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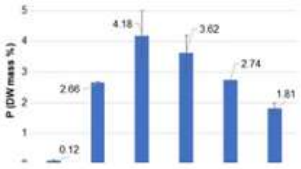


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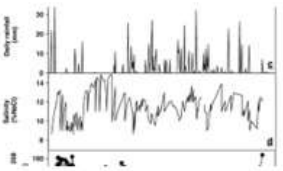

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## Abstract

The microalgal industry as a source of high-value products (i.e. β-carotene, astaxanthin) was established over 50 years ago. However, only a very small number of species have been commercialised. There is a need for new species and new products to expand this industry. The objective of this study was to examine the reliability and productivities of long-term outdoor culture of a newly isolated halophilic diatom, *Amphora* sp. MUR258 (Bacillariophyceae), in raceway ponds in Perth, Western Australia. The *Amphora* sp. was grown in outdoor raceway ponds as a semi-continuous culture for about 13 months at a culture salinity between 8.6 and 14.9% (*w*/*v*) NaCl. The highest cell density ($167 \times 10^4$ cells $mL^{-1}$), specific growth rate (0.29 $day^{-1}$) and biomass and lipid productivities (24 and 6.8 g $m^{-2}$ $day^{-1}$, respectively) were achieved in summer. The annual average of biomass (ash-free dry weight) and lipid productivities was 7 and 2.2 g AFDW $m^{-2}$ $day^{-1}$, respectively. Minor contamination by a *Navicula* sp. was seen during winter, but was not a significant problem. No major protozoan contamination was seen. These results indicate the potential of reliable large-scale cultivation of *Amphora* sp. MUR258 as a potential source of diatom lipid and/or fucoxanthin.

# Long-term reliable culture of a halophilic diatom, *Amphora* sp. MUR 258 in outdoor raceway ponds

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## Abstract

The microalgal industry as a source of high value products (i.e. β-carotene, astaxanthin) was established over fifty years ago. However, there is a need for new species and new products to expand this industry. The objective of this study was to examine the reliability and productivities of long term outdoor culture of a newly isolated halophilic diatom, *Amphora* sp. MUR 258 (Bacillariophyceae), in raceway ponds in Perth, Western Australia. The *Amphora* sp.was grown in outdoor raceway ponds as a semi-continuous culture for about 13 months at a culture salinity between 8.6 and 14.9% (w/v) NaCl. The highest cell density ($167x10^4$ cells.$mL^{-1}$), specific growth rate (0.294 $d^{-1}$) and biomass and lipid productivities (24 and 6.8 g.$m^{-2}$.$d^{-1}$, respectively) were achieved in summer. The annual average of biomass and lipid productivities were7 and 2.2 g ash-free dry weight.$m^{-2}$.$d^{-1}$, respectively. Minor contamination by a *Navicula* sp. was seen during winter, but was not a significant problem. No major protozoan contamination was seen. These results indicate the potential of reliable large-scale *Amphora* sp. cultivation as a suitable source of diatom lipid.

**Key words**: Bacillariophyceae, halophilic diatom, long-term culture, raceway pond, productivity

## Introduction

Microalgae have become of particular interest over the last few decades due to their capabilility to synthesize a range of valuable compounds,making them a potentially important source of chemical products than can be used in the feed, food,nutrition, cosmetics,pharmaceuticals and biofuels industries (Gong et al. 2011). Moreover, microalgae are very diverse (estimated several million species) compared with about 250,000 species of higher plants, around 77,000 fungal species and 2,500 bacterial species,and they are an untapped resource of natural compounds waiting to be discovered (Radmer and Parker 1994).

Currently, only a few microalgae species such as *Chlorella, Spirulina, Dunaliella* and *Haemotococcus*are being successfully grown commercially at large-scale (Olaizola 2003; Milledge 2011).Succesful commercial large-scale microalgae production depends on many factors one of which is the development of cost effective large-scale culture systems for the algae (Borowitzka 1999). There are two main types of cultivation systems currently available, open ponds and closed photobioreactors(Borowitzka and Moheimani 2013a; Zittelli et al. 2013). To date, open ponds are more economical to build and operate compared to closed photobioreactors. This makes open cultivation systems such as paddle wheel-driven raceway ponds as the most widely used cultivation system for large-scale microalgae production (Borowitzka and Moheimani 2013a).

