al. (2007) reported that survival rate, feeding activity and growth were significantly reduced at ammonia concentrations as low as 0.5ppm for late juveniles (4.13-4.49g). Ponds at NZPL are often stocked with prawns smaller than this, (although sometimes even less than 1 gram). As ammonia has been recorded at levels higher than 0.5ppm on some occasions at NZPL there is the potential for juveniles (especially *late* juveniles) being stocked into water that is going to be very toxic to them. I would recommend that ammonia levels are monitored especially closely prior to stocking, especially when stocking with *late* juveniles. Ammonia must remain closely monitored even if stocking with early juveniles, as they will of course have to pass through the size range Naqvi et al. (2007) report as being sensitive to ammonia before reaching marketable size. Therefore there is real potential for ammonia to reduce survival, feeding, and growth rates at NZPL.

Ponds were not harvested at the same time during the sampling period either which creates difficulties when trying to make direct comparisons between ponds. Unfortunately, due to the nature of the operation at NZPL there is no way around this problem other than to drain all ponds, refill and restock them at the same time. This is not feasible for a number of reasons:

- Only a small fraction of the ponds contain marketable sized prawns at any one time. Draining all the ponds simultaneously would incur a severe economic loss for the farm.
- The capacity of the settling pond is not great enough to receive the water from all 19 grow out ponds. Draining all 19 ponds simultaneously would require emptying nutrient enriched water into the Waikato River.
- Harvesting all 19 ponds at once would require an upgrade of harvesting facilities (bigger processing area, more casual workers for harvest, increased freezer capacity etc).
- Even if production at NZPL was synchronized, the majority of each harvest would have to be frozen to prevent spoilage, and fresh prawns would only be available for a few days immediately after harvest. This would reduce the value of the prawns as fresh product is of higher value than frozen product.

Unfortunately (from a scientific point of view) there were none of the problematic blooms of benthic algae at NZPL over the sampling period. Pond 13, one of the shallowest ponds with an average depth of 900mm came close. Filamentous benthic algae could be seen around the shallower edges of the pond and also in deeper areas where it had grown long enough to be visible at the surface. Farm managers decided to manually remove the benthic algae before it got too out of hand. No other ponds

had benthic algal blooms and even if more had it would be impossible to conclusively prove that this reduced yield because of the low number of harvests during the sampling period.

It would also have been difficult to determine the severity of benthic algal blooms. Turbidity means that the bottom of ponds is almost never visible from the surface. Ponds that may have bloomed could potentially have had the proportion of their bottom covered by algae measured after being drained for harvesting. That may not necessarily have been sufficient though as that would only have allowed us to know how much benthic algae was present in the pond at the very end of the grow out period, not the entire period, which is what we'd need to know in order to know how long prawn foraging (and therefore nutrition and growth) may have been inhibited.

Budget constraints also prevented the sampling from being carried out more often and for a longer period of time. Many farm managers report observing very rapid, even daily changes in algal conditions in ponds (Burford 1997). Burford recommends daily sampling for extended periods of time to accurately reflect the rapid changes in chlorophyll *a* that can occur.

The homogeneity of the variables measured may also be due to the gradation of prawn ages blurring any differences, not just the fact that the water is recycled amongst all the ponds. While a number of variables *might* be vastly different between a pond that has just been stocked compared to one that is about to be harvested, in between are another 17 ponds that cover the whole spectrum of grow-out stages between stocking and harvest. Over two separate grow out seasons in a

penaeid prawn farm, Burford (1997) found that in both cases, ammonia increased during the season as prawn biomass increased. If prawns at NZPL have a similar effect on ammonia concentration, or are able to influence other water quality variables we may just see a blur that statistical analyses wouldn't be able to detect any differences in due to the gradation of production stages between ponds.

5.17 The ideal scenario in a perfect world…

In an ideal situation, the following conditions would be permissible for an experiment.

1) All ponds would be drained and prawns removed regardless of size.
2) All ponds would be refilled and restocked at the same time with juveniles of the same size from the hatchery.
3) All relevant variables would be measured *daily*.
4) Farm managers would not alter any management practices for the duration of the study, and any benthic algal blooms would be allowed to occur.
5) Phytoplankton identification would be improved by identifying phytoplankton down to species level wherever possible.
6) All ponds would be harvested at the same time.
7) A method of quantifying benthic algae for each grow out cycle would be developed.
8) Several grow out cycles for each pond would be carried out in order to compare differences in yield between ponds and over time.

5. 18 Conclusions

The impetus for this study was the occurrence of benthic algal blooms which farm managers believed caused greatly reduced yields of prawns through foraging impairment. Unfortunately during the study period there were no such blooms of benthic algae so the cause(s) of benthic algal blooms cannot be determined. Had there been more benthic algal blooms the study would have required a longer duration to gain a larger sample size for yield in order to determine whether or not benthic algae does in fact have an impact on yield.

Based on observation of Pond 13, which came closest to having a benthic algal bloom, I suggest that such blooms are due to excess light reaching the benthos, allowing benthic algae to photosynthesize and proliferate. The conditions stimulating this appear to be:

- Shallower pond depths.
- Decreased shading of the benthos by phytoplankton, possibly due to lower orthophosphate.
- As past blooms have been observed to occur in summer the intensity and duration of sunlight also probably contributes to the proliferation of benthic algae. As had been found for phytoplankton, solar irradiance was found by Thom & Albright (1990) to trigger the build up of benthic vegetation in Puget Sound, USA, with a decrease in irradiance in autumn seeing a die-back of benthic vegetation.

Despite this though we cannot conclude that benthic algal blooms cause a reduction in prawn yield. The short time frame of the study meant that the sample size for yield

was small. During the study Pond 13 was harvested once, producing 4.05 marketable tonnes per hectare which is only slightly less than the mean yield of 4.18 for the farm as a whole during the study. There would also need to be a higher number of benthic algal blooms as well as a bigger sample size for yield before we can start to conclude that the former impacts the latter. Based on the information available though I would recommend that the shallower ponds have their depths increased to match those of Ponds 1-5 which are observed to very rarely (if ever) suffer benthic algal blooms. Pond 18, the deepest pond should be made shallower. Another alternative for shading the benthos is to stimulate phytoplankton blooms by fertilizing the water (Burford 1997), although this is not without its own problems if excess phytoplankton cause conditions to become anoxic at night. The use of shade cloth to prevent sunlight reaching the benthos would also reduce the sunlight available for phytoplankton.

Changes over time in water quality parameters could not be analysed statistically due to gaps in the data cause by normal farm operations (i.e. harvesting the product). This highlights the difficulty of combining controlled science with practical commercial operations.

Aquaculture can have detrimental impacts on the environment and there is an increasing worldwide demand of aquaculture species as wild stocks decline. Due to its semi-intensive nature and use of freshwater the farming of *Macrobrachium rosenbergii* has a low environmental footprint compared with other aquaculture species. The use of geothermal water to heat water at NZPL means that energy requirements also have a low impact. The prawns are a high value product at NZD

$50 per kilogram and the farm provides high levels of employment. Due to its popularity as a tourist destination (with an estimated 90,000 visitors per annum) there are further economic spin-offs for the farm beyond the sale of the product which also benefit the economy of Wairakei and the nearby tourist town of Taupo. As such I believe that the culture of *Macrobrachium rosenbergii* should be encouraged in New Zealand (and elsewhere in the world), culture practices refined and further research be put into optimizing production.

APPENDIX 1: Morphology and morphotypes of *Macrobrachium rosenbergii*.

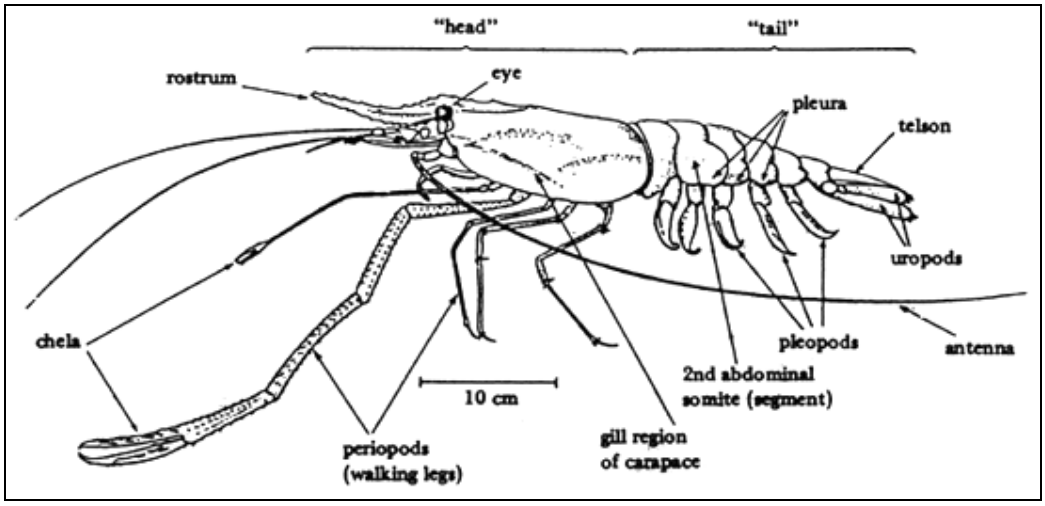


Diagram 1 – anatomy of *Macrobrachium rosenbergii*.
Diagram: http://www.fao.org
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Photo 1 – small males (SMs).
Photo: http://aquaculture.ako.net.nz/?cat=9&paged=2

Photo 2 – orange claw male (OC).

