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Growth performance of the marine microalgae *Pavlova salina* and *Dunaliella tertiolecta* using different commercially available fertilizers in natural seawater and inland saline ground water

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Abstract

Pavlova salina and *Dunaliella tertiolecta* were cultivated using batch culture techniques to examine their growth performance with commercial agricultural fertilizers compared to the commonly used but expensive f/2 algae culture media. *P. salina* demonstrated significantly higher growth rates (as measured by determination of average cell densities) when provided with f/2 media, but no growth when cultured using commercial fertilizers, namely Miracle-gro and Maxicrop. In contrast, the average cell density of *D. tertiolecta* was found to be significantly higher ($P < 0.05$) when cultured using commercial fertilizers, as opposed to f/2 media. Significant differences in lipid content were demonstrated in the Maxicrop 2.25 ml fertilizer media compared to the other treatments ($P < 0.05$). Finally, comparisons between natural sea water (NSW) and inland saline ground water (ISGW) have resulted in slightly higher biomass increases being observed for the NSW treatments than the ISGW treatments ($P < 0.05$), but also demonstrated the lucrative potential of ISGW towards aquaculture applications.

Introduction

The role of microalgae in the primary productivity and the ecology of the planet and is well studied (Andersen, 2005), and consequently their essential roles in the aquaculture industry, where they are used directly for the feeding of molluscs, crustaceans and fish is likewise well documented (Guedes *et al.* 2010; Brown, 2002; Lavens and Sorgeloos, 1996; and Lananan *et al.* 2013; and Richmond, 2004). Further, microalgae can provide several different types of biofuels (Chisti, 2007), and they are also widely cultivated commercially for human nutritional products. The wide variety of roles that the microalgae can fulfill therefore explains why they are produced around the world in small- to medium-scale production systems, producing anywhere from tens to several hundreds of tons of biomass annually (Benemann, 2008).

Valenzuela-Espinoza *et al.* (1999) stated that many culture mediums that contain an adequate concentration of nutrients have been developed to attain optimum growth rates of microalgae. These mediums have been investigated and developed as a result of many research projects in previous years by Guillard and Ryther (1962), Walne (1970), Provasoli (1971), and Guillard (1975), with the aim of finding a low-cost alternative combination of nutrients that engenders optimum growth characteristics.

Although there have been a variety of different media utilized for the culture of microalgae (including the exploitation of aerobic digests of cow and hen excretions, liquid extracts of reagent-grade marine salts and the use of agricultural fertilizers) (Valenzuela-Espinoza *et al.* 1999), by definition chemically defined fertilizers such as the commonly used f/2 solution are relatively expensive to formulate, and therefore are a somewhat limiting factor in the development and maintenance of large scale algal production, which is necessary for commercial viability in a variety of contexts. It is therefore clear that less expensive nutrient sources are desperately needed. Cultivating live feed such as microalgae and zooplankton such as rotifers and artemia comes with high establishment and maintenance costs, and can contribute up to 30% of an aquaculture facility's total production budget, and is therefore considered one of the essential obstacles that mollusc and crustacean larval production facilities face (Coutteau and Sorgeloos, 1992). Live feed costs could be minimized by administering a suitable high nutrient commercial

fertilizer, which can be applied to the culture of various species of marine microalgae. Further, this implementation can enhance the growth of microalgae and live feed in aquaculture facilities and reduce the consumption of traditional media such as analytical grade inorganic salts.

This study analyses the performance of two commercial agricultural-grade fertilizers that are readily available and relatively cheap as culture mediums for microalgae, and compares them with the f/2 medium. The study also aims to demonstrate the relationship between culture conditions, nutrient uptake, lipid content and biomass production of the marine microalgae *P. salina* and *D. tertiolecta* using different commercial fertilizers. Further, the study will also investigate the growth performance of the best performed fertilizer from all microalgae treatments and compare it to the growth performances witnessed with Natural Seawater (NSW) and inland saline ground water (ISGW) extracted from a bore.

Materials and Methods

All cultures were grown under identical conditions at the Epping Campus of Northern Melbourne Institute of TAFE's (NMIT) Live Feeds Production Laboratory from August to October 2013. Axenic cultures of *Pavlova salina* and *Dunaliella tertiolecta* were sourced from the Australian National Algae Culture Collection (CSIRO), and stock cultures were kept in full strength (33.6ppt salinity), non-axenic natural seawater.