One of the main challenges for large-scale culture is contamination (e.g. by other algae, grazers) making it difficult to operate a reliable mono-species culture for a long term. Most of current commercially produced microalgae (i.e. *Spirulina, Chlorella* and *Dunaliella*) have a common characteristic which is able to grow in highly selective environments allowing them to grow in open air systems and still remain relatively free of contaminantion by other algae or protozoa (Borowitzka 2013). For instance, *Chlorella* prefers nutrient-rich media for optimum growth, *Dunaliella salina* grows at very high saltconcentrations (up to 35% NCl), *Spirulina* requires a high alkalinity (Borowitzka 2005). Therefore, selecting the right algal species for large-scale outdoor culture is a critical factor in the development of successful mass microalgal cultures for any particular purpose. The ability to tolerate a wide range of salinity is one of the desirable characteristics for sustainable growth of microalgae in outdoor open ponds(Borowitzka and Moheimani 2013a). Furthermore, microalgae species able to grow over a wide salinity range are preferred as this reduces the need for freshwater to replace losses due to evaporation and permits recycling of the medium for sustainable use of nutrients (Fon Sing et al. 2014).. Since not many organisms can tolerate hypersaline condition, this increase in salinity also will reduce the contamination by other organisms (Matsunaga et al. 2009).

Commercial algal biomass production also requires that the alga can be cultured for long periods, preferably the whole year, so as to reduce production costs. *Amphora* sp. MUR 258 is a newly isolated halophilic diatom (Bacillariophyceae) that was observed as a major contaminant in a *Dunaliella salina* culture grown in a $10m^2$ raceway pond at the Algae R&D Centre, Murdoch University, Perth, Western Australia, in April 2011. Preliminary laboratory studies indicated that this alga could grow well over a wide range of salinities (6-12% w/v

NaCl) and temperatures (19-36$^{o}$C) and had a high lipid content of up to 67% of ash-free dry weight (un-published data). Therefore, in order to assess the potential of this alga for commercial culture this study investigated the reliabilty and biomass and lipid productivities of *Amphora* sp.MUR 258 grown in a 1 $m^2$ outdoor raceway pond over a whole year.

**Materials and Methods**

***Algal strain and inoculum preparation*:** *Amphora* sp.MUR 258 was originally isolated from an outdoor raceway culture of *Dunaliella salina* grown in 2F medium [Guillard (1975) F medium with 2x concentrations of N and P] at 20±2% NaCl in a 10 $m^2$ paddle wheel driven raceway pond at the Algae R & D Center at Murdoch University, Perth, Western Australia (31.9554° S, 115.8585° E) in April 2011. The alga was isolated using the agar plating technique (Andersen and Kawachi 2005) in F+Simedium (Guillard 1975) at three different salinities (10, 12 and 15%NaCl). Pure unialgal colonies were obtained after repeated streaking on fresh agar media at 10% salinity.

The inoculum for the raceway pond was was grown indoors in3 x 20 L carboys(Moheimani et al. 2011) usingF+Si medium at 10% salinity, at 26±1$^{o}$Cwith a 12 h : 12 h dark and light cycle,at a light intensity of 200-250 µmol photons.$m^{-2}$.$s^{-1}$. These cultures were then used as inoculum for the outdoor pondgiving an initial cell concentration of about $20x10^4$ cells.$mL^{-1}$.

***Outdoor cultivation**:* The outdoor cultivation was conducted at the Algae R & D Centre at Murdoch University using a 1 $m^2$ fibreglass paddle wheel-driven raceway pond at 15 – 20 cm depth.The medium was untreated natural seawater collected from Hillary's Beach (Perth, Western Australia)and adjusted to 10% salinity by the addition of NaCl was enriched with nitrate ($17.64x10^{-4}$ M), phosphate ($7.24x10^{-5}$ M), silicate ($2.12x10^{-4}$ M) and iron concentration ($1.17x10^{-5}$ M) as for the F+Si medium. The culture was initially operated in batch mode. Once the culture reached stationary phase, a semi-continuous regime was initiated by periodically harvesting a certain amount of the culture (20-50% depending on the specific growth rate) and replacing the harvested volume with the same amount of fresh medium. The culture was run for about 13 months.

***Growth measurements and analytical methods**:* the growth of the cultures was monitored by counting the numbers of microalgae cells every second day using Neubauer haemocytometer. Specific growth rate was determined at the log phase of growth using the following equation :

$$\mu = \frac{\mathrm{Ln}\left(\frac{N2}{N1}\right)}{t2 - t1}$$

Where *N1* and *N2* are the cell density at times 1 (*t1*) and 2 (*t2*), respectively.

