Assessment of a pond for oyster hatchery development in South Africa

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Abstract

This study assessed a man-made salt-water pond (saltwater pond 1 SP1) as a potential site for the development of an oyster hatchery in Velddrif, South Africa. Over the study period it was observed that the site was not suitable for oyster larvae culture due to the following factors: temperature spikes during the summer months, low dissolved oxygen levels, high salinity levels, and the proliferation of the filamentous green algae (FGA), mainly the sp. *Rhizoclonium implexum*. Widespread patches of *R. implexum* were observed within SP1 and increased in biomass over the study period. Biomass patterns were not measured within this study, however microcosm experiments directed at nutrient depletion rates caused by FGA proliferation assessed the effect of the FGA on the system. Within microcosm experiments with and without FGA, nitrite within the system was significantly lower in the FGA inclusion treatment. Pond nutrient dynamics within the system indicated that widespread nutrient depletion occurred between the incoming water and the rest of the pond, and it was clear that the inflow station had significantly higher nutrient concentrations than all the other stations within SP1. Phytoplankton concentrations were extremely low and could be attributed to the FGA dominated state within SP1. Taken together with the fact the water levels within SP1 were not stable, the data suggested that SP1 was not an optimal source of seawater for either algal or larval oyster culture, and an alternative oyster hatchery site should be assessed.

Chapter 1: Introduction and Literature review

Current trends of commercial oyster culture in South Africa

Aquaculture in South Africa (SA) is still regarded as a developing sector, particularly marine aquaculture (mariculture) (Griffiths and Branch, 1997; Haupt *et al*., 2010). This is largely due to the exposed nature of the South African coastline, which has few sheltered bays suitable for mariculture. In 2011 marine invertebrates, particularly high-value exported abalone, contributed the most value to South African commercial production. Total sales of abalone (93.9 %), oysters (3.8 %) and mussels (2.4 %), generated R3.5 billion in export revenue during that year (Haupt *et al*., 2010; DAFF, 2012).

The commercial culture of oysters in SA began in 1948 (Haupt *et al*., 2010). Pioneer oyster farmers at the time cultured the indigenous South African common rock oyster, *Striostrea margaritacea*, but insufficient knowledge of culture techniques with regards to growth, fattening and reproduction as well as environmental change associated with freshwater intrusion, and shifting sand were the major problems that led to the discontinuation of these ventures. The failed cultivation of *S. margaritacea* lead private culture operations to opt for another candidate oyster species that could tolerate these particular environmental perturbations (Haupt *et al*., 2010).

The Pacific Oyster, *Crassostrea gigas*, a temperate species with broad salinity preferences was imported into RSA during 1973 and was initially cultured in the Knysna Lagoon (Griffiths *et al*., 1992; Sandifer and Hopkins, 1996; Robinson *et al*., 2005; Haupt *et al*., 2010). *C. gigas* attained faster growth rates than the indigenous *S. margaritacea*, and reached market size within less than a year, compared to three years for *S. margaritacea* (Haupt *et al*., 2010). Due to its fast growth, *C. gigas* was chosen as the oyster species used in the commercial oyster farming sector in South Africa (Flores-Vergara *et al*., 2004; Dégremont *et al*., 2007; Haupt *et al*., 2010).

The focus of commercial oyster culture is currently concentrated within the Western Cape, specifically the Saldanha Bay area where *C. gigas* is farmed on long lines in Saldanha Bay (Haupt *et al*., 2010). Olivier *et al*., (2013) discussed how the Saldanha Bay area offers a competitive advantage for South African oyster farmers due to the rich upwelling system and high phytoplankton abundance on the West Coast. The nutrient-rich water surrounding the Saldanha Bay area provides higher growth rates and better oyster meat quality than any other site within South Africa used for oyster production (Pieterse *et al*., 2012). Therefore the commercial culture of oysters within Saldanha Bay has shown consistent growth in production and provides improved job security within the region (Olivier *et al*., 2013). There have been questions raised about the translocation of alien species and disease with the increase in oyster production. This is due to the fact that the industry relies on imported oyster spat to sustain its production capacity (Haupt *et al*., 2009; 2010).

Worldwide, the oyster industry has been responsible for the introduction of alien marine epifaunal and infaunal organisms, microorganisms and multicellular parasites (Ruesink, 2005). By law South Africa did not follow the ICES (the Code of Practice of the International Council of the Exploration of the Sea) with regards to oyster spat imports. Within South African operations it was not necessary to quarantine, disinfect or treat imported oyster spat (Haupt *et al*., 2009). Oyster spat imports from France were banned to prevent the introduction of the contagious Ostreid Herpes Virus (OsHV-1) (Haupt *et al*., 2010), highlighting the risks of importing alien *C. gigas* spat.

Crassostrea gigas itself has also become invasive in approximately a third of the countries to which it has been introduced for aquaculture (Ruesink 2005). Introduced populations of *C. gigas* have been identified in three estuaries along the South African coast (Robinson *et al*., 2005; Haupt *et al*., 2010). It was believed that *C. gigas* spat importation did not lead to establishment of natural populations into South Africa (Griffiths *et al*., 1992). However, Keightley *et al*., (2015) reported that there were populations of *C. gigas* within the Swartkops and Kaaimans estuaries, and were now not found within the Knysna and Goukou estuaries, previously reported in 2005 (Robinson *et al.,* 2005). Biosecurity (control of pathogen introductions) is regulated by the import permitting system of the South African Department of Agriculture, Forestry and Fisheries (DAFF) (Haupt *et al*., 2009), which ensures that imported oyster spat have been thoroughly assessed for any contamination before transportation to the prospective destination.

Reduction of both biosecurity risks and costs can be achieved by the importation of juvenile *C. gigas* to oyster nurseries that house spat less than 5 mm in diameter (Haupt *et al*., 2010). The oyster spat is then kept in upwellers until they reach approximately 20-25 mm in length, after which they are then moved into plastic mesh cages and placed in the water column of salt-water dams or ponds until they reach a suitable size for them to be moved. This nursery process takes 3-8 months dependent of the site and seasonal environmental conditions. There are two established oyster nurseries in Southern Africa, located in Kleinzee (Haupt *et al*., 2010). Oyster spat reared within a nursery environment have a lower susceptibility to alien species contamination since the oyster spat are reared in a controlled hatchery environment. However these nurseries are sometimes unable to meet demand from the farms that grow oysters out for market. Therefore it has been highlighted that the establishment of South Africa’s own oyster hatchery will both mitigate biosecurity risks and provide adequate oyster spat supply for the commercial sector (Haupt *et al*., 2009; 2010).

The economics and biological requirements of microalgae production in oyster hatcheries

Currently there are no operational commercial oyster hatcheries in South Africa (Haupt *et al.*, 2010). Spat production in oyster hatcheries worldwide has increased in recent decades to become an economically important part of the culture process (Flores-Vergara *et al.*, 2004). On the West Coast of the United States, the total spat production reached a level of 80% of the total spat quota to supply the growing commercial sector (Ponis *et al.*, 2003).

Bivalve hatcheries require a large amount of infrastructure development and are associated with high production costs (Alagarswami *et al.*, 1987; Ponis *et al.*, 2003). The primary requirement for an oyster hatchery is an uninterrupted supply of pollutant-free seawater that contains the appropriate nutrient concentrations and ratios for the production and maintenance of live microalgae (Alagarswami *et al.*, 1987; Ponis *et al.*, 2003). Microalgae are essential food for broodstock conditioning and larvae and juvenile feeding (Muller-Feuga, 2000; Ponis *et al.*, 2003; Dégremont *et al.*, 2007). A production cost of 30% is attributed solely to microalgae production in oyster hatcheries (Hemaiswarya *et al.*, 2011).

The worldwide production of microalgae for aquaculture purposes is estimated at one million kg.year^{-1} (Muller-Fuega, 2000). The cultivation of microalgae generally occurs within the oyster hatchery (Algarswami *et al.*, 1987), in tanks under controlled light, temperature, carbon dioxide and nutrient ratios regimes to ensure high quality (Hemaiswarya *et al.*, 2011). Microalgae supply and temperature are considered the key factors that affect the physiological processes of *C. gigas* oyster larvae (Flores-Vergara *et al.*, 2004; Rico-Villa *et al.*, 2006).

Oyster spat require specific microalgae and diatoms within their diet to ensure adequate growth. Naturally occurring microalgae species may be utilized within an oyster hatchery, although the chosen species need to fulfill the following criteria to ensure that the oyster spat can utilize them; (1) the microalgae have to be of an appropriate size and shape for ingestion (optimal size range is between 2-5 µm), (2) the cell wall of the microalgae must not inhibit digestion, (3) the microalgae must have a high nutritional content (*i.e.* must be high in high unsaturated fatty acids, (4) the microalgae cultures must be easily produced in different systems and scales within oyster hatcheries (Ponis *et al*., 2003, 2006; Rico-Villa *et al*., 2008).

Multi-species diets in oyster hatcheries have shown that balancing the essential compounds including proteins, lipids and fats from each microalgae species ensures high growth and survival rates of oyster spat (Ponis *et al*., 2003; Hemaiswarya *et al*., 2011). Common microalgae genera used in oyster hatcheries are *Chlorella* (Chlorophyte), *Tetraselmis* (Prasinophyte), *Isochrysis* (Prymnesiophyte), *Pavlova* (Prymnesiophyte), *Chaetoceros* (diatom), *Nannochloropsis* (Eustigmatophyte), *Phaeodactylum* (diatom), *Skeletonema* (diatom) and *Thalassiosira* (diatom) (Ponis *et al*., 2003; 2006, Rico-Villa *et al*., 2008; Hemaiswarya *et al*., 2011). Hatchery feeding strategies range from mono-specific to multi-species to optimize oyster spat nutrition.

Nutrient requirements for a successful oyster hatchery

Monitoring of the nutrient content, especially for limiting nutrients in a particular system, allows a hatchery manager to assess whether nutrient enrichment is necessary (Butzler and Chase, 2009). Limiting nutrients particularly (N, P, Si) influence algal productivity, and particularly Si for diatom growth (Liu *et al*., 2004). There is a clear link between limiting nutrients and productivity of phytoplankton. Liu *et al*., (2004) stated that "Ultimate limiting nutrients (ULN) are limiting nutrients in surrounding water bodies that restrict short-term phytoplankton productivity and are able to control long-term phytoplankton productivity of the whole system by their supply rate." Oyster hatcheries use seawater with the appropriate nutrient concentrations and ratios in upwelling systems to ensure the successful larval rearing process of oyster spat (Haupt *et al*., 2010). Productivity of microalgae is directly related to nutrient content of the water (Turpin *et al*., 1999; Lušić *et al*., 2008).