Photo: Daniel MacGibbon

Photo 3 – blue claw male (BC).
Photo: Daniel MacGibbon

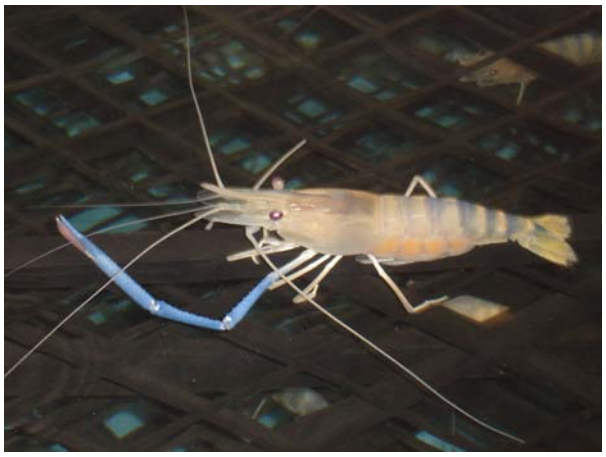
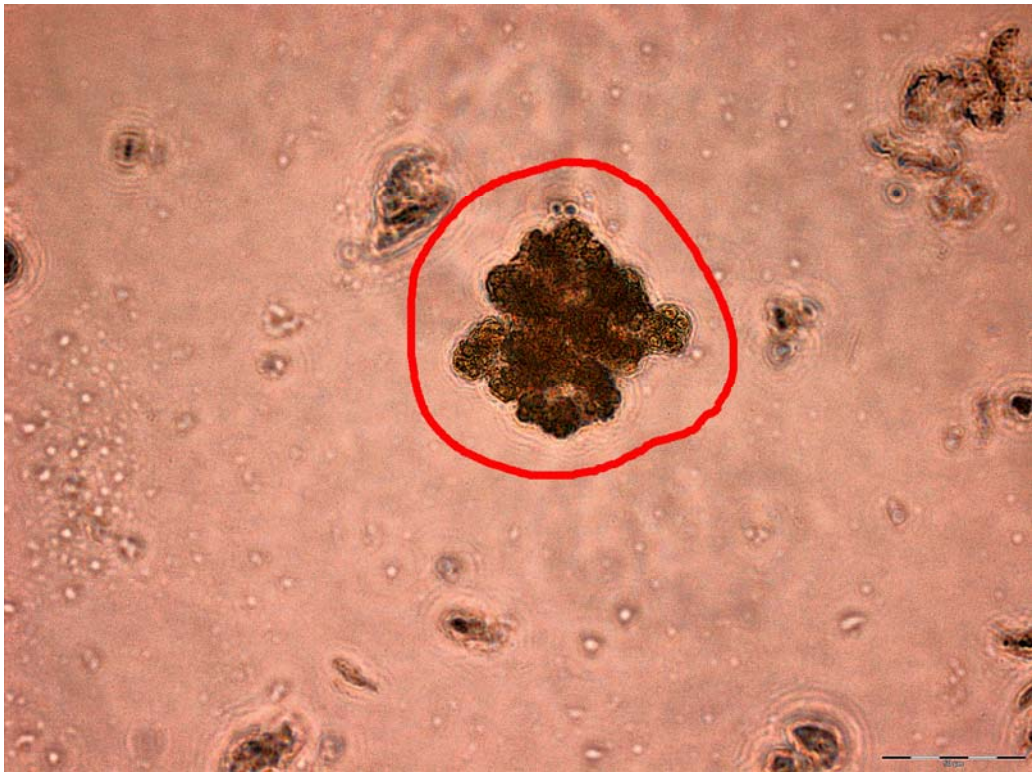


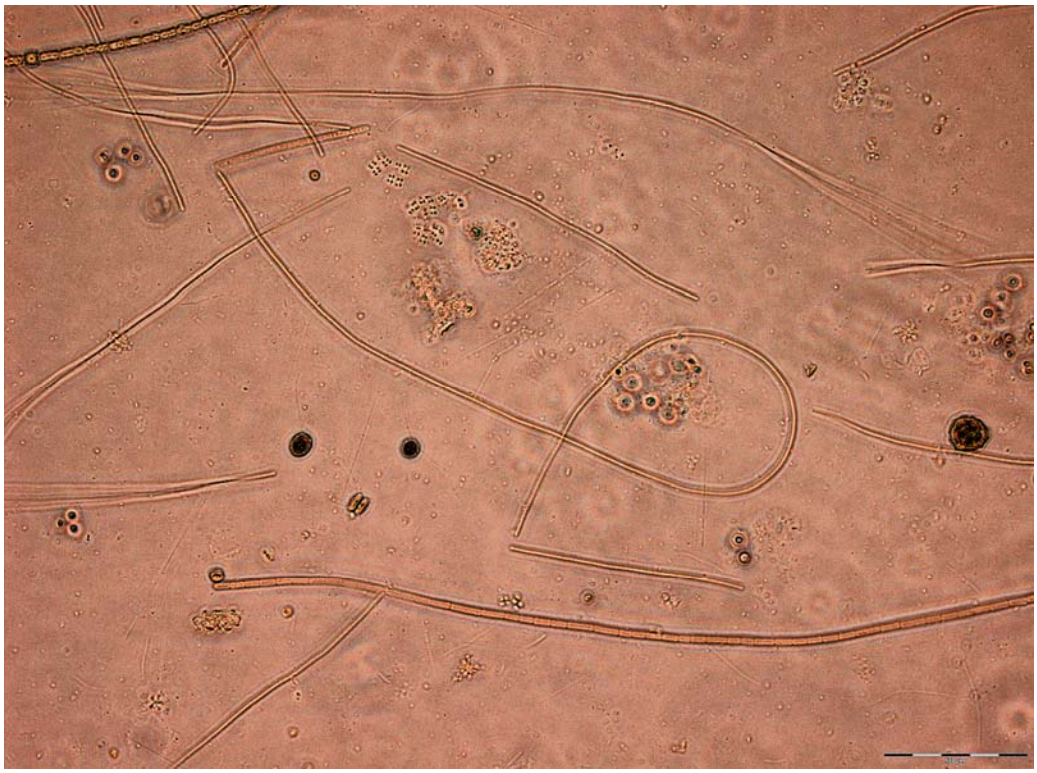
Photo 4 – female.
Photo: Daniel MacGibbon

APPENDIX 2: Phytoplankton identified during the study.

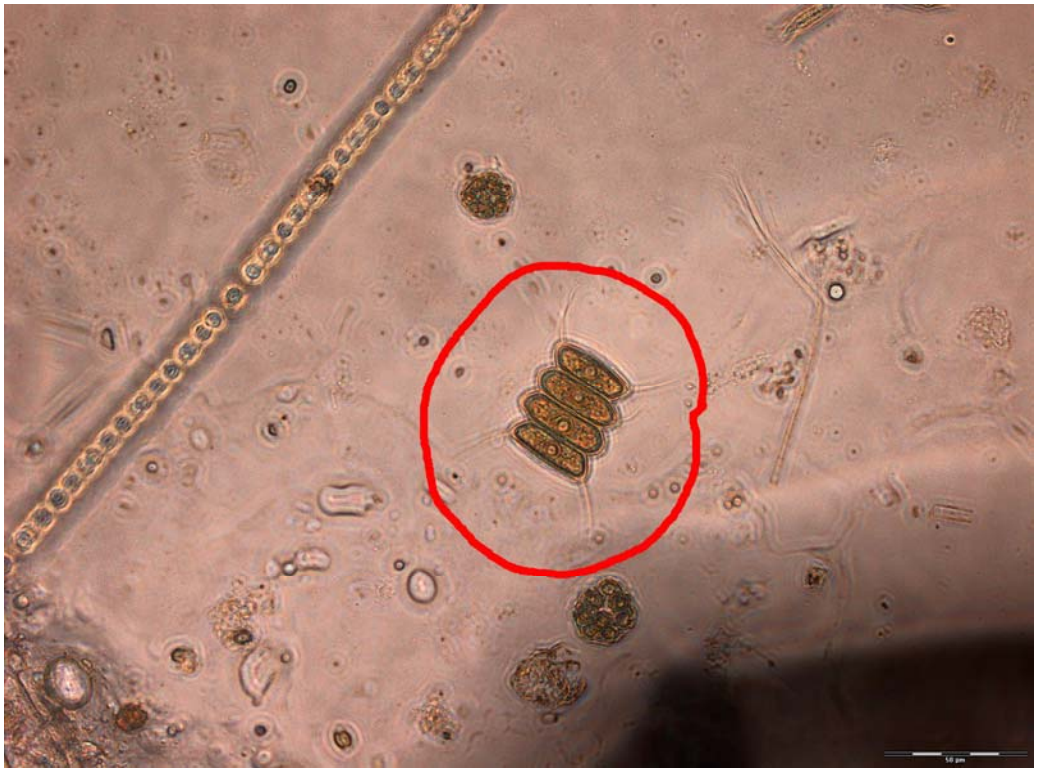
Coccoid unicells
Photo: http://www.ndsu.nodak.edu/instruct/fawley/coccoids/Itasca/newspp.htm