Dry weight (DW), ash-free dry weight (AFDW) and lipid content were determined using the methods described by Moheimani et al. (2013). Productivity was calculated using the equation $Pr = \mu \times Y$ (Moheimani et al. (2013), where *Pr* is the productivity in $g.L^{-1}.day^{-1}$ for volumetric productivity or $g.m^{-2}.day^{-1}$ for areal productivity, μ is the specific growth rate and *Y* is the yield of the culture in $g.L^{-1}$ or $g.m^{-2}$ for volumetric and areal yield, respectively.

Air temperature and irradiance data were obtained from the Bureau of Metereology (http://www.bom.gov.au/). Salinity was measured using a digital hand salinometer (Atago).

***Statistical analysis*:** Significant differences on the growth, DW, AFDW, lipid content, biomass and lipid productivities between seasons were analysed with repeated measures one-way analysis of variance (Rm ANOVA). Wilks' Lambda test was used to compare means. All statistical analysis was performed using SPSS22 (IBM, USA).

**Results**

***Growth and productivities.*** Semi-continuous *Amphora* sp. MUR 258 culture was possible in the 1 $m^2$outdoor raceway pond over one year period between 5/Dec/2011 and8/Jan/2013 (**Error! Reference source not found.e**). The Daily solar irradiance ranged between 2.5 MJ $m^{-2}.d^{-1}$ (on a cloudy day in early winter) to 34 MJ $m^{-2}.d^{-1}$ (on a clear sunny day in summer) (**Error! Reference source not found.a**). The daily air temperature ranged from -0.7 $^oC$ in winter (July 2012) to 42.2$^oC$ in summer (December 2012) (**Error! Reference source not found.b**). The highest (34.4 mm) and lowest (0.0 mm) monthly total rainfalls were observed in June and March 2012, respectively (**Error! Reference source not found.c**). Salinity range of the culture was between 8.6-14.9% NaCl (**Error! Reference source not found.d**). The salinity was adjusted to about 10% after each harvest. However, the salinity dropped to below 9% due to dilution on rainy days and increased to over 14% on hot sunny days during summer due to evaporation. It is to be noted that the use of seawater for replacing the evaporation losses resulting in a gradual increase in the salinity.