Small-scale batch cultures were maintained throughout the experiment. The investigation included two different readily available commercial fertilizers ("Miracle–gro" and "Maxicrop") that were sourced from a hardware store, while f/2 algal boost was supplied by AusAqua in South Australia. The nutritional value of each of the mentioned fertilizers and the algae boost are described in detail in Table 1. The best performing algae treatment was further investigated by culturing algae in two different natural water sources; Natural Seawater (NSW) collected from the Hobsons Bay precinct within Port Phillip Bay at Port Melbourne, and inland saline ground water (ISGW), which was sourced from a groundwater bore at Donald, 283 kilometers north west of Victoria.

Table 1: Composition of each of the products used to enrich the culture.

Component	Maxicrop (mg/ml)	Miracle-gro (mg/ml)	Algae Boost f/2 (mg/ml)
Total Nitrogen (N)	18.4	242	75
Phosphorous (P)	4.8	56	5
Potassium (K)	12.4	117	0
Sulfur (S)	0.24	36	0
Magnesium (Mg)	21.3	8	0
Iron (Fe)	0.2	1.7	0
Copper (Cu)	0.2	0.7	0.0098
Zinc (Zn)	0.12	0.6	0.022
Manganese (Mn)	0.12	0.5	0.018
Cobalt (Co)	0	0	0.01
Boron (B)	0.04	0.2	0
Molybdenum (Mo)	0.0008	0.005	0.0063
Sodium meta-silicate	-	-	0
Biuret (Max)	0.16	0	0
Total Dissolved Solids	210	0	-
Added Water	790	0	0

Five different treatments were used as part of this experiment, including one concentration ($2.5g^{-l}$) of Miracle-gro, two different concentrations of Maxicrop (consisting of 5.5 $ml.l^{-1}$ as described by the manufacturer and a lower concentration of 2.25 $ml.l^{-1}$ to maximize transparency and examine the growth performance at a non-recommended concentration), one concentration of f/2 (at

1mL per flask) and a control, in which no additives were provided. All treatments (with the exception of the lower concentration of Maxicrop that was utilized) were according to manufacturer's instructions. Triplicate cultures of each species for each treatment were grown in 2L Erlenmeyer flasks using autoclaved natural sea water.

Cultures were kept indoors continuously under artificial day light supplied by cool-white fluorescent tubes at an intensity of 4000 Lux, which is the recommended light intensity for laboratory-based microalgal cultivation (FAO, 1996; Andersen, 2005; and Lananan *et al.* 2013). Continuous aeration was provided by an outdoor compressor (Esam, Australia) to deliver optimum levels of natural air for faster algal growth (Coutinho et al. 2006) and to provide mixing and the elimination of deadspots. All flasks were maintained at a pH level of 8 – 8.5 using sodium bicarbonate (as recommended by Coutinho et al. 2006) and checked by using a calibrated Hanna Instruments pH 211 Microprocessor/pH meter. Since most types of algae grow well at temperatures in the range of 17-22°C (Laing, 1991), an electrical air heater and thermometer were used to maintain and monitor temperature within the constant temperature room that the algae were stored in.

Aeration of receiving water supplies took place for a period of at least two hours before inoculation, after which 40 ml of stock culture at a density of approximately 6×10^6 cells ml^{-1} was added and allowed to mix.

Evaporation was corrected every 48 hours by the addition of diluent water, and salinity levels were maintained at 25ppt using a refractometer (FAO, 1996; Andersen, 2005; and Chipchase & Awal, 2012;) and additions of measured amounts of sodium chloride (Cheetham Salts, Geelong, Victoria).

Cultures were assessed every 48 hours by obtaining two counts of each flask, counting the number of cells per ml for each flask using a Neubauer hematocytometer slide under a compound microscope to obtain the most accurate average culture biomass, in accordance with suggestions from several authors (Fulks & Main 1991; FAO, 1996; Andersen, 2005; and Lananan *et al.* 2013).All data were recorded on Microsoft Excel (Seattle, Washington) spreadsheets to be analyzed later using analysis of variance (ANOVA).