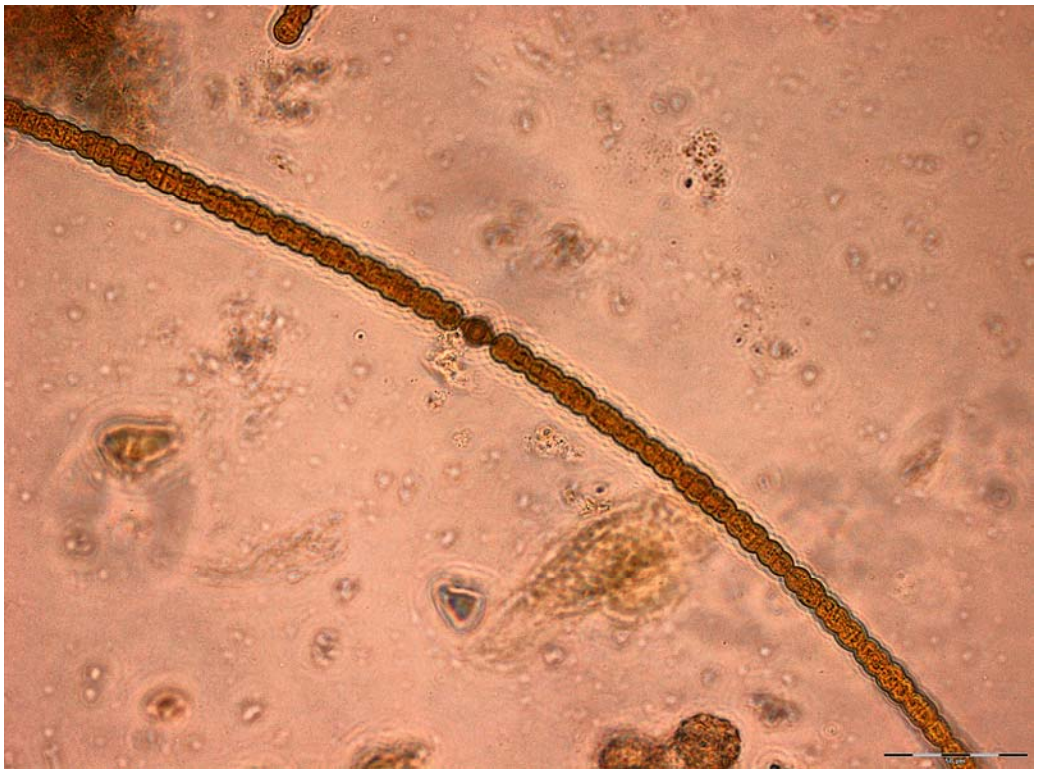
Coelastrum sp.
Photo: Daniel MacGibbon



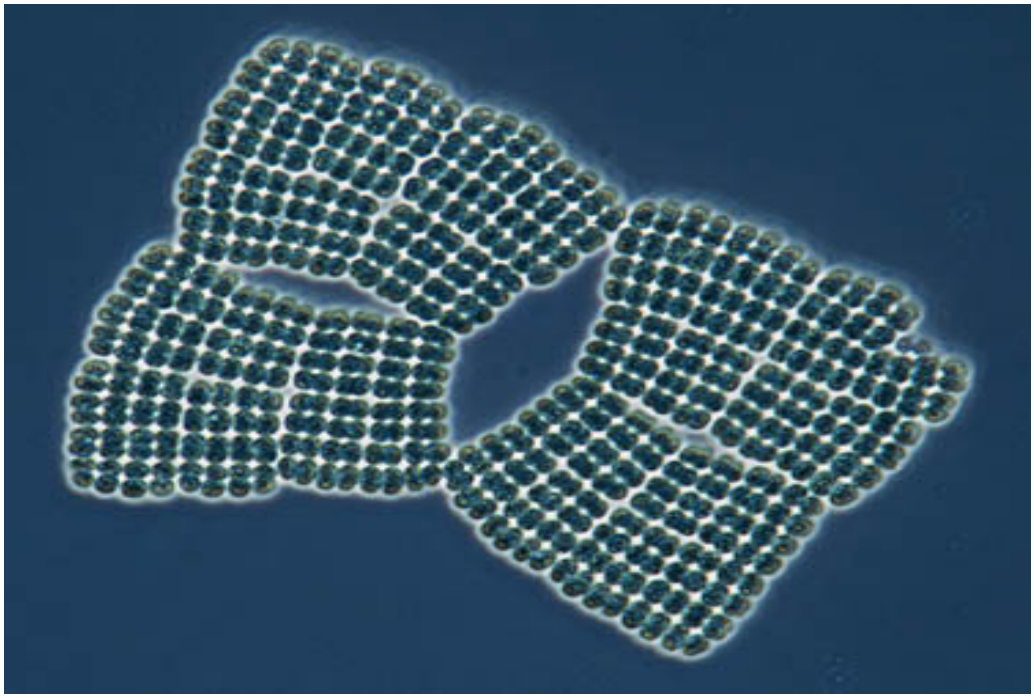
Filamentous cyanobacteria
Photo: Daniel MacGibbon