Nitrogen is often a limiting nutrient in temperate coastal waters, for example in Marennes-Oléron Bay (France) and Rijeka Bay (NE Adriatic Sea) (Tilman *et al*., 1982; Turpin *et al*., 1999; Lušić *et al*., 2008). In the Bay of Bourgneuf (France, Atlantic coast), nitrogen limited the growth of *Haslea ostrearia, Phaeodactylum tricornutum, Skeletonema costatum* (Turpin *et al*., 1999). Nitrogen supplementation experiments conducted on both natural populations and algal cultures have shown that addition of nitrogen alone stimulates growth (Fong *et al*., 1993), and that nitrogen was the principle nutrient limiting marine algal growth under controlled light and temperature regimes (Maestrini *et al*., 1997).

Phosphorus limitation is system-dependent, but generally experienced by phytoplankton within freshwater systems (Liu *et al*., 2004; Ren *et al*., 2009), but experimental evidence suggests that it may also be significant in marine systems such as the northeastern Adriatic Sea (Lušić *et al*., 2008). Phosphorus limitation is system-dependent and varies between locations (Liu *et al*., 2004; Lušić *et al*., 2008; Ren *et al*., 2009), and seasons (Sarnelle, 1992). Total phosphorus (P), is the sum of inorganic and organic phosphorus (Lušić *et al*., 2008). In marine systems, organic phosphorus may exceed inorganic phosphorus, with the former more available for phytoplankton utilization (Lušić *et al*., 2008).

Silicon may affect the species composition of microalgal communities, particularly diatoms, since silica is an essential for the formation of the cell wall or frustule (Tilman *et al*., 1982; Turpin *et al*., 1999; Lušić *et al*., 2008). Silicon supply significantly increased cell numbers in specific diatoms and is a limiting nutrient for growth of the diatom, *H. ostrearia* (Turpin *et al*., 1999). Both in the NE Adriatic and Northern Atlantic, N/Si ratios of 0.4: 1 and

0.1:2 both induced Si limitation in monocultures of the diatoms *P. tricornutum* and *H. ostrearia* respectively (Lušić *et al.*, 2008; Turpin *et al.*, 1999), suggesting considerable reduction in diatom growth when N: Si >1.

The relationship between macroalgae and microalgae

Filamentous green algae (FGA) is the collective term used to group filamentous macroalgae species (Thybo-Christesen *et al.*, 1993; McGlathery *et al.*, 1998; Sundbäck *et al.*, 2003) and generally includes *Cladophora*, *Ulva* and *Chaetomorpha* spp. Proliferation of FGA in a particular system is directly related to nutrient availability, and other environmental factors such as light and temperature. High level nutrient depletion, environmental augmentation and overall aesthetics have labeled FGA a problematic component of aquatic systems in certain cases (Thybo-Christesen *et al.*, 1993; McGlathery *et al.,* 1998; Havens *et al.*, 1999; Eyre and Ferguson, 2002; Sundbäck *et al.*, 2003).

Nutrient removal by FGA affects the overall nutrient dynamics of a system (Thybo-Christesen *et al.*, 1993; Butzler and Chase, 2009). For example, the FGA, *Cladophora* spp. accounted for up to 95% of available N and 85% available P consumption within a shallow Danish Bay, with high nutrient depletion rate creating clear, nutrient poor, water directly beneath the FGA mats (Thybo-Christesen *et al.*, 1993). Indeed, nutrient uptake from both the water column and sediment, particularly by *Chaetomorpha linum*, significantly reduced sediment nutrient fluxes into the surrounding water column in controlled laboratory experiments (McGlathery *et al.*, 1998). Within shallow marine systems localized depletion of surround nutrients leads to and consequently, competition for nutrients with other primary producers such as seagrasses, microphytobenthos and phytoplankton (Sundbäck *et al.*, 2003).

The relationship between FGA and microalgae is highly complex and influenced by environmental change, nutrient flux and allelopathy. *In situ* and laboratory experiments demonstrated that a shallow temperate lake in Central Jutland, Denmark was dominated by two general states, a clear-water state resulting from high FGA biomass and suppression of microalgae, or a turbid state dominated by microalgae (Trochine *et al*., 2011). Experimental trials within a fresh water man-made system on *Sprirogyra* spp. showed that FGA removed nutrients faster than microalgae (Irfanullah and Moss, 2004). In semi-closed saltwater ponds where nutrients are quickly removed from the system, development of microalgae is often limited due to the high rate of nutrient removal by FGA (Dupuy *et al*., 2007). Butzler and Chase (2009) suggested that algae (macro and micro) were able to absorb nutrients much faster than macrophytes. Competitive exclusion within a system may not be solely related to nutrient introduction, but rather a combination of abiotic factors such as nutrient addition, wind stress and temperature (Butzler and Chase, 2009).

Distribution of FGA within shallow saltwater habitats is seasonal, for example, in shallow bays on the west coast of Sweden the FGA (*Ulva* and *Cladophora* spp.) covered approximately 30 to 50% of shallow (0-1 m) soft-sediment habitats, only during the summer months (Sundbäck *et al.,* 2003). Furthermore the temporal and spatial distributions of FGA mats are related to wind and wave exposure, and the total organic content of the sediment (Sundbäck *et al*., 2003). These distribution patterns increase the effect of abiotic factors such as light on these particular systems. Dense floating FGA mats may reduce up to 60-90% of light reaching the bottom sediment, and self-shading may limit uptake of benthic flux which could result in the release of nutrients into the water column (Thybo-Christesen *et al.,* 1993). The effect of light penetration on bottom sediment could result in the competitive exclusion of macrophytes resulting in a FGA dominated system (Butzler and Chase, 2009).

Areas that are subjected to proliferation of FGA are generally associated with high nutrient loads (Dupuy *et al*., 2007). Particularly, shallow coastal areas such as lagoons and in this case a shallow salt-water pond, are susceptible to nutrient enrichment due to limited tidal flushing and freshwater intrusion (Eyre and Ferguson, 2002). Some mitigation measures to limit the proliferation of FGA include the reduction of nutrient run-off from land sources, and the removal of fixed nutrients by manually harvesting the FGA (Sundbäck *et al*., 2003). Mitigation measures are site specific and therefore are dependent on the overall use of the system.

It is often necessary to investigate natural system dynamics within a laboratory context to further understand the relationship between FGA and microalgae in natural systems (Carter *et al*., 2005; Ren *et al*., 2009). Ren *et al*. (2009) suggested three generalized strategies to examine nutrient limitation on microalgae growth namely: (1) the establishment of nutrient uptake kinetics, (2) determination of nutrient criteria, and (3) experimental nutrient bioassays. Nutrient bioassays which include microcosm experiments, allow control of temperature, nutrient addition and species composition, helping to define the total nitrogen and phosphorus ratios that are critical for water management recommendations (Maestrini *et al*., 1999; Carter *et al*., 2005; Ren *et al*., 2009). Investigations of competition between macro and microalgae within a controlled experiment may link productivity changes to particular nutrient and chlorophyll concentrations. However, rapid nutrient mixing within microcosms may lead to abrupt transitions between limited states, whereas in the natural environment more gradual nutrient mixing leads to smoother transitions between states (Passarge *et al*., 2006). Photosynthetic rate increases may follow within 4-6 hours of the supplementation of potentially limiting nutrients (Lean and Pick, 1981), and nutrient uptake in microcosm experiments is highest during the first four days of an experimental trial (Turpin *et al*., 1999). Therefore microcosm experiments can be adapted to mimic specific environmental features that pertain to a particular site. For this present study, Saltwater Pond (SP1) (see below for description) was the model system.

My study site is the primary intake pond of a salt-works. It is 6 ha in area, 1.5m deep, and supplied with fresh seawater from a beach well-head in St Helena Bay through a 1.7 km-long pipeline at a rate of 250 to 500m^3.hr^{-1}. It has been identified as a possible site for an oyster hatchery, but its production potential has not been assessed since it was first filled with seawater approximately 2 years ago. With the exception of pilot data collected intermittently in the first half of 2013, including nutrient and phytoplankton analyses of the water, key management information for the pond's potential for oyster growth was not available before the study.

In summer 2012-13, the pond became heavily overgrown with FGA, and phytoplankton concentrations in the water apparently dropped. It was established that the summer period was crucial to the success of the oyster hatchery since previous *C. gigas* spat growth trials showed almost complete mortality during this period. Therefore it was imperative that this particular study focused on the summer period. In March 2013, approximately 250 young mullet (*Liza richardsonii*, Total Length 10-20 cm) were introduced into the pond to consume this plant material. Preliminary observations suggest that the benthic macrofauna is species-poor and its biomass is probably extremely low, given the recent filling of the pond. Burrowing sand-prawns *Callichirus kraussi* and mudprawns *Upogebia capensis*, important detritivores that turn over nutrients and oxygenate sediments, are absent from the study pond although present in the very similar primary intake pond of a nearby salt-works 9 km to the southwest.

The broad objective of this study was to assess an onshore saline pond, SP1 (Velddrif Saltworks Co., Velddrif, Western Cape, South Africa) as a suitable site for development of a commercial oyster hatchery, in light of heavy growth of FGA in the pond and likely nutrient depletion.

Chapter 2: Assessment of a pond for oyster hatchery development in South Africa

Introduction

The commercial oyster sector in South Africa has shown steady growth despite the absence of a hatchery. Reliance on spat imports from Chile in the commercial sector has raised concerns about the future viability of the sector (Haupt *et al.*, 2010). Risks of disease translocation (OsHV-1, protozoans such as *Perkinsus marinus*, and *P. olseni* and protists such as *Mikrocytos mackini*), and alien species introductions of epibionts such as shell burrowing polychaetes growing on juvenile *C. gigas*, have motivated research on the development of a bivalve hatchery in South Africa (Robinson *et al.*, 2005; Haupt *et al.*, 2009). Oyster farmers, researchers and government institutions such as DAFF and the NRF have identified development of a bivalve hatchery as a priority for the continued success of the South African commercial oyster sector.

It is imperative that bivalve hatcheries have a continuous supply of pollutant-free seawater with appropriate nutrient concentrations and ratios to ensure the successful production of microalgae on site (Algarswami *et al.*, 1987; Muller-Feuga, 2000; Ponis *et al.*, 2003; Dégremont *et al.*, 2007). Flow-through on-shore seawater ponds form good reservoirs for this supply, and one has been identified by industry for the purposes of starting a bivalve hatchery in the form of a man-made pond on the West Coast of the Western Cape, 6 km NE of Velddrif, selected for its location, pollutant free water supply and topography (Figure 2.1). Since its filling approximately two years ago, it has become overgrown by the filamentous green algae (FGA), *Rhizoclonium implexum* (Dillwyn) Kützing, hereafter referred to as simply “FGA” because it was the dominant macroalgae present in the system, smaller amounts of other *Ulva* sp. and filamentous cyanobacteria clusters were also present. A concomitant decline in the observed phytoplankton in the pond may have resulted from nutrient depletion by the FGA.