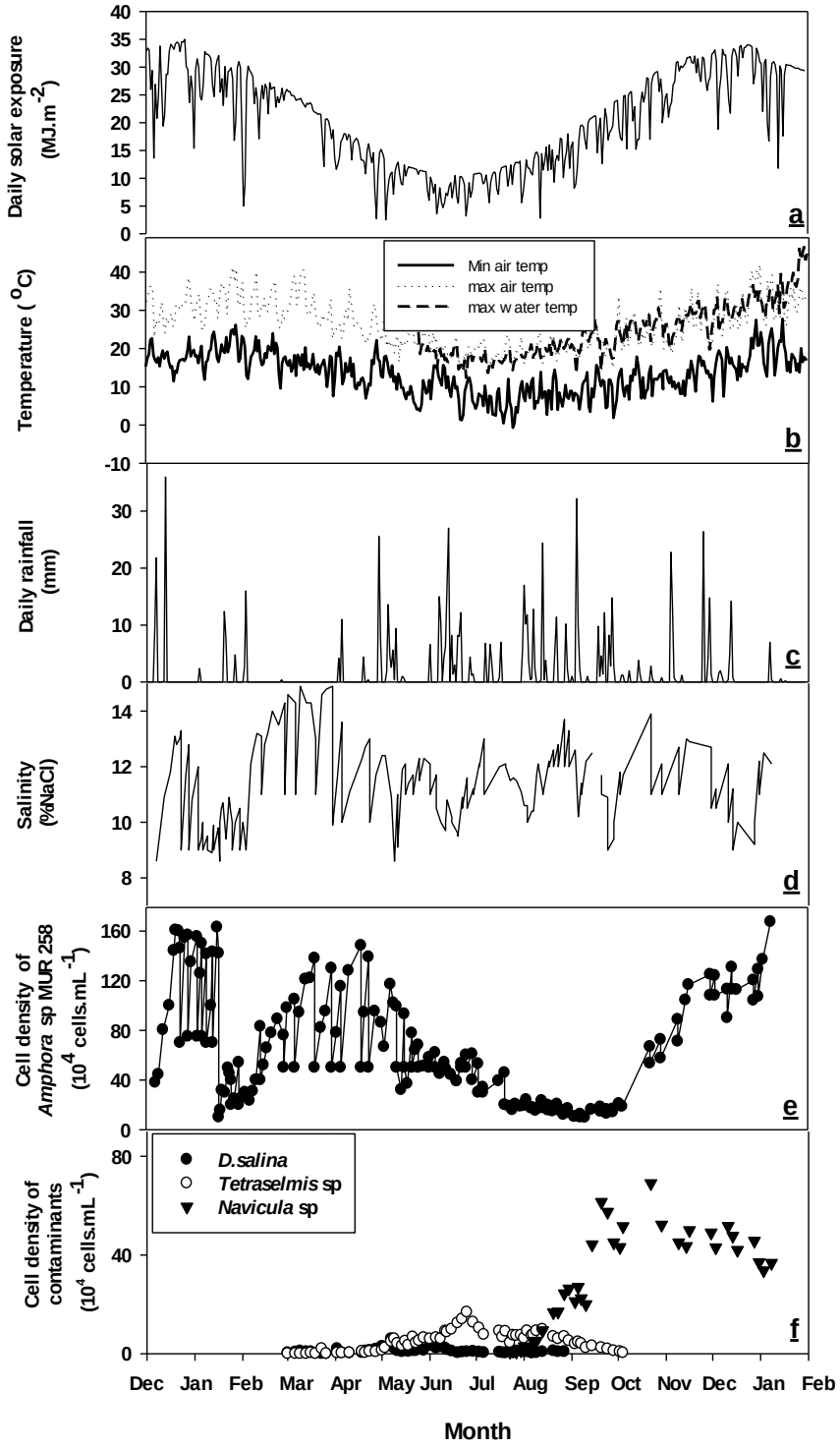


**Figure 1.Environmental conditions and growth characteristics of Amphora sp. MUR 258 cultured in a 1m$^2$ paddle-wheel driven outdoor raceway pond over one year period (December 2011-January 2013) : (a). Daily solar exposure ; (b). temperature; (c). Daily rainfall; (d). Salinity ; (e). Cell density of Amphora sp. MUR 258 and (f). Cell density of contaminants**

The culture was initially operated in batch mode for about two weeks until the culture reachedmaximum cell density ($160x10^4$ cells.$mL^{-1}$) before initiating the regular operation under semi-continuous regime (**Error! Reference source not found.e**). *Amphora* sp. MUR 258grew well over the whole cultivation period, although growth was very slow during winter (June-August). The cell density was higher in summer except for the short period from 17/Jan/12 to 10/Feb/12 (summer). During this 23 days period, the culture was operated at a very low cell density ($30\pm20x10^4$ cells.$mL^{-1}$) in order to see how *Amphora* sp. MUR 258 would respond to elevation in light intensity. From 13/Feb/2012, the culture was operated at higher cell density and the harvesting rate was adjusted to maintain a constant cell density of about $50x10^4$ cells.$mL^{-1}$. With the advent of winter, the maximum cell density decreased from about $60x10^4$ cell.$mL^{-1}$on 5/June/2012 to just below $10x10^4$ cells.$mL^{-1}$ on 7/Sep/2012. However, the cell density increased gradually and again reached its maximum at $167x10^4$ cells.$mL^{-1}$ in the following summer (early January 2013).

There was a significant difference in the dry weight, ash-free dry weight and lipid content per cell of the alga between season (Rm ANOVA, $P<0.05$). The highest dry weight per cell was achieved in winter and the lowest was obtained in Autumn (Rm ANOVA, $F_{(3,6)}=12.194$, $p=0.006$). Similarly, the ash-free dry weight and lipid per cell of the alga reached maximum in winter and lowest in autumn (Rm ANOVA, $F_{(3,6)}=7.706$, $p=0.018$ and Rm ANOVA, $F_{(3,3)}=21.499$, $p=0.016$, respectively) (**Error! Reference source not found.**). Significant difference in the specific growth rate (SGR) and biomass productivity of the *Amphora* sp. MUR 258 between seasons were also observed. The alga had the highest SGR in summer and the lowest in winter (Rm ANOVA, $F_{(3,2)}=71.295$, $p=0.014$). The highest biomass productivity was recorded in summer and the lowest biomass productivity was obtained in winter (Rm ANOVA, $F_{(3,2)}=30.998$, $p=0.031$) (**Error! Reference source not found.**). On the other hand, no significant difference on the lipid productivity of the *Amphora* sp. MUR 258 between seasons was observed (Rm ANOVA, $F_{(3,1)}=11.365$, $p=0.214$). The lipid productivity ranged from 0.05-6.8 $g.m^{-2}.d^{-1}$. The annual average biomass and lipid productivities were 7 and 2.2 g ash-free dry $weight.m^{-2}.d^{-1}$, respectively.