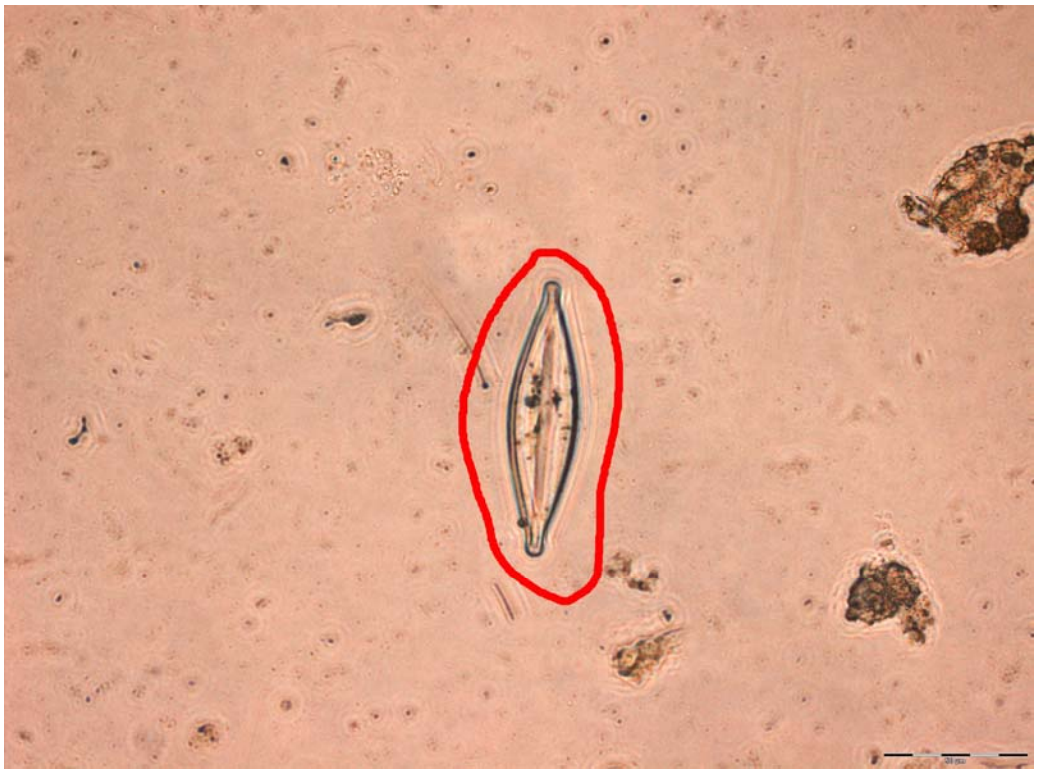
Scenedesmus sp.
Photo: Daniel MacGibbon



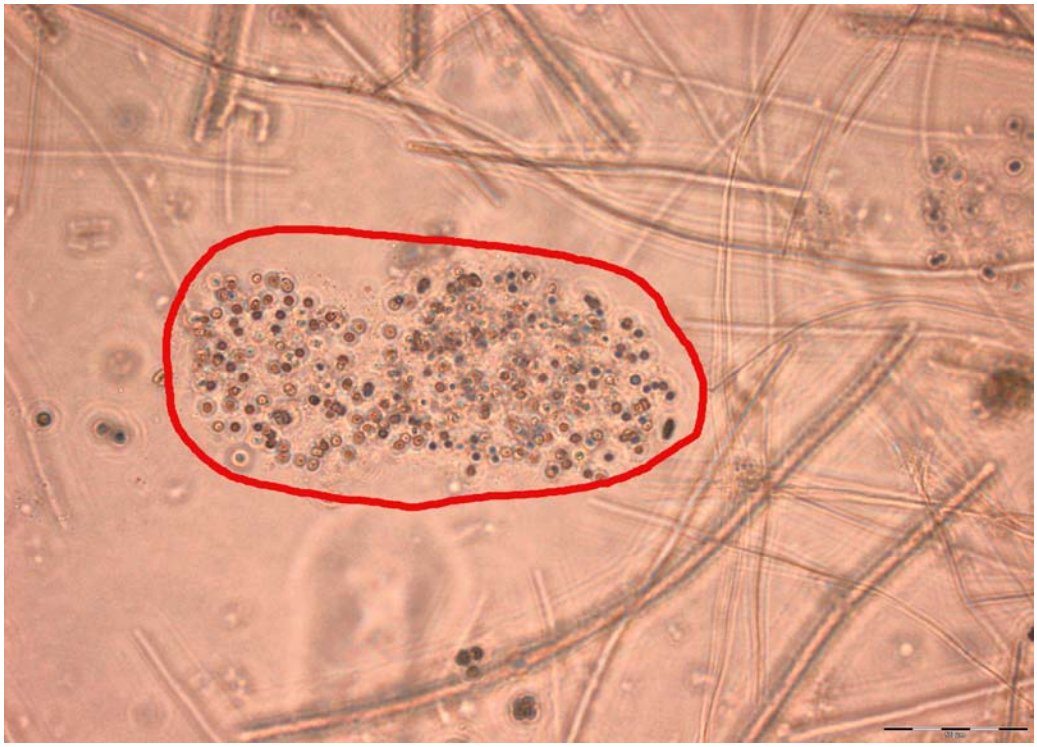
Anabaena sp.
Photo: Daniel MacGibbon



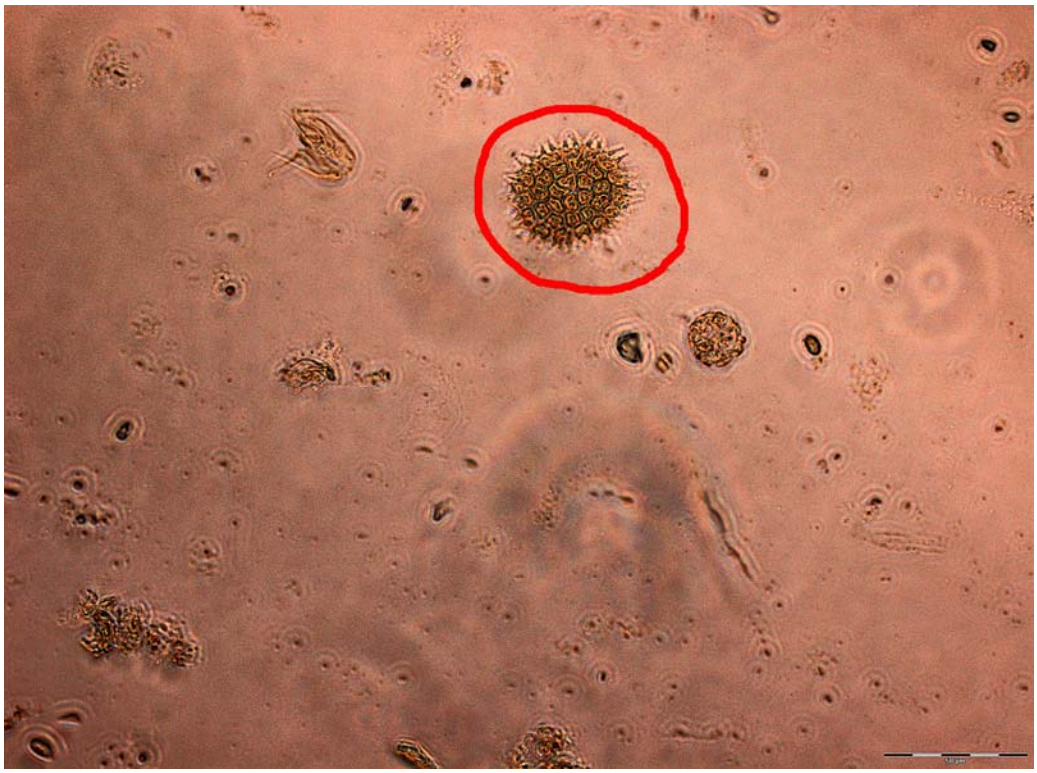
Merismopedia sp.
Photo: http://www.microscopy-uk.org.uk/mag/wimsmall/bacdr.html