Figure 2.1 SP1 showing patches of the FGA during the study period

The study site was overgrown by the FGA (Figure 2.1) throughout the study period from November 2013 to April 2014. It can be described as a green tidal-flat mat consisting of fine, loose entangled threads, generally found in calm water in the intertidal zone on sand or mud (Figure 2.2). This particular FGA is from the Phylum: *Chlorophyta*; Order: *Cladophorales*; Family: *Cladophoracaeae* and has a cosmopolitan distribution worldwide. It is characterized by light green unbranched threads with elongate cells approximately 16-22 μm wide (Silva *et al.*, 1996).

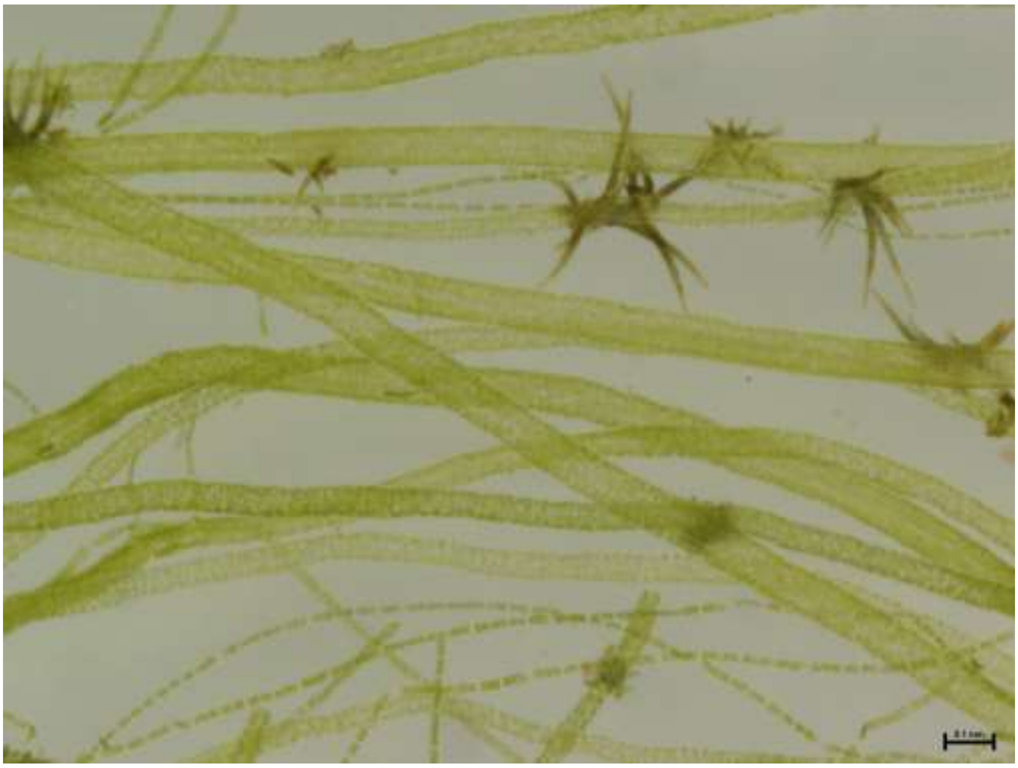

Figure 2.2 FGA sample taken from SP1, indicating the presence of *R. implexum* (fine unbranched threads), *Ulva* sp. and cyanobacteria clusters

My study investigated abiotic factors (wind speed and direction, water temperature, dissolved oxygen, and salinity) in SP1 to provide an understanding of the relationships among these variables to inform management decisions about the development of a bivalve hatchery at the site, using the pond water.

Temperature is a key factor affecting the physiological processes of *C. gigas* within a hatchery (Brzeski and Newkirk, 1996; Dégremont *et al*., 2007).. Environmental conditions caused increased mortality in this species during summer periods in La Tremblade, France. *C. gigas* larvae tolerate a temperature range of 17-32 °C. Temperatures below 17 °C decrease the growth of *C. gigas* larvae by inhibiting digestion (Dégremont *et al*., 2007). However, although *C. gigas* grows fast at temperatures lower than 24 °C, the larvae still survive at temperatures as low as 15 °C (Flores-Vergara *et al*., 2004). It was previously believed that temperatures exceeding 27 °C would cause high oyster mortality (Brzeski and Newkirk, 1996), but recent studies indicate that the optimal temperature for culturing *C .gigas* larvae is 27 °C, which provides the highest growth rate of 19.31 $\mu m.d^{-1}$ and an 87% settlement rate (Rico-Villa *et al*., 2008).

Dissolved oxygen and salinity affect growth, survival and reproductive activity in *C. gigas*. Dissolved oxygen concentrations between 2 and 5 mg/L are sufficient for oyster production (Neto *et al*., 2013). For *C. gigas* larvae, salinity 28 ppt ensures embryo development in the hatchery (FAO, 2004). Suitable salinity for the cultivation of *Crassostrea* spp. occurs between 25 and 35 % (Neto *et al*., 2013).

Nutrient-rich water is required for the successful larval rearing of *C. gigas* spat, because microalgal productivity is directly related to water nutrient content (Turpin *et al*., 1999; Haupt *et al*., 2010). Concentrations of limiting nutrients such as total nitrogen, phosphates and silicates influence the productivity of microalgae (Liu *et al*., 2004; Lušić *et al*., 2008). Monitoring of the nutrient content, especially for limiting nutrients, in a particular system allows a hatchery manager to assess whether nutrient enrichment is necessary to increase phytoplankton growth culture (Butzler and Chase, 2009).

The molar elemental ratio C: N: P in most phytoplankton is stated as the "Redfield ratio" 106: 16: 1 and can be used as a general standard when assessing nutrient limitation in phytoplanktonic cells (Redfield, 1985). When phytoplankton cells have a ratio that is different to the Redfield ratio they are said to nutrient limited for an element, and this is dependent on the particular phytoplankton species. The elemental ratio of seawater affects the type of phytoplankton species dominance because some species may be able to utilize the available nutrients better than others. Silicon only matters when diatoms are present and the Redfield-Brzezinski ratio proposed for nutrient limitation in diatoms is 106: 15: 16: 1 (Brzeski and Newkirk, 1996). Nitrogen limitation occurs when N: P <16, and N: Si <1;

phosphorus limitation occurs when N: P >16 and Si: P >16; and silicon limitation occurs when N: Si >1 and Si: P <16 (Dubravko et al., 1995).

Blooms of FGA are associated with high nutrient availability and are known to assimilate large amounts of nitrogen and phosphorus from the surrounding water column (Thybo-Christesen *et al.*, 1993). Steep vertical nutrient gradients occur within floating and benthic macroalgal mats in shallow systems (McGlathery *et al.*, 1998), creating competition between micro and macro algae. In semi-closed systems where nutrient uptake rates are high due to high FGA abundance, micro algae development is often limited (Dupuy *et al.*, 2007). Simulating the nutrient uptake dynamics may give insight into the rate at which specific nutrients are removed from a particular system (Maestrini *et al.*, 1997). The microcosm component in this study investigated the links between nutrient depletion and change in biomass measured as chlorophyll *a* over a controlled time period. This was conducted to quantify the relationship between FGA and other primary producers in the pond system, under controlled conditions.

Specific aims:

- To describe abiotic variables (wind strength and direction, temperature, dissolved oxygen, and salinity) influencing nutrient dynamics and water quality of SP1 over six months (November 2013 – April 2014);
- To ascertain nutrient concentrations of Total Nitrogen, Phosphorus and Silica of SP1; and
- For the two main primary producers in the pond, use of a microcosm experiment to quantify nutrient depletion (measured concentrations over time) and primary production (change in biomass or chlorophyll *a* concentrations) under standard conditions in separate monocultures,
- To assess whether the pond SP1 is an optimal candidate for oyster larval culture.

Methods and Materials

The study site was an onshore saline pond at the primary intake of a salt production works on the West Coast of South Africa (32°43'42.5"S 18°12'37.8"E) approximately 6 km NE of Velddrif. Sampling was conducted at six fixed stations (Figure 2.3) located in the primary intake pond, roughly 6 ha in area, 1.5 m deep, and supplied with fresh seawater from a beach well-head in St Helena Bay (Figure 2.3). Each station comprised a concrete weight that was placed on the pond bottom, supporting a vertical plastic rod reaching to the pond surface. Submersible data loggers were fixed to this rod at 25 cm and 100 cm from the bottom (respectively, "Bottom" and "Top" hereafter). These data were supplemented with twice-monthly water sampling trips from November 2013-April 2014 for nutrient analyses and temperature and salinity measurements.

Figure 2.3: Fresh sea-water is pumped from St Helena Bay at the beach well head situated at (1) and flows (blue arrow) towards SP1 (bottom left). Six sampling stations were established and were named as follows: (2) inflow; (3) outflow; (4) outflow bay; (5) centre; (6) left; (7) right (Source: Google Earth).

Pond management: time series of abiotic variables

A submersible RBR concerto data logger was mounted on the centre station rod from 01 January 2014 to 18 May 2014, providing estimates of dissolved oxygen, temperature and salinity at five-minute intervals, 75 cm from the pond bottom throughout this period. In addition *in situ* dissolved oxygen and temperature readings were taken at 0.5 m depth at each station using a YSI ProODO dissolved oxygen meter during the bi-monthly water sampling trips between November 2013 and April 2014.

From 27 November 2013 to 01 May 2014, two temperature loggers (Thermochron iButtons, Maxim, Dallas, Texas, USA) were attached to the top and bottom levels of each of the stations approximately 0.75 m apart. The temperature loggers were placed at each site and were fixed at 25 cm and 100 cm from the bottom, and provided half-hourly estimates of temperature. Data obtained was used to plot the temperature time series of the sample sites.

In situ hourly wind speed and daily wind direction data were obtained from the South African Weather Service for 01 November 2013 to 01 May 2014 for Cape Columbine. A wind strength time series for the study period was used to visually assess the influence of wind strength and temperature stratification in the pond.

Water samples were taken every two weeks for the duration of the study period for manual nutrient analysis in the laboratory. Approximately 100 ml water was taken from approximately 50 cm below the water surface at each site, filtered on-site using Whatman GF/F filters (0.45 µm) and stored in polypropylene sampling bottles on ice prior to nutrient analysis in the laboratory. Five nutrients were quantified using adapted methods for ammonium NH_4 (Grasshoff *et al*., 1976), phosphate PO_4 and silicate SiO_4 (Grasshoff *et al*., 1999), and nitrite NO_2 and nitrate NO_3 (Nydahl, 1976).

Water samples of 200 ml were taken from the surface of the centre station for phytoplankton identification and fixed in a buffered formalin solution and analysed using the Utermöhl method (Hasle, 1978). Before to counting, samples were gently shaken 50 times to fully suspend all cells, poured into cleaned sedimentation chambers of 25ml, and allowed to settle overnight at room temperature with no direct sunlight. The chamber bottoms with settled cells were viewed with a ZEISS 476100 inverted microscope, initially at 160x to assess cell density and distribution, then counted at 400x magnification. In each sample four diagonal transects were counted (Utermöhl, 1931; 1958). Species names and pictures were used to verify identification (Tomas *et al.*, 1997).