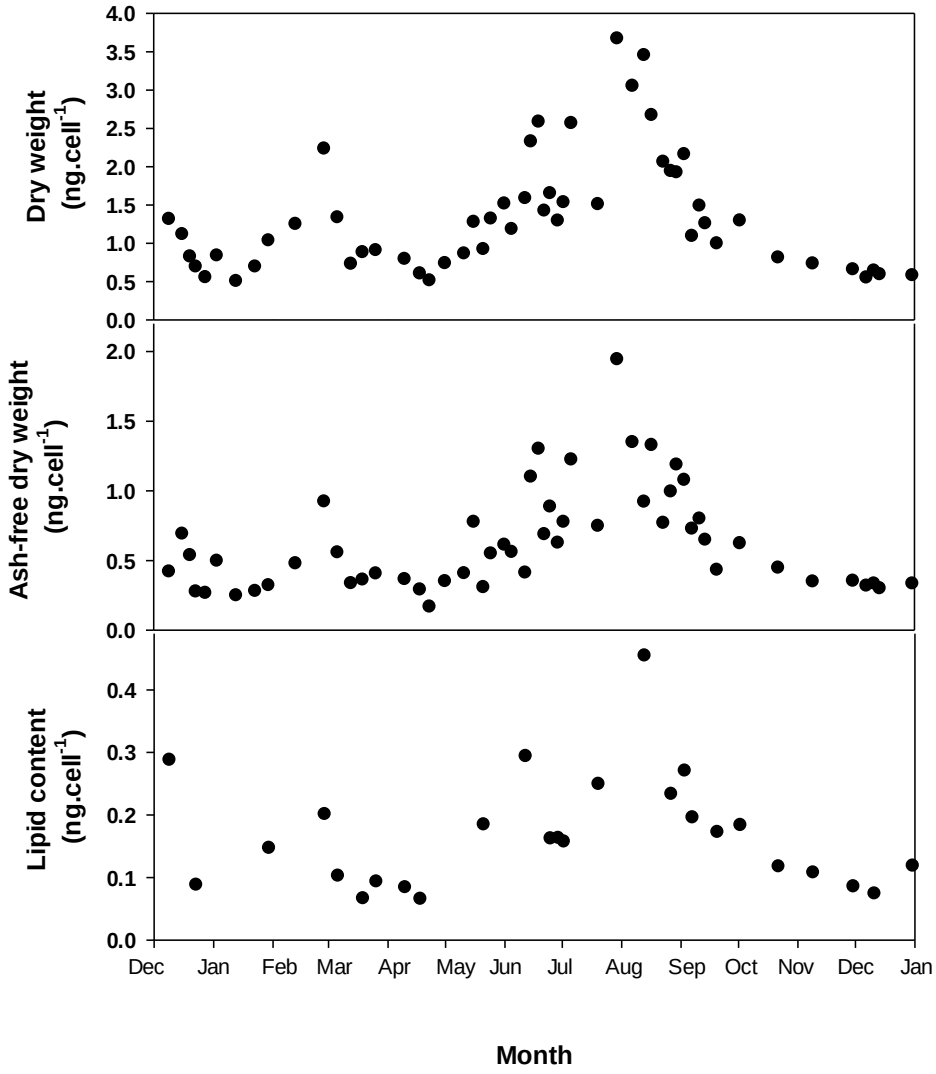


**Figure 2.Cell weight (dry weight and ash free dry weight)and lipid content of *Amphora* sp. MUR 258 cultured in a 1m$^2$ outdoor fibreglass paddlewheel-driven raceway pond over one year period (December 2011-January 2013)**