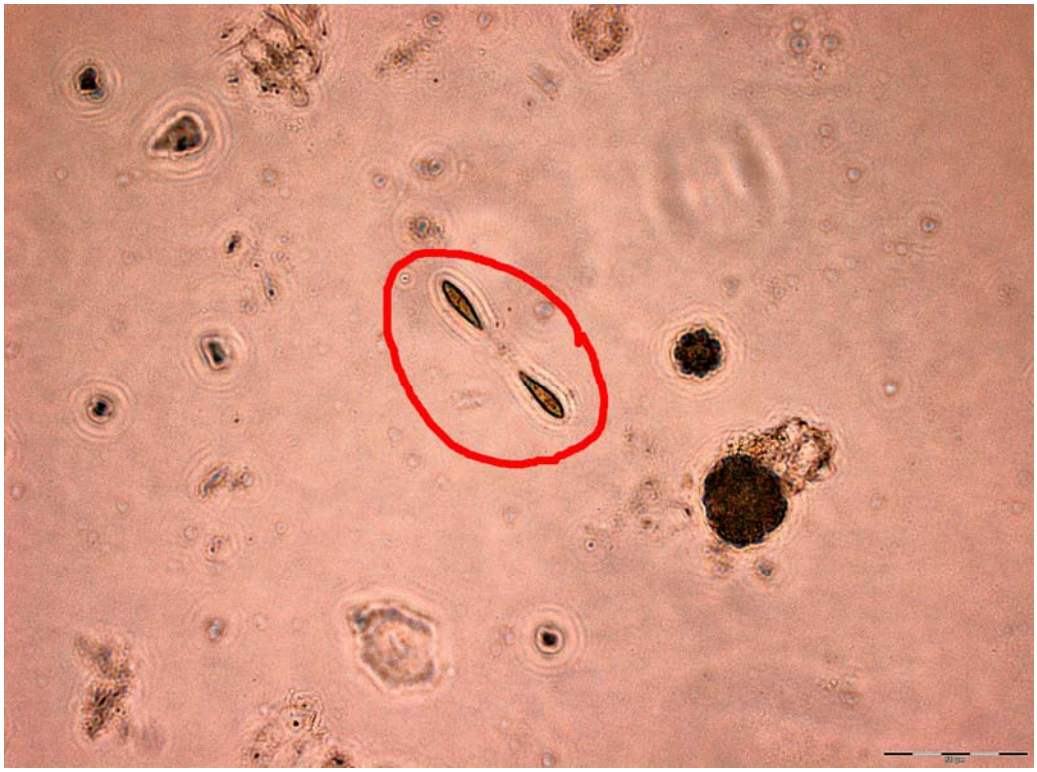
Pennate diatom
Photo: Daniel MacGibbon



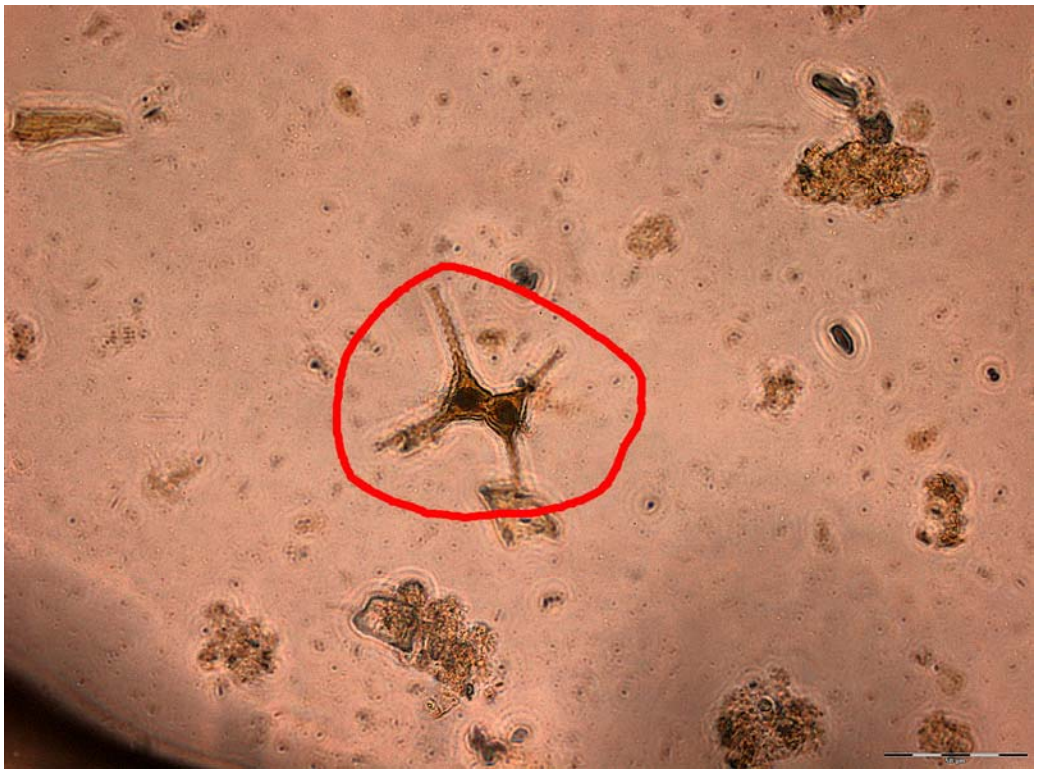
Dictyosphaerium sp.
Photo: Daniel MacGibbon



Pediastrum sp.
Photo: Daniel MacGibbon

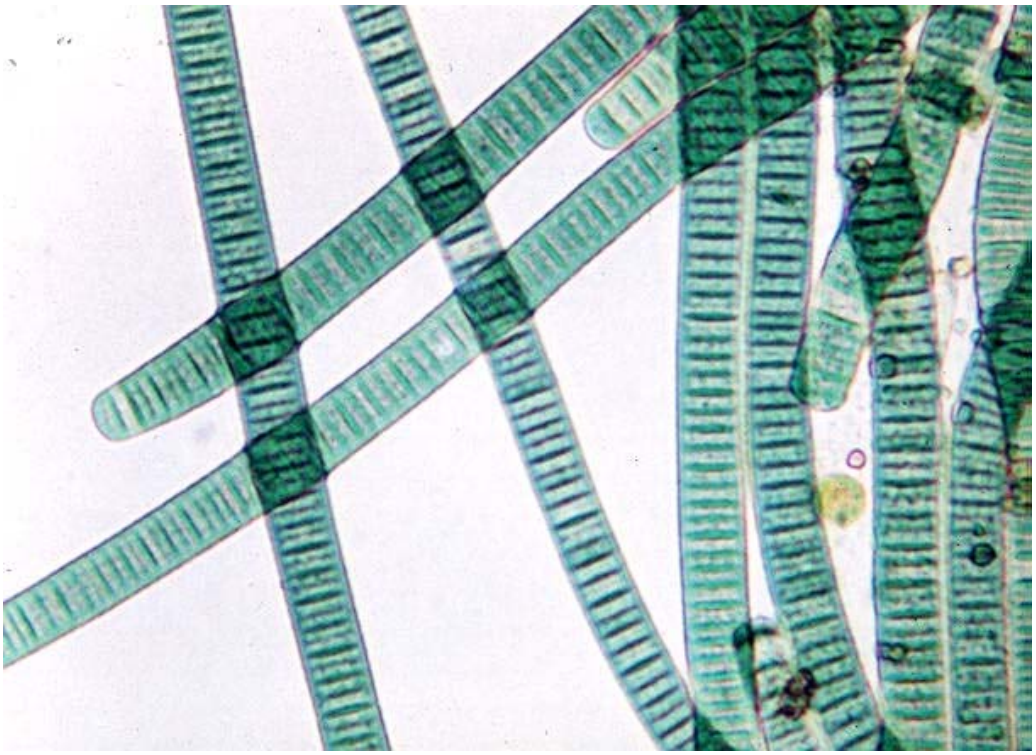


Eyes sp.
Photo: Daniel MacGibbon

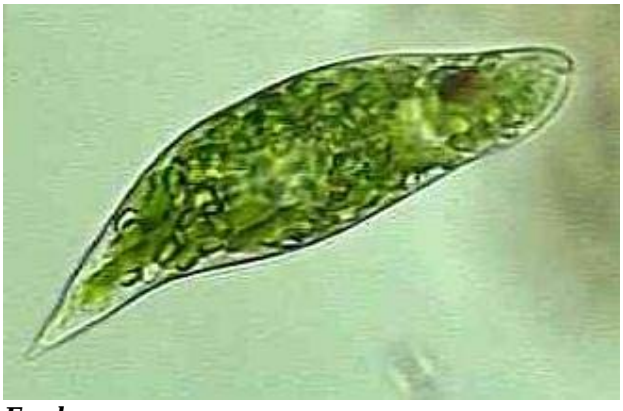


Staurastrum sp.
Photo: Daniel MacGibbon

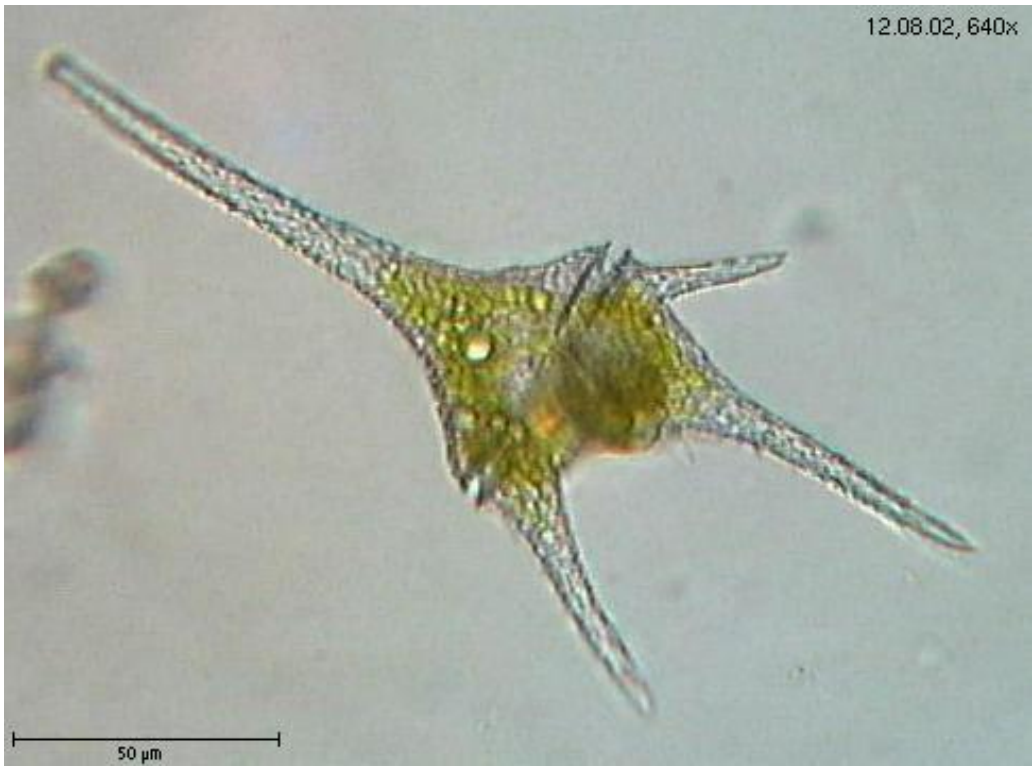
Golenkinia sp.
Photo: http://protist.i.hosei.ac.jp/PDB/Images/Chlorophyta/Golenkinia/index.html