The most highly dense replicas of each treatment of algae were selected to examine the lipid content. Initially, the microalgae from each treatment were harvested by centrifugation (FAO, 1996; Prommuak *et al.* 2012), and cells deposited on the walls of the centrifuge tubes were scraped as paste and placed into a separate tube for weighing. Harvested algae paste was then placed in an oven (Thermoline Scientific, Wetherill Park, New South Wales, Australia) at 60°C for 24 hours to dry. Each dry sample was then weighed and mixed at a rate of 5 ml of hexane to each 1g of dry cells. Homogenizers and ultrasonicators were also used to support the hexane in breaking down the cells before tubes were placed in a refrigerator. Tubes were still in the refrigerator to allow the hexane to breakdown the cells and separate the lipid from the dry algae. Lipid content was then calculated using the following formula (Lee *at al*., 1998):

Lipid content (%) = (Extracted lipid weight / Dried algal weight) × 100

The best performed algae from all treatments were isolated and a strain was taken and examined for growth performance in ISGW and natural seawater in order to test which medium was more applicable for the best algal growth.

As *P. salina* and *D. tertiolecta* were kept in culture units to grow, algae cells were counted randomly for each of the treatment replicas. Counts were obtained every 48 hours and means were calculated and recorded to be statistically analyzed with one or two way ANOVA and graphed using Microsoft Excel 2010 (Seattle, Washington).

Results

Natural seawater (NSW) and ISGW (inland saline ground water) ionic composition

The ionic composition and concentrations of the major chemical compounds for both waters used throughout this study are shown in Table 2. There were some significant and potentially very important differences in the samples analysed as part of this investigation.

Table 2: The ionic composition of NSW sourced from Port Phillip Bay and IGSW sourced from Donald in Victoria.

Parameter	NSW concentration (mg/L)	ISGW concentration (mg/L)
Sodium (Na)	10561	4520
Calcium (Ca)	400	326
Potassium (K)	380	28
Magnesium (Mg)	1272	607
Total Nitrogen (N)	0.38	0.6
Ammonium (NH_4)	0.05	0.09
Nitrate-Nitrite	0.26	0.41
Total Phosphorus	0.14	0.08
Cadmium (Cd)	2.5×10^{-4}	1.9×10^{-4}
Copper (Cu)	1.7×10^{-3}	9.0×10^{-4}
Mercury (Hg)	1.3×10^{-4}	8.0×10^{-5}
Nickel (Ni)	2.28×10^{-3}	1.9×10^{-3}
Zinc (Zn)	9.13×10^{-3}	8.0×10^{-3}

The mean growth performance of *P. salina* with f/2 media was significant ($P = 0.0001$), being considerably higher than Maxicrop and Miracle-gro commercial fertilizers, however the fertilizers demonstrated the same growth as the control, which was not significantly different ($P = 0.6756$), as shown in Figure 1.