The following formula was used to calculate cell concentrations for each sample.

$$Cell\ concentration\ (cells.ml^{-1}) = \frac{cell\ count\ \times area\ of\ chamber\ (mm^2)}{area\ counted\ (\mu m^2) \times \frac{1}{volume\ settled\ (ml)}}$$

Where:

Area of the chamber = 490.87 mm^2

Area counted (AC) = width of strip (e.g. 0.76 μm^2 at x16 and 0.31 μm^2 at x40 magnification) x length of strip (e.g. 15 mm) x number of strips (e.g. 4)

Volume settled (e.g. 25ml)

Microcosm experiment

50 L of seawater was collected from the inflow pipe of SP1 and stored in 5 L polypropylene bottles. The water was filtered within three hours of collection using a vacuum pump and Whatmann GF/F filters (0.45 μm), frozen in a blast freezer, stored at -18 °C and thawed one day prior to use in the experiments. This water served as the main culture medium for the experiments. Seawater collected for these experiments was not autoclaved due to possible PO_4 and Fe precipitation, and NH_4 release (Turpin *et al.*, 1999).

Treatment Groups

Three treatment groups were used in the microcosm experiments, Control (n=4), FGA present (n=10) and FGA absent (n=10). All experiments were conducted in 1 litre glass reagent bottles which were autoclaved prior to the start of the experiments to prevent introduction of extraneous bacteria, and filled with 700 ml of filtered seawater (culture medium) collected from the inflow pipe of SP1.

The FGA present treatment simulated a FGA-dominated state. It comprised the culture medium, approximately 0.45 g wet mass of *R. implexum* blotted with tissue paper to remove excess water weight, and an "inoculum" of 300 ml of unfiltered, seawater taken from the deepest region of the pond: namely the "outflow bay" site, during collection of FGA, a day before the experimented started. Inoculum was used to "spike" the filtered seawater with the pond's naturally occurring phytoplankton community. "Inoculum"-water was transported from the pond to the laboratory on ice.

The FGA absent treatment comprised the culture medium spiked with the same volume of inoculum as the FGA present treatment, without *R. implexum,* to quantify the productivity of microalgae in the absence of competition.

The control treatment comprised the initial culture medium spiked with 300 ml autoclaved seawater.

Since we were interested in the initial community responses, chlorophyll *a* concentrations and concentrations of NH_4, NO_3 and PO_4 were measured on day 1, 2, 3 and 8 for daily nutrient concentrations and day 0, 1, 2, 3 and 8 for chlorophyll *a*. Each experimental cycle ran for seven days or until chlorophyll *a* decreased or showed no increase from the previous day according to methods described in Ren *et al.* (2009).

The experiments were conducted in a controlled laboratory environment under a fixed 14 hour light and 10 hour dark regime with an ambient temperature of 16 °C. Airlines with glass pipette tips were used in all the experiments to ensure sufficient airflow and oxygen in the water.

Water samples were taken at the stipulated times for the duration of the experimental period for nutrient analysis. Approximately 50 ml water was taken from approximately each respective replicate and filtered using Whatman GF/F filters (0.45 μm) and stored in polypropylene sampling bottles in a chest freezer prior to nutrient analysis in the laboratory. Three types of nutrients were analysed using the adapted methods for PO_4 (Grasshoff *et al*., 1999), NO_2 and NO_3 (Nydahl, 1976). Nutrients were analysed manually by me under the supervision of Dr Trevor Probyn.

Chlorophyll *a* analysis was conducted by filtering 100 ml of water through a Whatman GF/F filter (0.45 μm) for each replicate. The filters were then stored in a chest freezer prior to analysis. For the chlorophyll *a* analysis, the filters were fixed in 5 ml of dimethyl sulfoxide–90% acetone (40:60 by volume) and allowed to extract over-night (Lohrenz *et al*. 1999). The extracts were then measured on a Turner Model 10 fluorometer before and after acidification with 10% HCl (Parsons *et al*. 1984). Calibration of the fluorometer was conducted using a known chlorophyll standard.

Statistical analysis

Microsoft Excel (2010) was used to store the time-series data for wind, temperature, dissolved oxygen and salinity. GraphPad PRISM 6 (GraphPad Software, Inc., 2012) was used to plot time-series' for wind, temperature, dissolved oxygen and salinity. GraphPad PRISM 6 was used to plot graphs for the nutrient data as well as calculate statistical differences using ANOVA's followed by Tukey's *post-hoc* tests to compare nutrient concentrations between the stations. For each nutrient curve, we ascertained which polynomial fit best using the extra sums of squares for the 2 models with the difference expected by chance. The result is expressed as an F ratio, used in combination with numerator and denominator degrees of freedom to generate a P value (Pieterse *et al.*, 2012). Unless otherwise stated, means are presented ± S.E.

Results

Temperature

Water temperatures at the bottom levels of each station were plotted from the 27 November 2013 to 23 April 2014 (Figures 2.4; 2.5, Table 2.1). Diurnal variation is evident (Figure 2.4; 2.5) as temperatures decrease during the night and increase during the day. Since the study was conducted during summer we see that temperature within SP1 gradually decreases as autumn approaches in 2014.

	Inflow	**Outflow**	**Outflow Bay**	**Left**	**Right**	**Centre**
Minimum	5.7	14.9	18.6	14.7	15.0	15.0
Maximum	43.1	29.6	28.2	29.4	30.1	30.3
Mean	20.1	23.0	23.7	22.7	23.0	22.9

Table 2.1 Means, minima and maxima of all half-hourly bottom temperature readings (°C) from 27 November 2014 to 18 May 2014 (n=7123).

Water level changes

From February 2014, the pond water level started to decrease due to leakages in the pond bottom and walls. Since we were measuring the water temperature differential between the "top" and "bottom" of the respective sites. All bottom data loggers, with the exclusion of the inflow site were submerged for the full study period. Water level changes could be seen when there were high fluctuations between top and bottom loggers.

At the inflow station the temperatures ranged from 5.7 to 43.1 °C with a mean of 20.1 °C ± 0.051 °C, where cold water minima were attributed to fresh seawater influx. Figure 2.4 (a) depicts the temperature time-series of the inflow site and the bottom data logger could be used to indicate periods of fresh saltwater pumped into SP1 from St. Helena Bay. Temperature readings taken from the inflow station were not consistent with the rest of the study site since the temperature loggers were not submerged for the entire study period and this explains the wide temperature range.

The bottom stations throughout the study period were fully submerged, with the right site (Figure 2.5 f) the warmest 23.0 °C ± 0.028 °C. Among the other stations, the centre site (Figure 2.4 b) had the largest range, with temperatures from 15 to 30.3 °C.

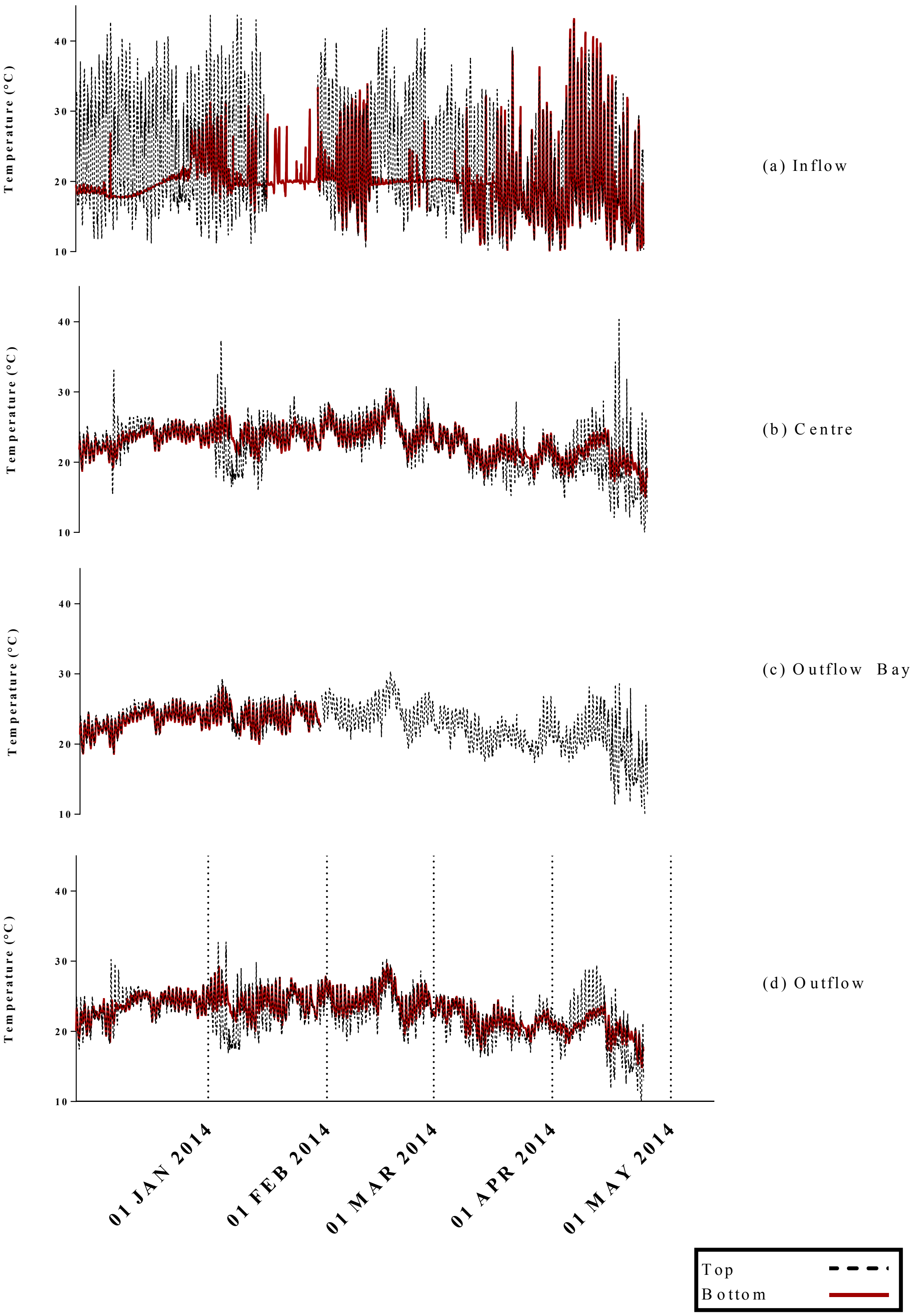

Figure 2.4.Half-hourly water temperature at the top and bottom positions for (a) inflow, (b) centre, (c) outflow bay and (d) outflow stations from 27 November 2014 to 24 April 2014.