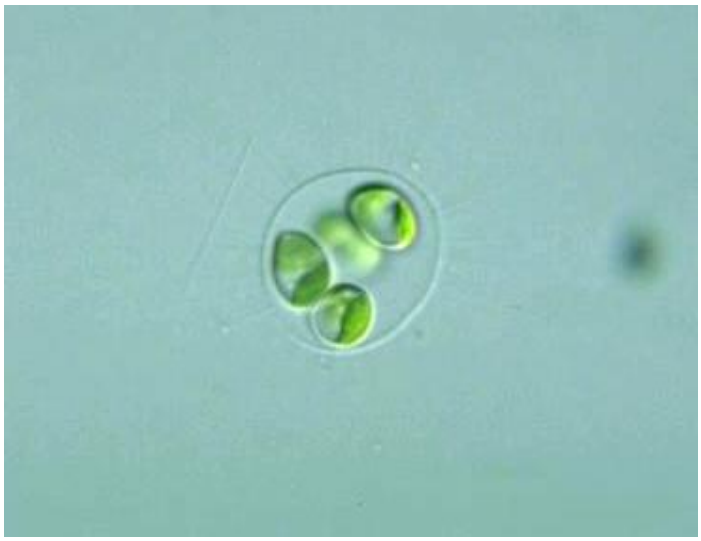
Oscillatoria sp.
Photo:
http://botit.botany.wisc.edu/images/130/Bacteria/Cyanobacteria/Oscillatoria/Oscillatoria_MC.html



Euglena sp.
Photo: http://www.xtec.cat/~jfarre13/hot-potatoes/cellula-1.htm

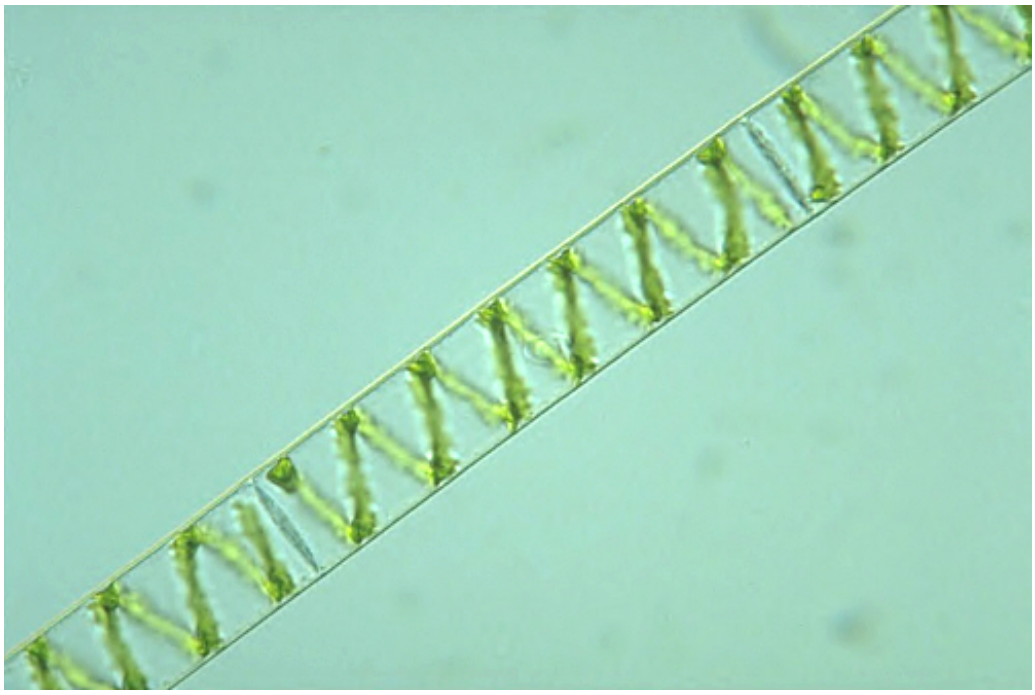


Ceratium sp.
Photo: http://blocs.xtec.cat/epsavidaalmar/42-els-dinoflagel%C2%B7lats/



Oocystis sp.
Photo: http://protist.i.hosei.ac.jp/PDB/Images/Chlorophyta/Oocystis/index.html

Lyngbya sp.
Photo:
http://microbes.arc.nasa.gov/images/content/gallery/lightms/preview/lyngbya.jpg\



Spirogyra sp.
Photo: http://www.biologie.uni-hamburg.de/b-online/library/webb/BOT311/Chlorophyta/SpirogyraBig500.jpg

APPENDIX 3: Water quality variables of all sampled ponds on all sampling dates.

The following bar charts are of each water quality variable for each pond on each sampling date. The sampling date numbers on the x-axis of each plot refer to the following actual dates:

Sampling date number	Actual date
1	8 November 2006
2	28 November 2006
3	8 December 2006
4	18 December 2006
5	28 December 2006
6	7 January 2007
7	17 January 2007
8	26 January 2007
9	5 February 2007
10	14 February 2007
11	25 February 2007
12	7 March 2007
13	17 March 2007
14	28 March 2007

Figure 1:Number of phytoplankton cells per ml for each pond on each sampling date