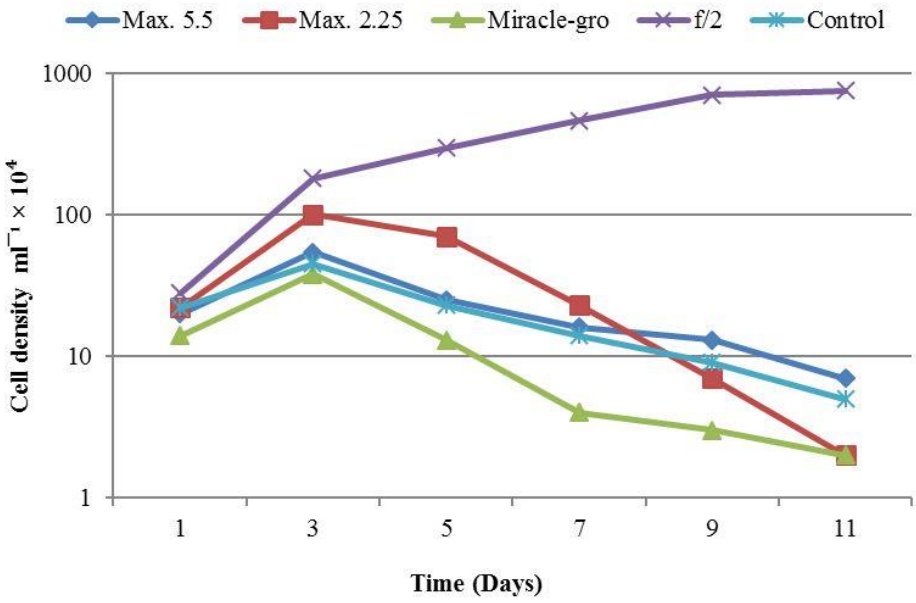

Figure 1: Average growth performance of *Pavlova salina* with different fertilizers in comparison to f/2 and control (ANOVA at 95% confidence $P< 0.05$ only with f/2 media).

In the second experiment, the average cell density growth of *D. tertiolecta* with Maxicrop 2.25 ml concentration was significantly higher ($P = 0.0103$) from day 9 onwards in comparison to other treatments, as demonstrated in Figure 3. In addition, growth was significant higher ($P = 0.0002$) for f/2 media, but perhaps not as much as expected, while Maxicrop performed considerably better after day 9, as shown in Figure 2.

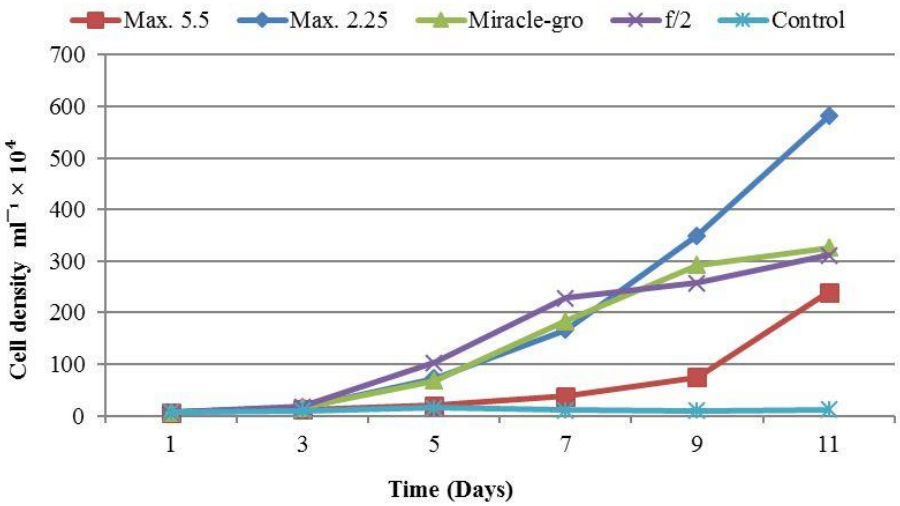

Figure 2: Average growth performance of *Dunaliella tertiolecta* with different fertilizers in comparison to f/2 media and control (ANOVA at 95% confidence *P*< 0.05 for both f/2 and fertilizers).

The distinct elevation of cell densities of *D. tertiolecta* was significant across all nutrient treatments. This proved the potential commercial viability of these fertilizers for algae production on medium to large scales, and provided strong incentive for lipid extraction. The highest level of lipid content was found to be significant (P = 0.0395) in the Maxicrop 2.25 ml concentration, while lowest levels were observed for f/2 media, as illustrated in Figure 3.

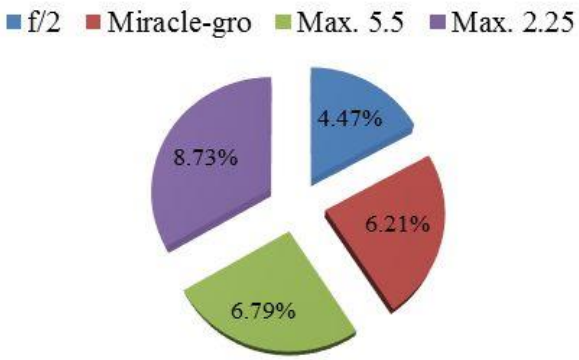

Figure 3: Lipid content of *D. tertiolecta*, expressed as a percentage of cell dry weight (ANOVA at 95% confidence *P*< 0.05).