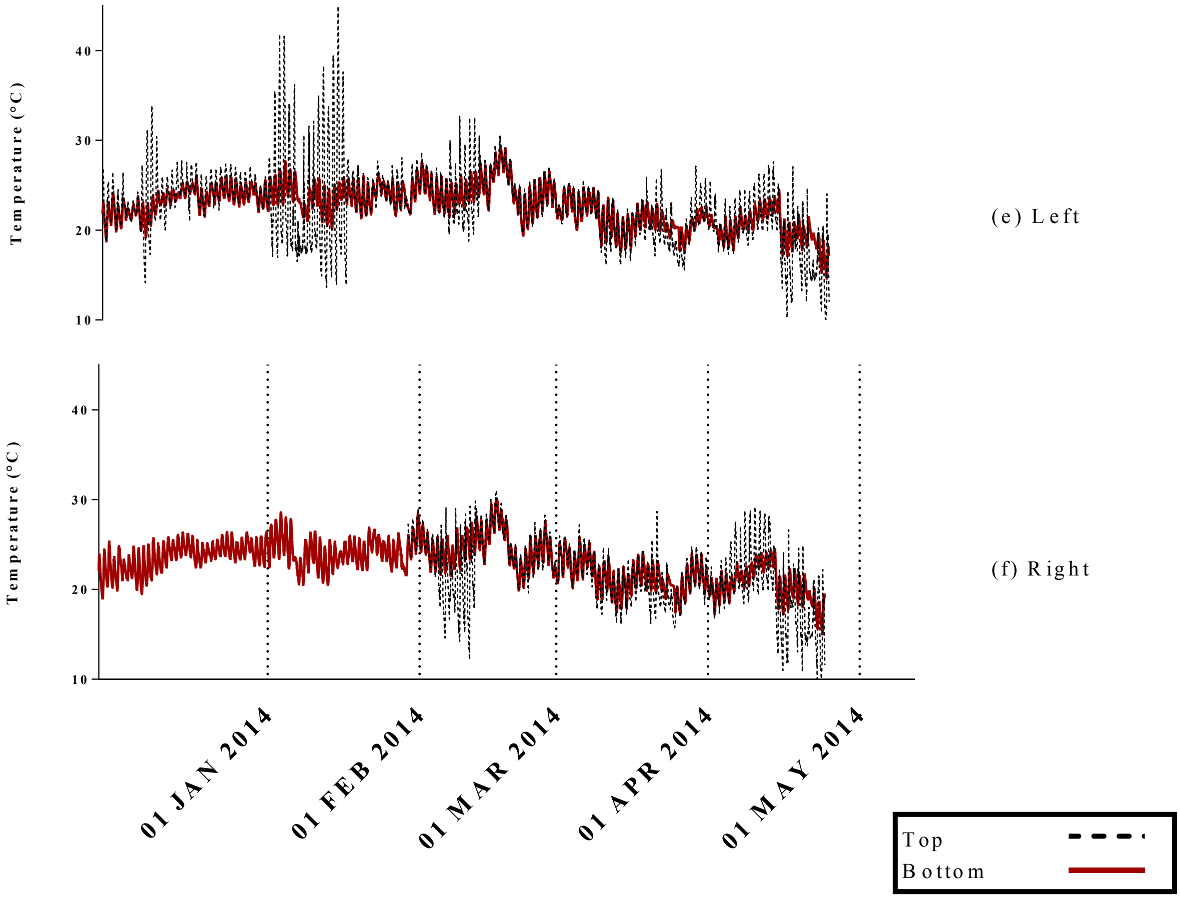

Figure 2.5.Hourly water temperature at the top and bottom positions for (e) left and (f) right stations from 27 November 2014 to 24 April 2014.

Wind Magnitude

Hourly wind magnitude was plotted over the period 27 November 2013 to 01 May 2014, (Figure 2.6), with magnitude omitted both because wind mixing of the water column in a small isolated water body such as the pond SP1 is independent of the wind direction, and for clarity. Wind data from Cape Columbine were plotted alongside the bottom temperature from the centre station. Some periods of relatively high wind speed appeared to be followed by water cooling, suggesting wind-induced mixing.

Wind induced mixing could be seen at five points during the study, indicated by the dashed lines on Figure 2.6. In these instances, average wind speed at Cape Columbine was over 13 m.s^{-1}. The third instance of wind mixing could be seen during the middle of December 2013, where a change of 4 °C was seen. Other periods of wind mixing occurred at the end of February 2014 and then again at the beginning of March 2014. Temperature at the centre site of SP1 in these instances dropped into the low 20 °C range and below at the fourth instance indicated with the second dashed arrow.

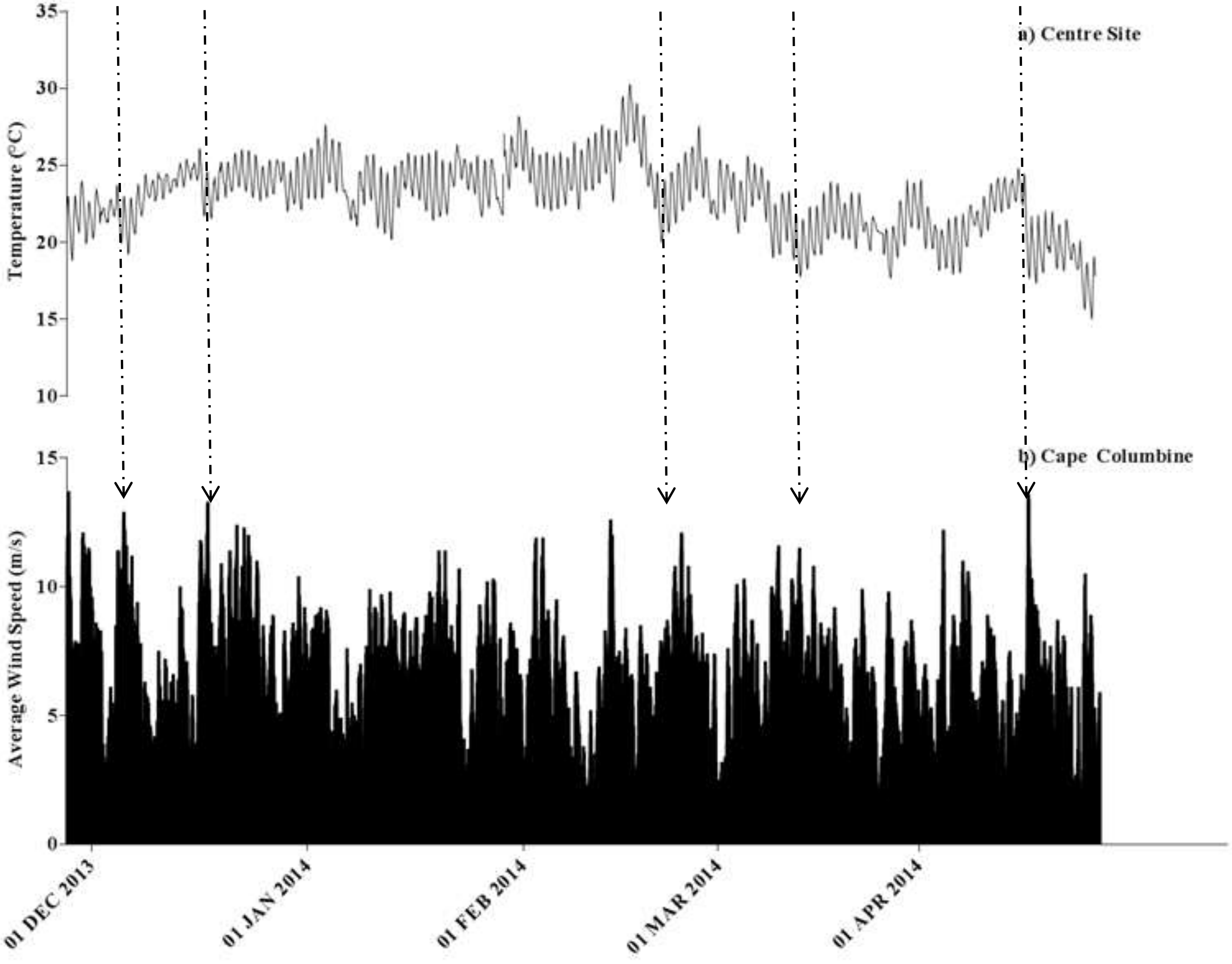

Figure 2.6.Half-hourly water temperature readings at the bottom position of the centre station (a) and hourly wind magnitude at Cape Columbine (b) from 27 November 2014 to 24 April 2014.Arrows indicate possible examples of wind mixing at SP1.

Dissolved Oxygen and Salinity

At the centre station from 12 February 2014 to 17 March 2014. DO ranged from 0 to 12.16 mg.L^{-1}with a mean of 3.49 ± 0.013 mg.L^{-1} (Fig. 2.7). Diurnal variation could be seen as DO levels increased in the day due to photosynthesis and decreased in the evenings.

Salinity measured at the centre site of SP1 from 12 February 2014 to 17 March 2014 ranged from 37.2 to 39.7 ppt with a mean of 38.8 ± 0.005 ppt.

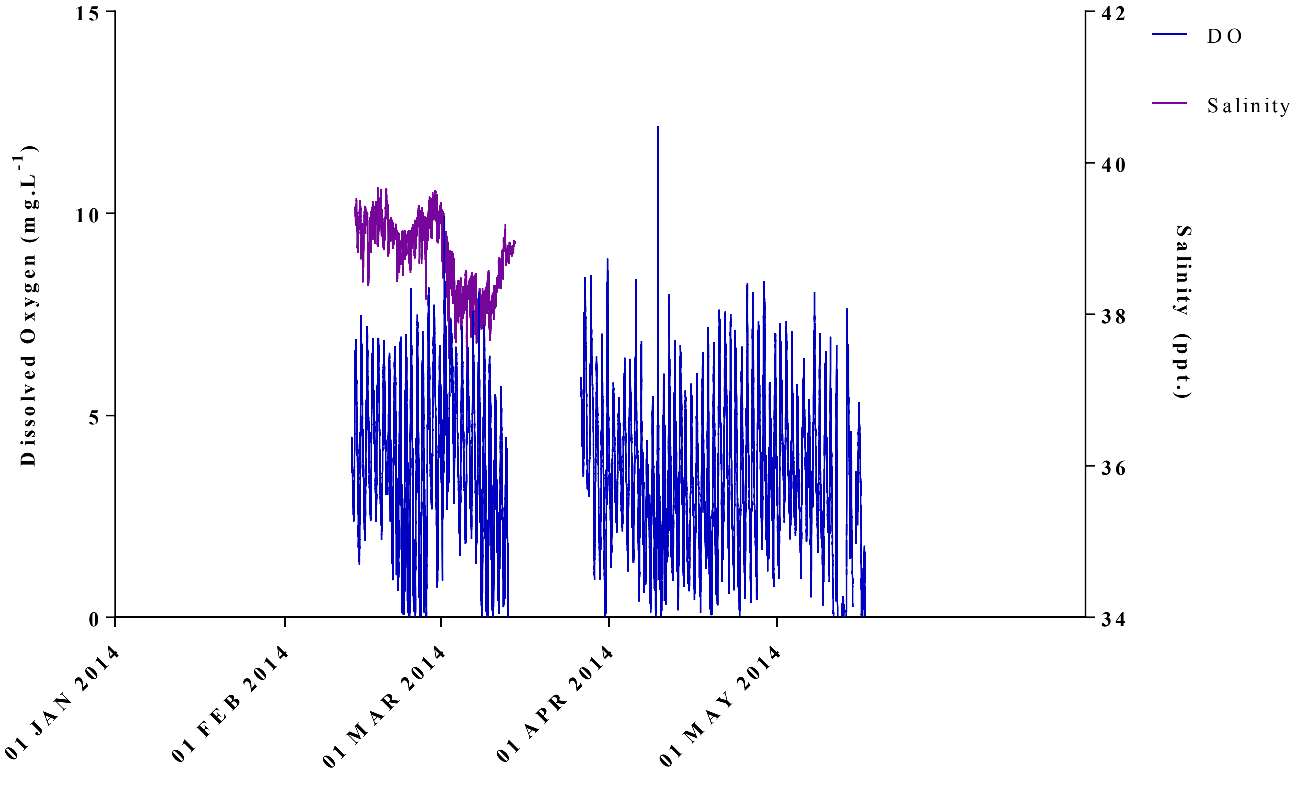

Figure 2.7 Dissolved oxygen and salinity at the centre site from 12 February 2014 to 17 May 2014 measured at five-minute intervals.