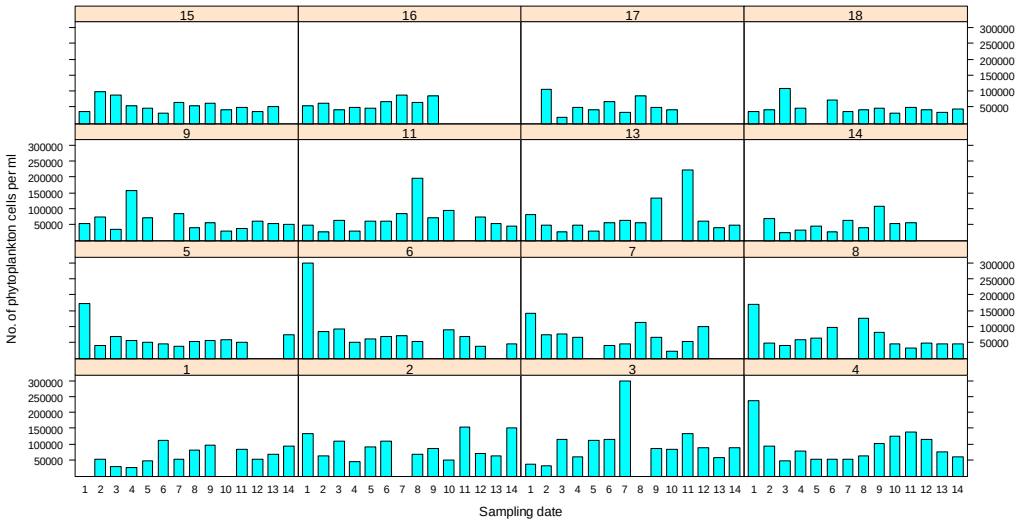


Figure 2:Shannon Index of Diversity (H') for each pond on each sampling date

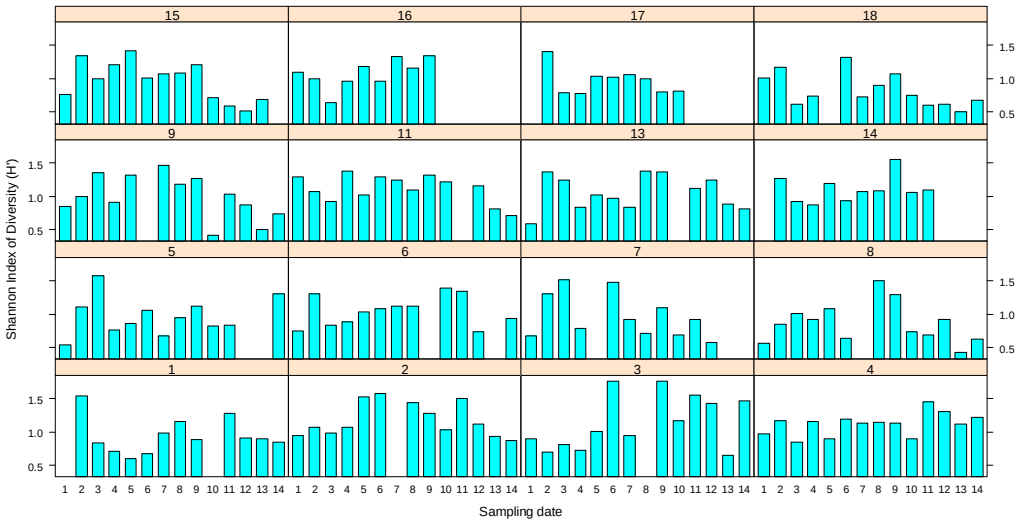


Figure 3: Chlorophyll a concentrations for each pond on each sampling date

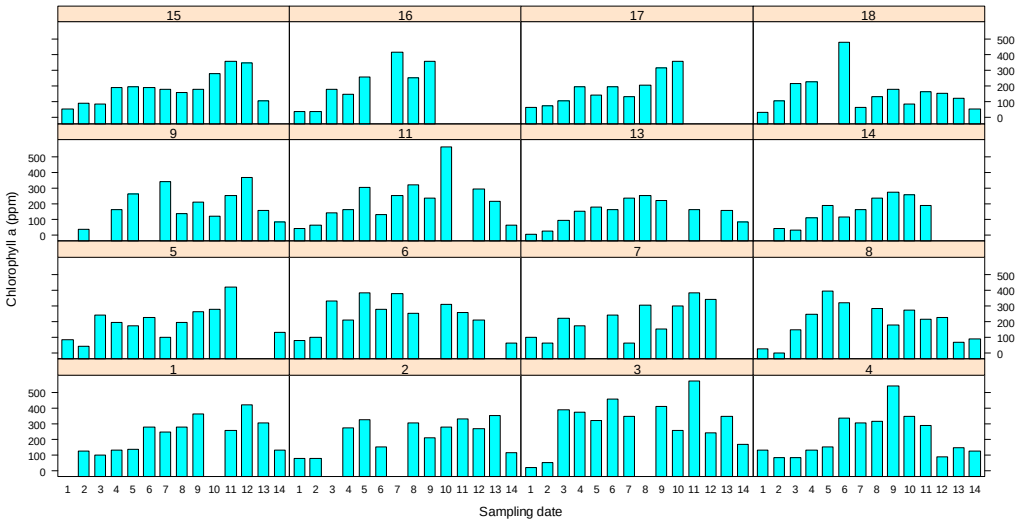


Figure 4: Ammonia concentrations for each pond on each sampling date

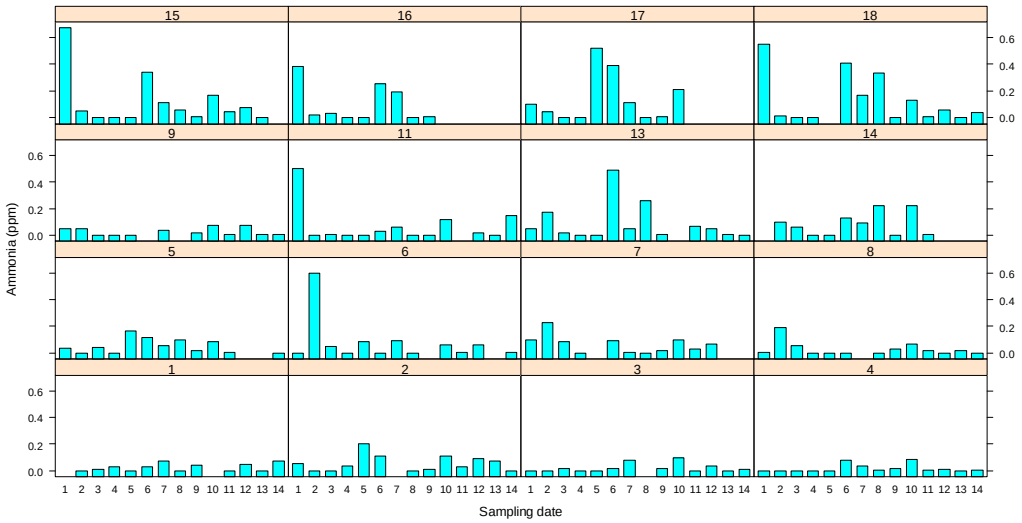


Figure 5:Nitrate concentrations for each pond on each sampling date

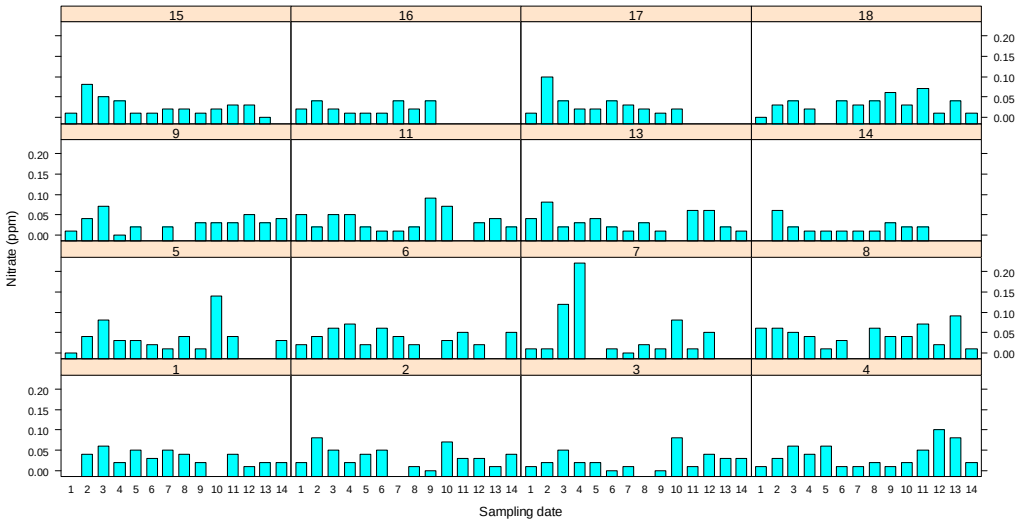


Figure 6:Orthophosphate concentrations for each pond on each sampling date

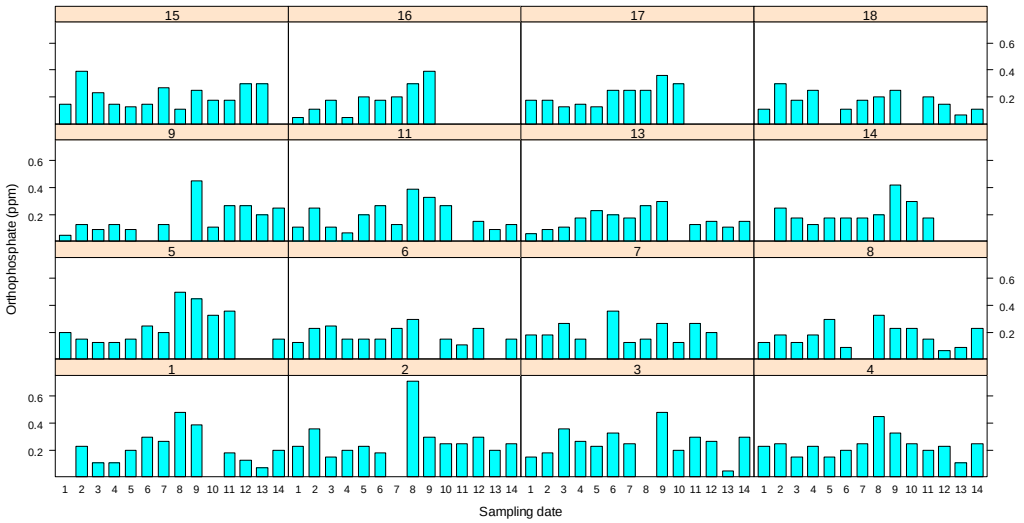


Figure 7:Temperatures for each pond on each sampling date

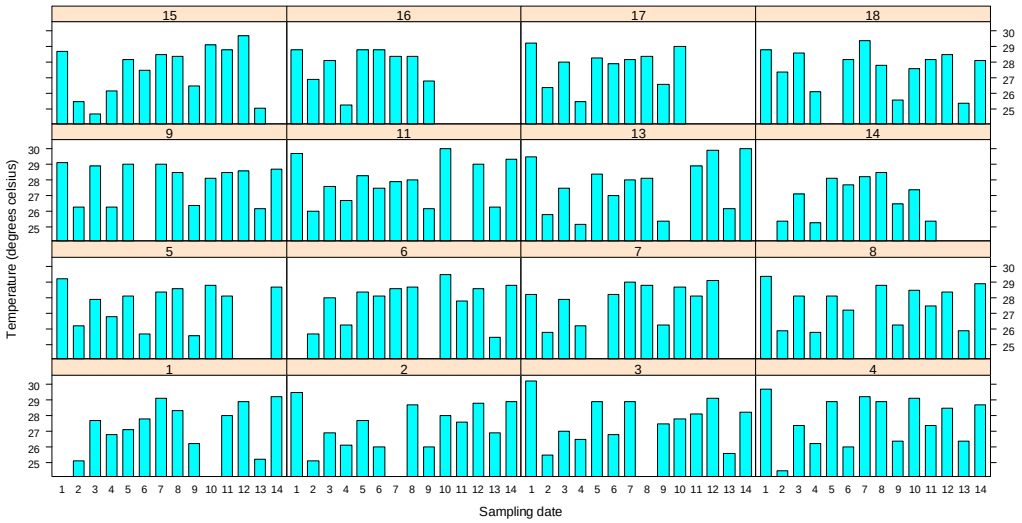


Figure 8:Dissolved oxygen concentration for each pond on each sampling date

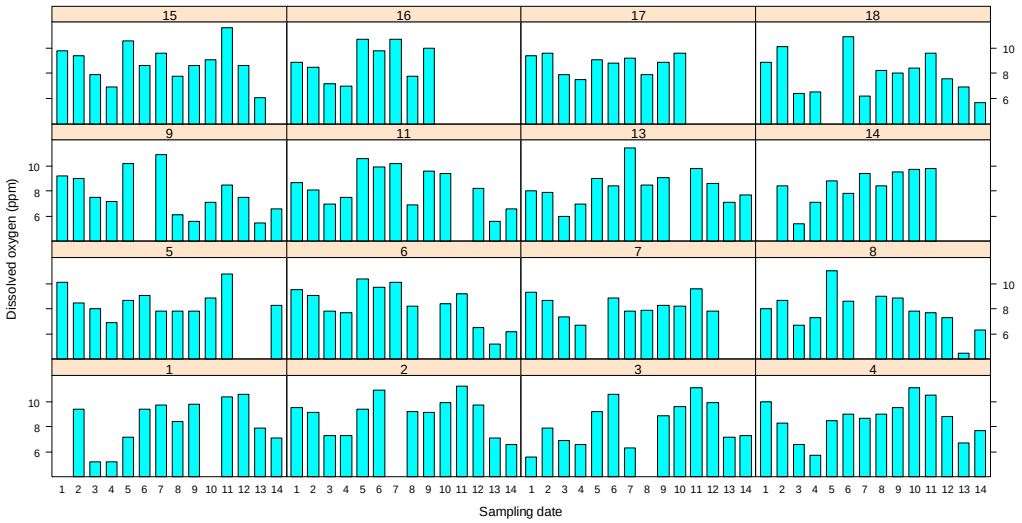
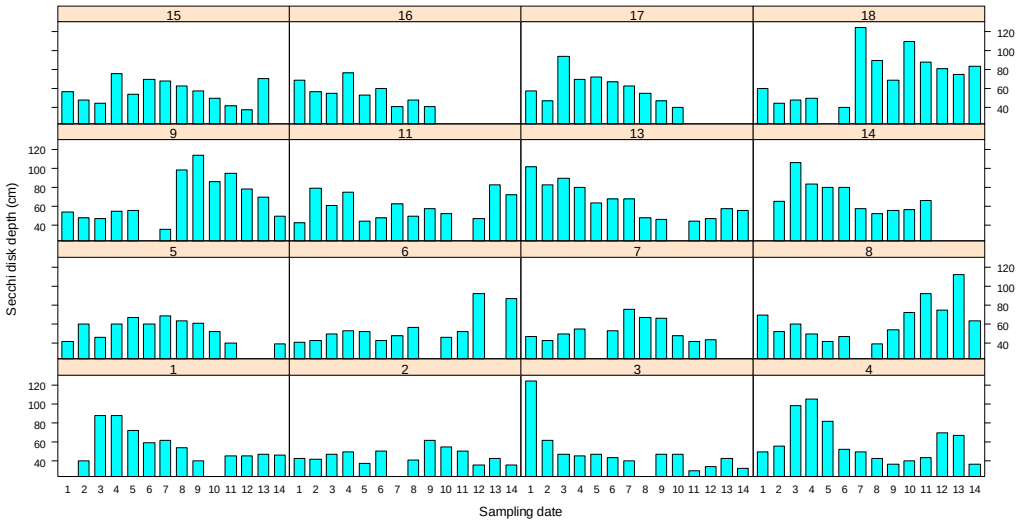