Enormous biomass growth of *D. tertiolecta* was revealed in the second experiment, and initiated further investigations to discover the viability of ISGW in comparison with NSW, considering each water sources' ionic composition. However, *D. tertiolecta* had resulted in significant (P = 0.0114) growth in ISGW, but NSW had an average of 20% higher production of cell biomass (as presented in Figure 4).

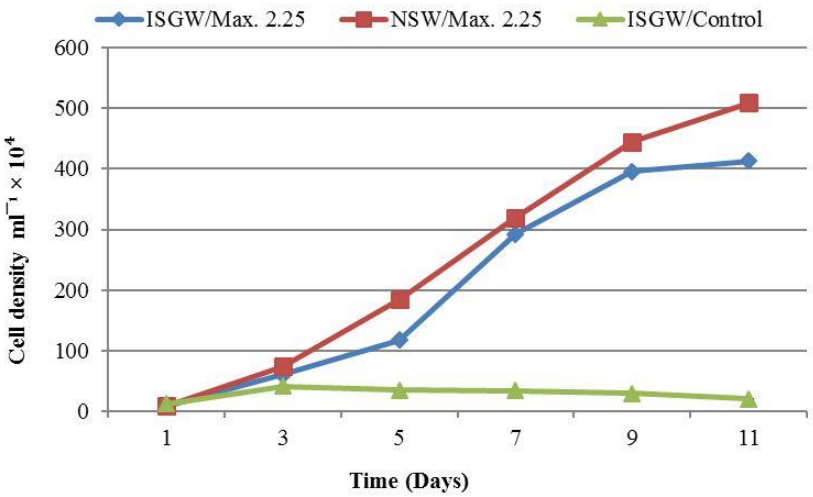

Figure 4: Average cell biomass of *D. tertiolecta* comparison between NSW, ISGW and control using fertilizer Maxicrop 2.25 ml concentration (ANOVA at 95% confidence *P*< 0.05).

Two-way ANOVAs comparing *P. salina* and *D. tertiolecta* growth with the same enhanced nutrient levels over 11 days demonstrated highly significant differences (P = 0.00216) of the various nutrients' impact on the growth of both marine microalgae species demonstrated in Figure 5.

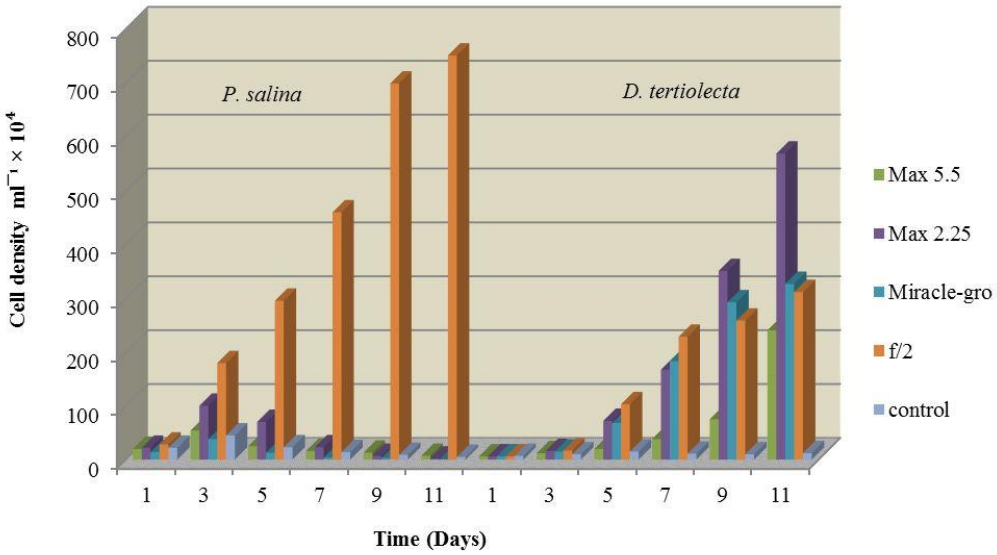

Figure 5: Relationship between *P. salina* and *D. tertiolecta* growth performance with different nutrients (ANOVA at 95% confidence *P*< 0.05).