Nutrient Concentrations

Concentrations of PO_4, SiO_4, NH_4, NO_2 and NO_3 showed a consistent pattern, with the inflow station having significantly higher values than the other stations, which did not differ from each other (Figure 2.8). The lowest PO_4 concentration of 1.58 ± 0.37 µmol.L^{-1} occurred at the left station, and the highest, of 16.39 ± 4.30 µmol.L^{-1} at the inflow, and this high concentration of P could have been attributed to high amounts of organic matter decomposition at the beach well head. PO_4 concentrations at the inflow station were significantly higher than at all other stations (one-way ANOVA $F_{5,65}$ = 10.79, $P < 0.0001$). The lowest SiO_4 concentration of 9.79 ± 1.369 µmol.L^{-1} occurred at the outflow bay station, and the highest at the inflow station 68.10 ± 20.46 µmol.L^{-1}. SiO_4 concentrations at the inflow station were significantly higher than at the other stations (one-way ANOVA $F_{5,65}$= 7.242, $P < 0.0001$).

The outflow bay station had the lowest NH_4 concentration 0.61 ± 0.15 µmol.L^{-1} and the highest at the inflow station 16.21 ± 6.67 µmol.L^{-1}. NH_4 concentrations at the Inflow station were significantly higher than at the other stations (one-way ANOVA $F_{5,65}$= 4.818, P = 0.0008). The lowest NO_3 concentration 0.290 ± 0.087 µmol.L^{-1}, occurred at the left station, and the highest at the inflow station 5.93 ± 3.01 µmol.L^{-1}. NO_3 concentration at the Inflow station were significantly higher than at the other stations (one-way ANOVA F5,65 = 2.924, P = 0.0192). Likewise, the lowest NO_2 concentration 0.15 ± 0.026 µmol.L^{-1}, occurred at the outflow station, and the highest at the inflow station 0.48 ± 0.12 µmol.L^{-1}. NO_2 concentrations at the inflow station were significantly different from the outflow station (one-way ANOVA F5,65 = 2.681, P = 0.029).

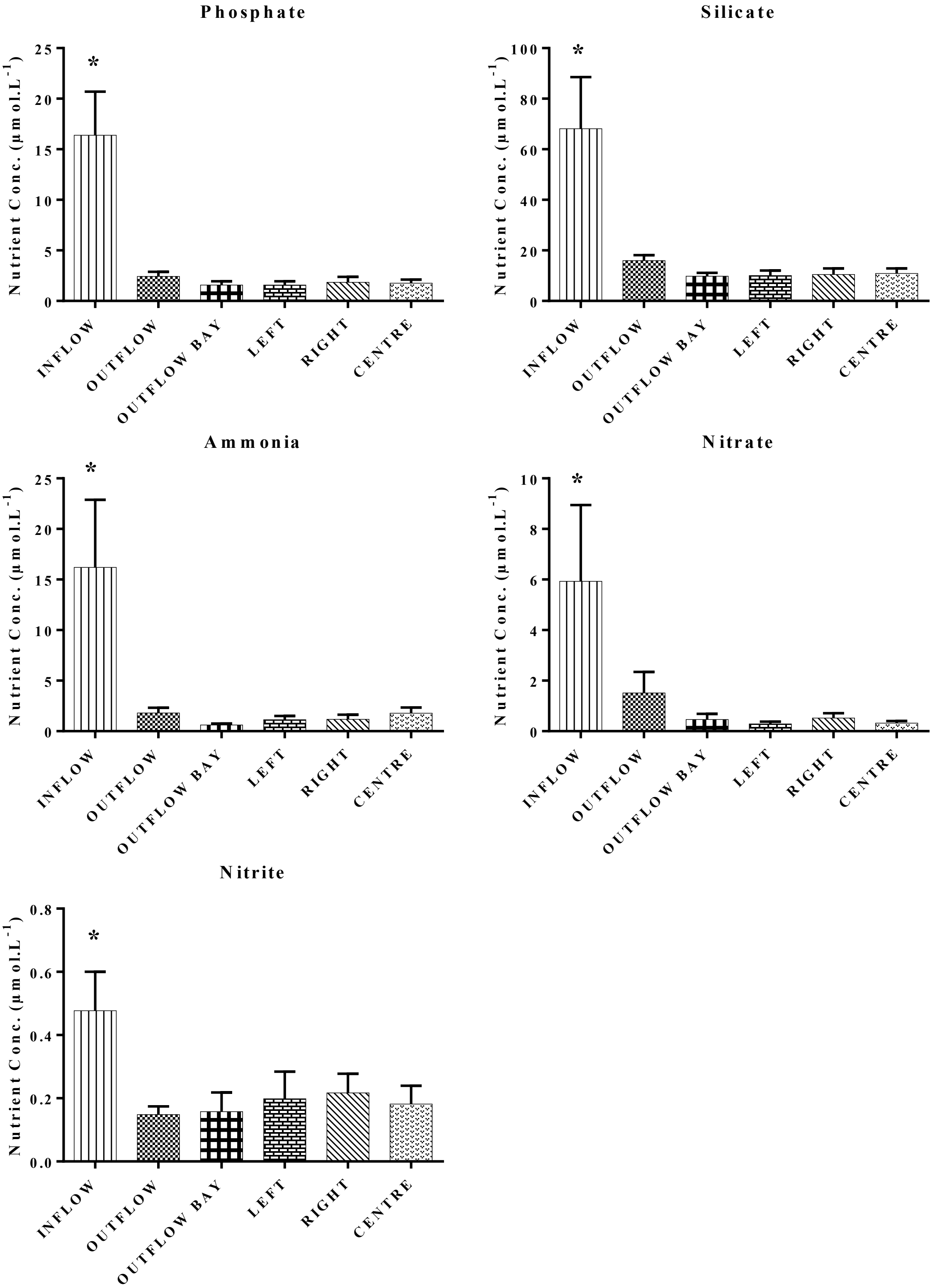

Figure 2.8 Mean nutrient concentrations of SP1 *denotes significant difference according to Tukey's multiple comparisons test (*P* <0.0001).

Nutrient Ratios

Concentrations of PO_4 and SiO_4 were plotted against TN (Total Nitrogen) of the inflow station to illustrate which particular nutrients were limiting in incoming water received from St Helena Bay (Figure 2.9, 2.10). Therefore it was necessary to calculate possible individual limiting nutrients in the incoming water measured at the inflow station. Nutrient ratio concentrations of TN vs. PO_4 and TN vs. SiO4 were calculated only when fresh seawater was pumped into SP1. The results indicated that nitrogen in the inflow from SP1 was not limiting with high overall nutrient ratios.

In Figures 2.9 & 2.10, nutrient ratios of TN: PO_4 of 16 and TN: SiO_4 of 1 are indicated by the dashed line. In comparison to the standard Redfield ratios both phosphates and silicates are not generally limiting nutrients in the inflow site.

Therefore it could be suggested that the nutrient ratios calculated for phosphates (TN: PO_4 = 1.11) and silicates (TN: SiO_4 = 0.17) may indicate that phytoplankton growth in the inflow water may be generally nitrogen limited.

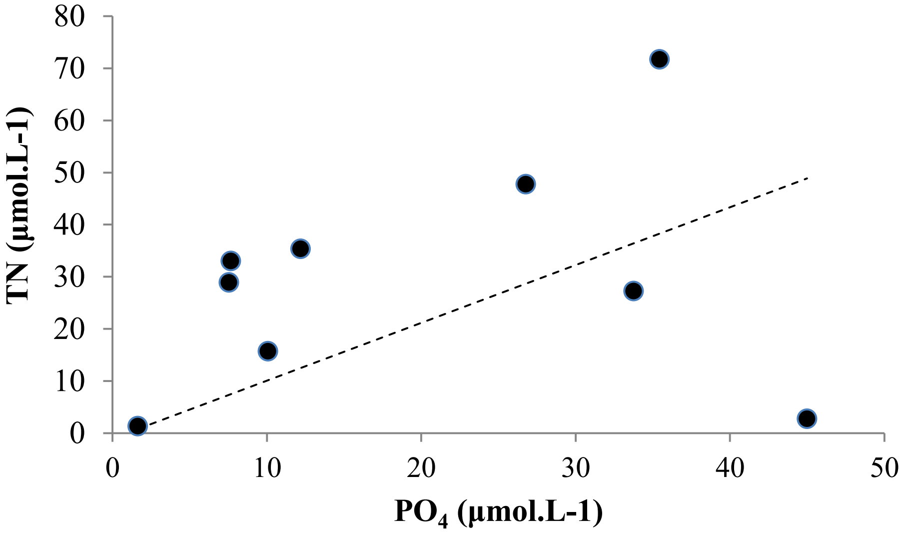

Figure 2.9.Scatterplot illustrating TN vs. PO_4 at the inflow, sampled only when fresh sea-water was flowing into SP1. Nutrient ratio (TN: PO_4 = 1.11) is indicated by the dashed line.