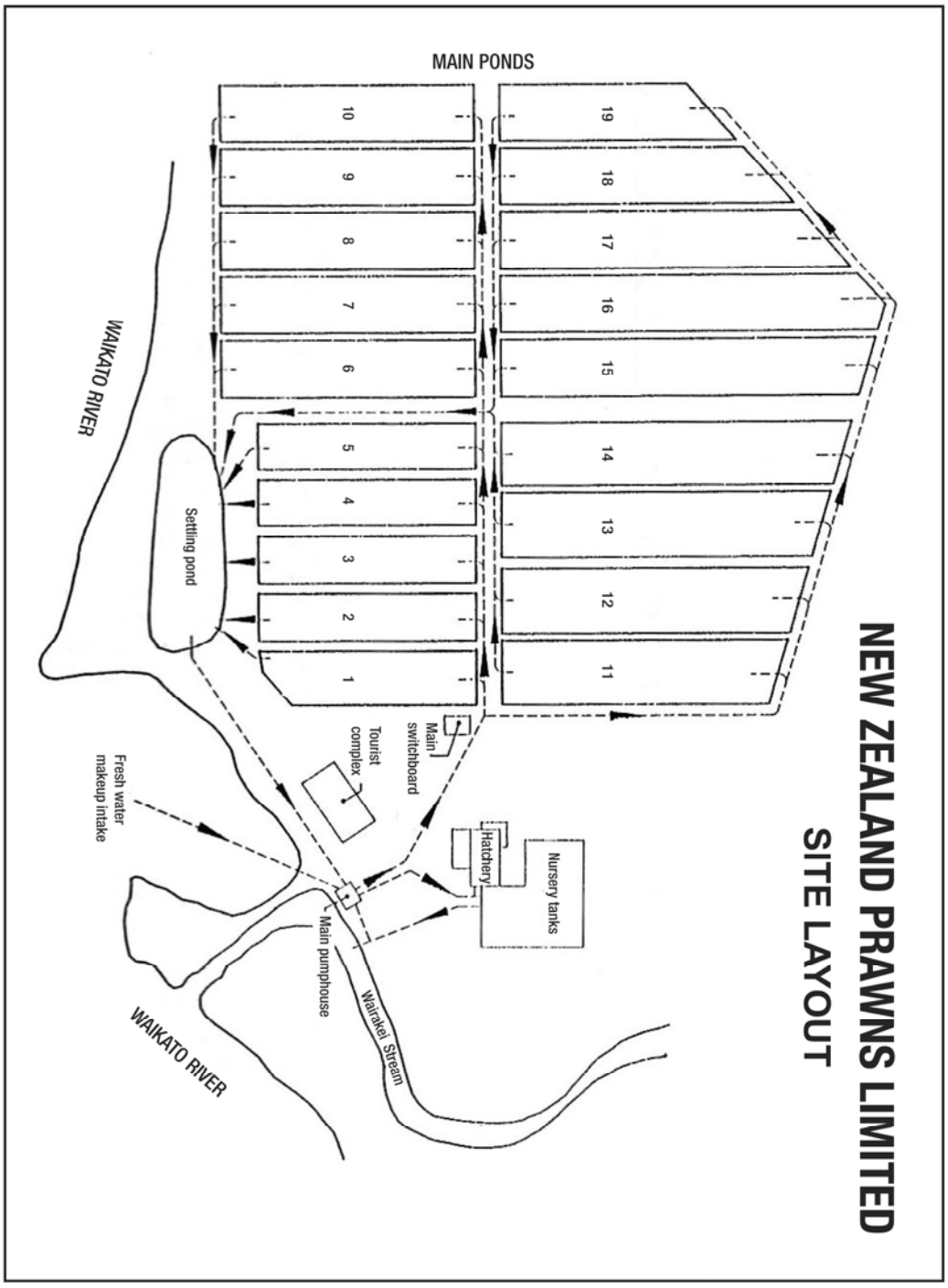


Figure 9:Secchi disk depths for each pond on each sampling date



APPENDIX4: Aerial diagram of New Zealand Prawns Ltd.



APPENDIX 5: Photos of New Zealand Prawns Ltd.

Photo 1: Grow out ponds (bottom series).
Photo: Daniel MacGibbon

Photo 2: Grow out ponds (top series).
Photo: Daniel MacGibbon

Photo 3: Settling pond.
Photo: Daniel MacGibbon

Photo 4: Heat exchange device.
Photo: Daniel MacGibbon

Photo 5: Drained pond (post-harvest).
Photo: Daniel MacGibbon

Photo 6: Harvesting of prawns.
Photo: Daniel MacGibbon

Photo 7: Sorting of harvested prawns.
Photo: Daniel MacGibbon

Photo 8: Artificial prawn habitats.
Photo: Daniel MacGibbon

Photo 9: Post-larvae nursery tanks.
Photo: Daniel MacGibbon

Photo 10: Post-larvae.
Photo: Daniel MacGibbon

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