Discussion

The results of this investigation demonstrated significant differences between the growths of *P. salina* treatment replicas with f/2 media and the commercial agriculture fertilizer media. This indicates that the ionic composition of fertilizers, as could be expected, differ significantly from the f/2 media, as the marine microalgae *P. salina* required specific concentrations of

macronutrients and trace metals. However, most of the media tested during the course of this investigation have not been formulated with microalgal culture in mind, and therefore no attempt has been made to balance the relative concentrations of macronutrients needed specifically for algal growth (Andersen, 2005). The growth performance of *P. salina* with f/2 media was significant ($P < 0.05$), which has proven that a nitrogen:phosphorus (N:P) ratio of 16:1, with low concentrations (as described previously in Table 1) is required for optimum growth. This indicates that agricultural fertilizers with N:P ratios at an average of 4:1, with lower or higher concentrations of macronutrientsm, may have limited the growth of *P. salina*, and yield results that were not significantly different ($P > 0.05$) to that of the control treatment (as shown previously in Figure 1).

Lopez-Ruiz *et al.* (1999), Valenzuela-Espinoza *et al.* (1999), and Andersen (2005) have stated that lower nutrient levels have been found to enhance the growth of some marine microalgae species, but often quantities of major nutrients are reduced in medium formulations. In contrast, Berges et al. (2001) noted that higher levels of nitrogen and ammonium can be toxic to some marine microalgae, and speculated that this could be another reason that *P. salina* didn't grow well in Miracle-gro. On the other hand, Maxicrop nitrogen and ammonium levels were much lower than f/2, which also could have limited the growth of *P. salina*. This may be overcome by dilution factor of the concentrated fertilizer media. If Miracle-gro nitrogen concentrations were diluted (as the level of nitrogen present is much higher than recommended) this could be a possible solution. In contrast, Maxicrop, which was diluted by 50%, caused reductions in the level of nitrogen far below the physiological requirements of *P. salina*. This revealed that levels of macronutrients, trace metals and salts found in the f/2 media were well within the optimum range for this particular algal species, unlike the situation with commercial fertilizers.

Maxicrop 2.25 ml concentration showed the highest population cell count and growth rate in comparison to other cultures enriched with Miracle-gro and f/2; and the higher concentration of Maxicrop at 5.5 ml (as recommended by the manufacturer) did not perform as expected. However, this could be because the nutrient requirement for *D. tertiolecta* is found in the diluted Maxicrop 2.25 ml media solution. In contrast to *P. salina*, this has proved that *D. tertiolecta* has no specific requirements, and it can tolerate a wide range of N:P ratios, but maximum cell biomass was obtained with Maxicrop 2.25 ml concentration with an N:P ratio at 4:1 ($P < 0.05$). On the other hand, growth performance was also significant ($P < 0.05$) for Maxicrop 5.5 ml, Miracle-gro and f/2 mediums (clearly illustrated in Figure 2). The reason for this could be the absence of a rigid polysaccharide cell wall in *Dunaliella*, allowing it to tolerate extracellular osmotic pressure and a wide range of salinities (Wang and Lan, 2010). Further investigations were therefore conducted by harvesting algal biomass on the 11^{th} day of culture and extracting lipid contents from the biomass treatment of each medium. This resulted in various lipid percentages being elucidated, with the largest lipid content of 8.73% of dry weight (Figure 3) being obtained from 6 L Maxicrop 2.25 ml media. Previous studies from Guschina and Harwood (2006), Solovchenko *et al.* (2008), and Pal *et al.* (2011) have shown that lipid content of marine microalgae is highly influenced by external and internal environmental parameters such as light intensity, nutrient availability (mainly nitrogen), salinity, temperature, pH, and culture age. This indicates that all parameters were at the perfect levels when considering the harvested biomass; nutrient availability could be considered an exception, though, since there was such variability between treatments resulting in different lipid percentages.

The significant ($P < 0.05$) lipid content extracted from *D. tertiolecta* cultivated in Maxicrop 2.25 ml media is potentially beneficial to aquaculture hatcheries, biodiesel manufacturers and the pharmaceutical industry, as it can cut down the cost of live feed production in aquaculture hatchery operations and the other aforementioned industries.