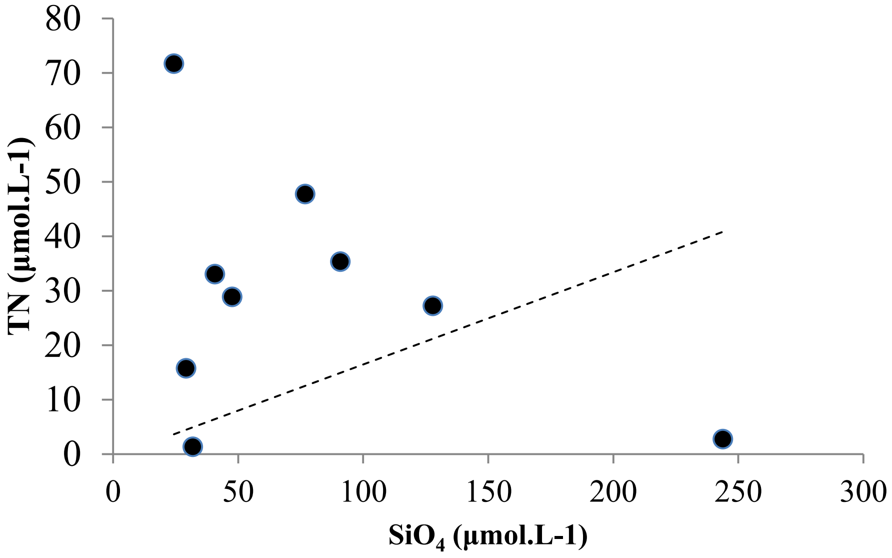

Figure 2.10.Scatterplot illustrating TN vs. SiO_4 at the inflow, sampled only when fresh sea-water was flowing into SP1. Nutrient ratio (TN: SiO_4 = 0.17) is indicated by the dashed line.

Microcosm experiment

Nutrients

Nutrient concentration time series curves of PO_4 and NO_3 were not significantly different between the three treatment groups, $F_{2, 66}$=1.35 and $F_{2,66}$ = 0.42 respectively. There was a significant difference between the three treatment groups in NO_2 (Fig 2.11), $F_{2, 66}$= 6.46 ($P < 0.005$).

PO_4 concentration in the FGA inclusion treatment showed a decrease within the first three days and was lower than both other treatments at the end of the experiment (Figure 2.11). Day “1” concentration in the FGA inclusion treatment was 7.39 ± 1.96 µmol.L^{-1}, Day “3” 3.12 ± 0.98 µmol.L^{-}1. FGA exclusion treatment showed no change within the first three days but showed a decrease in nutrient concentration from the beginning of the trial to 11.83 ± 2.11 µmol.L^{-1} on Day “3. The control treatment showed no significant change in nutrient concentration during the experiment.

NO_2 concentration in the FGA inclusion treatment was significantly lower than that of the other two treatments for the duration of the experiment. Nutrient concentrations for the FGA exclusion and control treatment both increased along a similar trajectory for the duration of the experiment and were both significantly higher than the FGA inclusion treatment at the end of the experiment.

NO_3 concentrations in both the FGA inclusion and exclusion treatments were below detectable levels for the duration of the experiment. Nutrient concentration in the control treatment decreased slightly during the initial three days of the experiment, 2.42 ± 0.22 $\mu mol.L^{-1}$ on "Day 1" to 1.39 ± 0.51 $\mu mol.L^{-1}$ on "Day 3", and was marginally higher than both the other treatments. This elevated nitrate concentration could be due to bacterial growth within the microcosm.

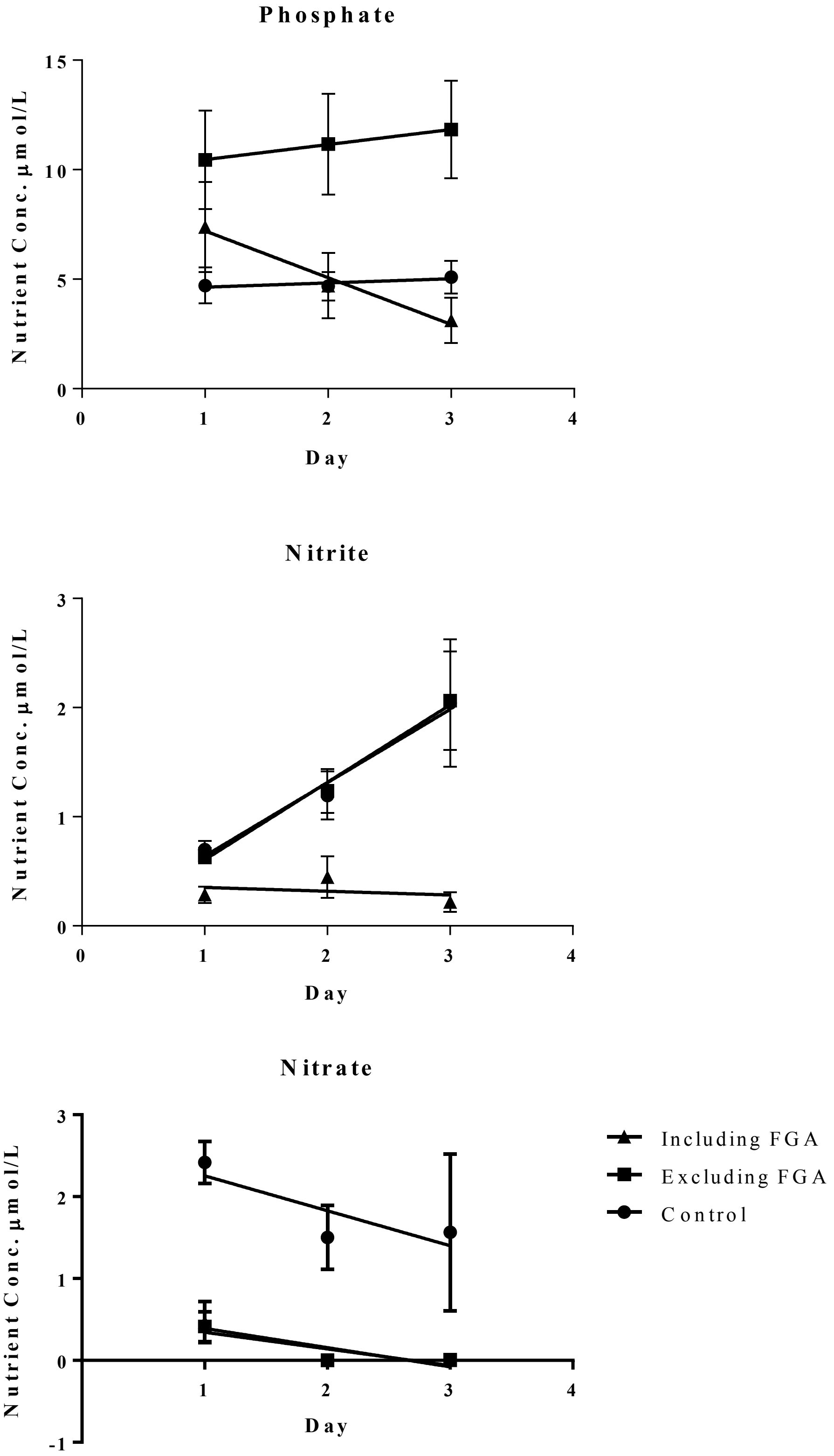

Figure 2.11. Mean nutrient concentration as a function of time: curves fitted to linear regression model and error bars indicate 1 S.E.

***Chlorophyll* a**

Chlorophyll *a* (Chl-*a*) concentration curves for the three treatment groups were significantly different from each other ($F_{6,111} = 17.29$, $P < 0.0001$). For the comparison, the best-fit curves were second-order (quadratic) polynomials (Figure 2.12) (See Appendix 3).

The FGA inclusion treatment was significantly different from the other treatments, showing the highest initial Chl-*a* concentration of 24.28 ± 5.33 $\mu mol.L^{-1}$and decreasing throughout the duration of the experiment to 3.56 ± 1.047 $\mu mol.L^{-1}$ on the final day (Figure 2.12). The FGA exclusion treatment was significantly higher than the other treatments at the end of the experiment with a Chl-*a* concentration of 15.21 ±. 2.50 $\mu mol.L^{-1}$. An increase in Chl-*a* concentration could be witnessed throughout the duration of the experiment within the FGA exclusion treatment group. Chl-*a* concentration in the control treatment was significantly lower than the other treatments and showed consistent low values between 0.042 $\mu mol.L^{-1}$ ±. 0.093 and 0.54 ±. 0.093 $\mu mol.L^{-1}$ (minimum and maximum) throughout the duration of the experiment.

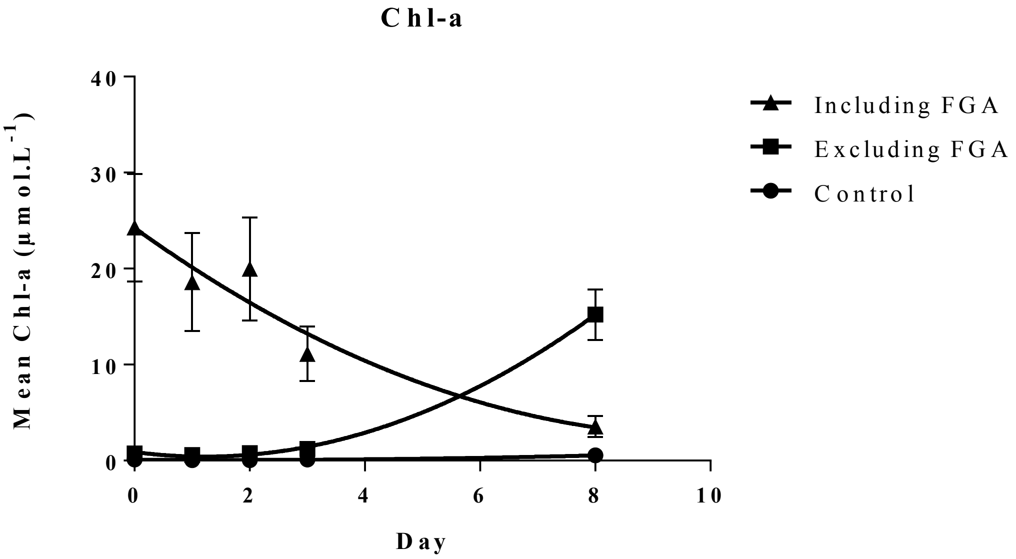

Figure 2.12 Mean Chl-*a* as a function of time: curves fitted to second-order polynomials and error bars indicate 1 S.E.

Phytoplankton analysis

No phytoplankton specimens were seen or identified in the water samples taken from SP1, which contained detritus, and broken segments of FGA. Preliminary phytoplankton analysis in 2012 indicated that SP1 was populated by the following microalgae: *Thalassiosiradecipiens* (diatom), *Nitschia sigma* (diatom), *Protoperidinium* sp. (dinoflagellate), and *Prorocentrummicans*(dinoflagellate).

Discussion

Abiotic Variables

After six months of observation during summer, a period previously known to have caused mortality in *C. gigas* spat during preliminary growth trials (Antonio Tonin and Sue Jackson, unpublished data), it seems likely that the study site might induce the syndrome known as "summer mortality" in oysters grown in the pond itself. This syndrome was characterised by Cheney *et al*. (2000), and was associated with high mortalities in *C. gigas* during summer months due to pathogens, elevated temperatures, low levels of dissolved oxygen, and xenobiotic and physiological stress (Flores-Vergara *et al*., 2004). Although we did not assess possible pathogens and stressors in this study, we would assume that our observations of physical and chemical variables allow us to still diagnose this particular site in terms of viability.

Dissolved oxygen measured at the centre station of SP1 indicated that oxygen levels gradually decreased over the duration of the study period. Dissolved oxygen levels were highest in January 2014 and gradually decreased to anoxic levels in May 2014. Water levels in SP1 decreased as leakage from the pond did not subside, and it was found that dissolved oxygen concentrations were below 2 $mg.L^{-1}$ 23% of the time. Suggested levels of dissolved oxygen for oyster production are between 2 and 5 $mg.L^{-1}$ (Neto *et al*., 2013), and indicate that dissolved oxygen levels within the pond would have to be augmented to sustain production of oyster larvae and additional aeration provided by means of paddle wheels or oxygen diffusers would need to be implemented.

A possible alternative could be to build a hatchery onsite, diverting water from the inflow pipe to the hatchery. Conditions within SP1 itself were less than favourable for the cultivation of *C. gigas* larvae. Unfavourable conditions such as high water temperatures, low dissolved oxygen levels and the FGA proliferation in SP1 were experienced within the pond. The initial idea for the oyster hatchery incorporated cost-effective upwellers that could be placed within the pond to grow the oyster larvae. The upwellers would decrease the overall running costs of the hatchery since the pond itself would control the environmental conditions within the upwellers. An on-site hatchery facility built next to the pond may be the only option, however the infrastructure costs become increasingly higher as this option takes shape. Sea-water from the Inflow pipe would have to be diverted to the hatchery building but could be manipulated by heating and aeration to provide the optimum environment for *C. gigas* larvae culture within the hatchery facility. The facility would have to store and heat sea-water within the hatchery facility because incoming water had an ambient temperature of ±18 °C. With the right infrastructure and planning a hatchery built on-site could be a feasible option.

Nutrient concentrations and phytoplankton culture for oyster larval feeding

As pointed out above, microalgae productivity is directly related to water nutrient content (Turpin *et al.*, 1999; Lušić *et al.*, 2008). SP1 is a shallow salt-water pond that showed signs of nutrient limitation and high nutrient depletion. The Inflow station was significantly higher in PO_4, SiO_4, NH_4, NO_2 and NO_3 than all the other stations, and this pattern is probably related to the proliferation of FGA in the system. In a shallow Danish bay, 95% of available N and 85% available P were consumed by FGA (Thybo-Christesen *et al.*, 1993). It could be suggested that SP1 was nitrogen-limited, as low levels of total nitrogen were recorded within the pond and Redfield ratios calculated for the inflow sea-water TN vs. PO_4 and TN vs. SiO_4, confirm this.

The inflow water could be used to feed a possible oyster hatchery at SP1, thus nutrient concentration of the incoming water would need to have a similar nutrient profile as commercial nutrient mixes used in the production of microalgae (Ponis *et al.*, 2003).

According to the FAO (2007) F/2 nutrient medium used in the production of microalgae in bivalve hatcheries is a common nutrient recipe that can be applied to assess the nutrient profile of SP1. F/2 medium comprises of the following nutrient concentrations: $NaNO_3$ = 88.2 µmol.L^{-1} (TN), $NaH_2PO_4H_2O$ = 36.2 µmol.L^{-1} (TP), $Na_2SiO_39H_2O$ (Si) = 10.6 µmol.L^{-1} (Guillard and Ryther, 1962). Concentrations of TN (38.86 ± 9.14 µmol.L^{-1}) and TP (16.39 ± 4.30 µmol.L^{-1}) measured at the inflow station were below those in F/2 medium, but inflow Si (68.10 ± 20.46 µmol.L^{-1}) was higher than the culture medium. SP1 (all stations excluding inflow) mean nutrient concentrations were considerably lower than both F/2 medium and the inflow, concentrations of 1.27 ± 0.31 µmol.L^{-1} (TN), 1.77 ± 0.45 µmol.L^{-1} (TP) and 8.42 ± 2.63 µmol.L^{-1} were recorded. Thus augmentation of nitrogen and phosphorus, but not silicon would be necessary for the production of microalgae within an on-site hatchery building (Maestrini *et al.*, 1997).

The failure to represent phytoplankton composition of the water in SP1 may have compromised the interpretation of N: Si ratios. Since silicon only matters when diatoms are present, phytoplankton data would have clarified the necessary Redfield ratios for microalgal growth (Redfield, 1958). Phosphorus is more available for phytoplankton utilisation as organic phosphorus (Lušić et al., 2008). Grasshoff et al. (1998) measured inorganic phosphorus, therefore not providing us an indication of the phytoplankton P requirement. There is strong interaction between P and sediments, combined with the method used to determine soluble P within SP1 may have caused challenges in determining the true Redfield ratios required for phytoplankton growth.

Microcosm experiments

These experiments suggested that the FGA in SP1 had an affect on the nutrient dynamics of the system. In semi-closed systems similar to SP1, such as the “Marais du Plomb” on the French Atlantic coast, nutrient utilisation was rapid due to high trophic complexity and the development of microalgae was limited (Dupuy *et al*., 2007). Competition between micro and macroalgae has been related to a combination of (1) nutrient addition, (2) wind stress and (3) temperature (Butzler and Chase, 2009). The proliferation of FGA could have led to the competitive exclusion of microalgae within SP1. Microcosm experiments permit us to assess the rate of nutrient depletion due to FGA proliferation.

Nutrient depletion of NO_2 in the presence of FGA was significantly higher than in the other two treatments, in the microcosm experiments. The FGA present treatment showed that nutrient removal was significantly higher when macroalgae were present. Trochine *et al*., (2010) demonstrated that nutrient concentrations of TN, TP, and NO_3-N decreased after 5 days when FGA (*Cladophora* and *Spirogyra* sp.) was present, and FGA present treatment within the current study showed a similar pattern. Nutrients received into SP1 from the inflow pipe would be utilised by FGA at a much faster rate than by microalgae, signifying the suppression of microalgae production within SP1. Nutrient reduction levels were up to 97% (TN), 89% (TP) and 88 % (Si) between the inflow and the rest of SP1. Microalgae suppression within SP1 decreases the viability of the pond as a nursery for *C. gigas* larvae, and the pond would have to be seeded with commercially available phytoplankton species once all FGA is removed to ensure survival of the larvae (Ponis *et al*., 2003).

When FGA was absent from the microcosms, phytoplankton growth occurred, as chlorophyll *a* levels were higher at the end of the trial. In shallow eutrophic systems that support large macroalgae densities, and in the case of SP1, nutrient concentrations and phytoplankton populations can be low, despite high nutrient loadings rates within the system (McGlathery *et al*., 1998). Shading due to high densities of FGA may have also increased the competition between macro and microalgae as light penetration could be diminished when large floating mats of FGA were present (Sündback *et al*., 2003; Trochine *et al*., 2010).

Therefore in a system without the FGA, phytoplankton growth would proliferate and could possibly provide the right environment for the culture of *C. gigas* larvae within SP1.

Chapter 3: Conclusion and management recommendations

Management decisions made in the preparation of a bivalve hatchery includes aspects such as costs, logistics, and abiotic and biotic parameters of the species being cultivated. It was evident that SP1 was initially thought to be the ideal candidate for a oyster hatchery, however observations made during the six month study period indicated that this particular site may not be as suitable as previously thought.

The main running cost associated with a bivalve hatchery is fresh seawater supply. Bivalve hatcheries require a constant supply of pollutant-free seawater with sufficient nutrient concentrations and ratios (Algarswami *et al.*, 1987). A constant supply of fresh seawater is required for the production of microalgae within the hatchery (Ponis *et al.*, 2003), and water sampled from the inflow of SP1 indicated that the water had insufficient TN and TP, but sufficient Silicon concentrations in relation to F/2 medium (Guillard and Ryther, 1962; Turpin *et al.*, 1999). Nitrogen limitation was witnessed within the incoming seawater; therefore nutrient spiking processes could be used to ensure the correct nutrient balance for microalgae production in a hatchery (Maestrini *et al.*, 1997). It must be noted that fresh sea-water flow during the study period was not continuous due to leakages within SP1. Management considerations from the Saltworks would be required to provide the hatchery with a constant supply of fresh sea-water. A possible alternative would be to create a reservoir header tank to store fresh sea-water. Daily filling of the reservoir could be incorporated into the pumping schedule of the Saltworks, and would ensure a constant supply of fresh sea-water if managed properly. Another major cost associated with the production of a bivalve hatchery is water temperature. Water received from St. Helena Bay through the beach well-head would have to be heated to obtain the optimal growth rates for *C. gigas* larvae. Water temperature from the inflow pipe of SP1 was <20 °C when the pipe was flowing and the optimal temperature for growth of *C. gigas* larvae has been reported to be 27 °C (Rico-Villa *et al*., 2008). Low cost solar heating could be used to heat up the incoming water, and this could be conducted by pumping the water through black pipes, this would lower the overall electricity usage.

Before data collection for this project SP1 was considered an ideal candidate for an oyster nursery, but after the assessment the pond was deemed not suitable for *C. gigas* larvae. Observed issues regarding the pond included: temperature spikes during the summer months, low dissolved oxygen levels, high salinity levels, and proliferation of FGA. Anoxic levels were witnessed at the centre station of SP1 in the middle of February 2014 and dissolved oxygen decrease was linked with high biomass of FGA and high levels of FGA decomposition within SP1.

The proliferation of FGA was the main issue in SP1. The growth of FGA increased throughout the study period, although biomass patterns were not assessed, future studies could assess the rate of biomass increase over time. FGA played a major role in the nutrient limitation of SP1. Microcosm experiments indicated that nutrient depletion was much higher when FGA was present, and macro-nutrient concentrations at the inflow station when compared to the rest of the pond were significantly higher. The competition for resources was evident as SP1 could be classified as a seasonally FGA-dominated system in the summer, when resource utilisation by FGA suppressed microalgal growth within the system. None of the analysed water samples contained identifiable phytoplankton cells.

FGA within the system would have to be removed in order for the system to be utilised. Manual harvesting methods could be implemented (Sündback *et al*., 2003), however this would be costly as a manual labour force would have to be used. Furthermore, FGA proliferation could have increased the rate of anoxia within the system as decomposing FGA within the shallow reaches of the pond may have added to eutrophication of the system.

In conclusion, SP1 is not a suitable site for oyster culture. Observations over the six month period indicated that SP1 was sub-optimal in terms of what is required for a successful bivalve hatchery. Alternatively, fresh sea-water taken from St Helena Bay could be utilised within an on-site oyster hatchery next to SP1. Since the hatchery would be situated on a salt-works, water waste and possible contamination would be avoided since no water returns to the ocean in the salt making process. Nonetheless, high infrastructure costs including a new building, electricity lines and water heat exchangers would be have to be incurred. Logistically an operation like this could be placed closer to the hub of oyster culture in Saldanha Bay, and would allow the oyster farmers to have a constant supply of *C. gigas* spat. This could be a possible solution contingent on the availability of clean seawater in Saldanha Bay.

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