Wang and Lan (2010) stated that lipid yields in microalgae cells did not significantly increase with higher phosphorus concentrations in the growth media. This statement may justify why the concentration of phosphorus within the Maxicrop 2.25 ml treatment was lowest compared to all other treatments. Wang and Lan (2010) shown that optimal lipid extraction was obtained with the following optimal macronutrient concentrations (mg/ml) in the fertilizer; sodium nitrate (0.35), potassium phosphate (0.46), magnesium sulfate (0.0823) and iron chloride (0.0068). These figures show very low levels compared to the concentrations found in this study, however Maxicrop has been shown to have the lowest levels of macronutrients but the highest levels of salts. This indicated that lower levels of macronutrients didn't influence lipid production but higher levels of salts can increase lipid yields.

The significant results for the growth of *D. tertiolecta* in experiment 2 and the potential of inland saline ground water have initiated interest for further examinations. Inland saline ground water (ISGW) and natural seawater (NSW) were comparable in terms of growth performance using the best performed media (Maxicrop fertilizer at 2.25 ml concentration). However, the growth pattern of *D. tertiolecta* has shown similar or close growth profiles in both waters. There was significant ($P < 0.05$) growth in both water treatments, with Maxicrop 2.25 ml nutrient comparing favourably to control treatments without nutrient.

It was expected that NSW will demonstrate better growth than ISGW because of the considerably higher levels of N, P and K typically encountered in this water source. Chipchase and Awal (2012) reported that artificial seawater with higher levels of macronutrients and lower levels of sulphate than ISGW enhanced the growth performance of marine microalgae. However, the

growth of *D. tertiolecta* in NSW was 19% higher than ISGW by day 11, as shown in Figure 5, which revealed that the ionic composition (such as salts and macronutrients) was the key difference.
Smith and Barlow (1999) stated that ISGW has a potential advantage over NSW as the quality of seawater often changes significantly due to pollution and global warming, which would be a major influence to the sterility of the microalgae culture media. These comparisons determined that *D. tertiolecta* can reach maximum cellular densities in ISGW when compared to NSW, considering the cultivation period would be longer.
ISGW has a wide range of potential advantages for the aquaculture of true marine microalgae. The fact these sources of ISGW are generally found in low rainfall regions, with inherent long sunny periods and warm environments, mean that they easily fulfill most of the requirements for algal growth. In contrast, the high cost of near shore sites, limited availability and maintenance may not be as viable for the microalgae culture facility of the future, which makes ISGW a viable and adequate source for the aquaculture industry.
Valenzuela-Espinoza *et al.* (1999) reported that the production of *Isochrysis aff. galbana* biomass and nitrate, phosphate and ammonium uptake were determined by using batch culture techniques for 7 days, comparing commercially used fertilizer and f/2 media. It was found that the average cellular density of the algae when grown in the agricultural fertilizer showed no significant difference from that produced by the f/2 media. Another study by Lananan *et al.* (2013) investigated the effect of Conway and f/2 media on the growth of various microalgae genera (including *Chlorella* sp., *Dunaliella* sp., *Isochrysis* sp., *Chaetoceros* sp., *Pavlova* sp. and *Tetraselmis* sp.). It was found that the reproduction of *Tetraselmis* sp., *Dunaliella* sp. and *Pavlova* sp. could be sustained longer in the f/2 medium. Higher cell densities were achieved by the microalgal genera *Dunaliella*, *Chlorella* and *Isochrysis* in Conway medium. Different genera of microalgae had a preference for different types of cultivation media.

The current study encountered a negative significant difference ($P < 0.05$) in the relationship between f/2 and the different agricultural fertilizers used in this study for the growth of *P. salina*, thus resulting in the rejection of the null hypothesis, and a clear difference when compared to previous studies that have been conducted on various marine microalgae species. However, this has highlighted the highly significant ($P < 0.05$) difference between *P. salina* and *D. tertiolecta*. The green pigments of *D. tertiolecta* have shown their high tolerance to varying levels of nutrients as well as variable levels of lipid production. In contrast, the golden brown *P. salina* revealed much lower levels of tolerance to various concentrations of macronutrients. This could have been due to the lightly silicified cell wall found in *P. salina* (Wang and Lan, 2010), which differentiates it from *D. tertiolecta* cell structure and biology, as *D. tertiolecta* features crystalline glycoprotein walls and fibrillar cell walls, as described by Domozych *et al.* (2012). Other factors that could also have been involved are the chemical composition of the different fertilizers and the levels of tolerance of both algae.

Miracle-gro had higher concentration levels of N, P, K, Fe and S, and lower concentration levels of Cu, Zn, Mn, Co and Mo when compared with the commonly used f/2 media. However, regardless of the differences in macronutrient levels, similar amounts of *D. tertiolecta* biomass were produced in the same period of time. This has proven similar to previous studies undertaken on different marine microalgae species (Venkatesan, et al., 2013; Arun and Singh, 2013).

.On the other hand, Maxicrop had higher levels of K, Fe and S; approximately equal concentrations of phosphorous and lower concentrations of Cu, Zn, Mn, Co and Mo than the f/2 media; however, *D. tertiolecta* was successfully grown at two different concentration levels (5.5 ml and 2.25 ml) and had higher cell densities than the f/2 culture media.

Wang and Lan (2010) stated that Mg, S and Fe have significant effects on the metabolism and physiology of microalgae. Therefore, in the absence of these macronutrients *P. salina* can grow exceptionally well along with optimum levels of other macro and micro nutrients. This has been proven to be completely different in the case of *D. tertiolecta*, which can tolerate all of the mentioned concentrations except for Maxicrop 2.25 ml concentration, which has been exceptional, as it contained the optimum nutritional levels necessary for the production of the highest lipid content when compared to the other treatments.

Marine microalgae culture technologies (which are considered an essential part of the aquaculture and biodiesel industries) require further investigation and development to ensure that they become more cost-effective and efficient over time. In line with such developments are the requirements to grow commercially significant algal yields in the smallest amount of time possible. Similarly, research and development needs to focus in particular on producing the highest lipid content possible in algal cells, which then can be used for a multitude of purposes and can also help to reduce capital costs.

Conclusion

Maxicrop at different concentrations and Miracle-gro were found to have no effect on the growth of *P. salina*, which has led to acceptance of the null hypothesis, but the significant growth that resulted when cultivated with f/2 media led to rejection of the null hypothesis. In contrast, Maxicrop 2.25 ml concentration was found to have the highest significant effect on the growth biomass of *D. tertiolecta* and lipid production when compared with f/2 and Miracle-gro. However, treatments of Maxicrop at 5.5 ml concentration along with Miracle-gro had no significant negative effect when compared to f/2, and produced similar or even higher cell biomass in some replicates. It is the author's strong suggestion that *D. tertiolecta* can readily be cultivated with the use of Maxicrop as a cost-effective enhancement in the very least, since fertilizers are an economical and much cheaper method for commercial production of algae. Further analysis and manipulations could lead to further increases in cell biomass as various techniques and formulations are experimented with.

This study has also investigated the significance of inland saline ground water for the biomass production of the marine microalgae *D. tertiolecta* compared to natural seawater. This has revealed significant differences in the ionic composition of both water sources, but NSW has provided higher levels of biomass production (in the order of 19%). Since ISGW has potentially less contaminants introduced and probably experiences less quality fluctuations (certainly when compared to NSW), it could have outstanding potential for the aquaculture industries in countries with enormous areas of arid land and highly saline underground water bores, as are readily encountered in countries such as Australia.

Finally, the difference between *P. salina* and *D. tertiolecta* growth performance was determined to be significantly different, giving positive indications for the mass production of *D. tertiolecta* as opposed to *P. salina* when using different agricultural commercial fertilizers. This should lead to an increased research and development focus on the mass production of *D. tertiolecta*, which is a barely studied microalgae species, using ISGW and agricultural commercially available fertilizers, which could help to ensure the viability of sustainable aquaculture well into the future.

Due to limited research on the use of commercially agricultural fertilizers in the marine microalgae aquaculture industry, further research would be beneficial to support these conclusions. Researchers, government bodies and marine biologists should consider the development and implementation of these outcomes to develop economical, sustainable sources of nutrients for the production of live feed in aquaculture hatcheries, biodiesel and pharmaceutical medicine production

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