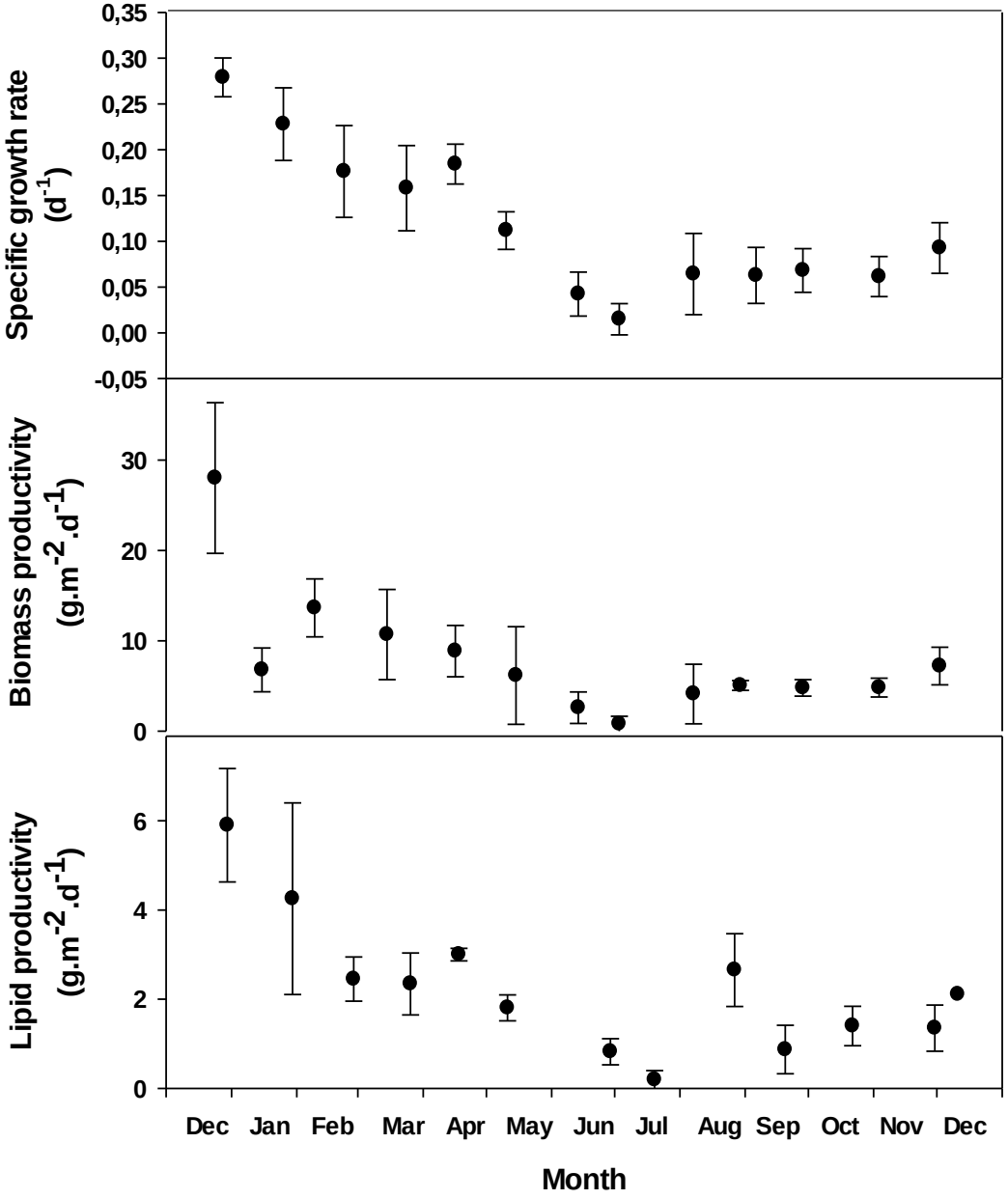


**Figure 3. Monthly average of specific growth rate, biomass and lipid productivity of *Amphora* sp MUR 258 cultured in a 1m$^2$ outdoor fibreglass paddle wheel-driven raceway pond over one year period (December 2011-January 2013). Values represent mean ± SD**

The specific growth rate of *Amphora* sp. was positively correlated with temperature and irradiance (**Error! Reference source not found.**). On the other hand,, *Amphora* sp. MUR 258 cell weight and cellular lipid content showed a curvilinear relathionship in which the cell weight decreased as the specific growth rate increased, but up to a certain point, the cells

ahowed a relatively constant weight even when the specific growth rate continued to increase (**Error! Reference source not found.**).

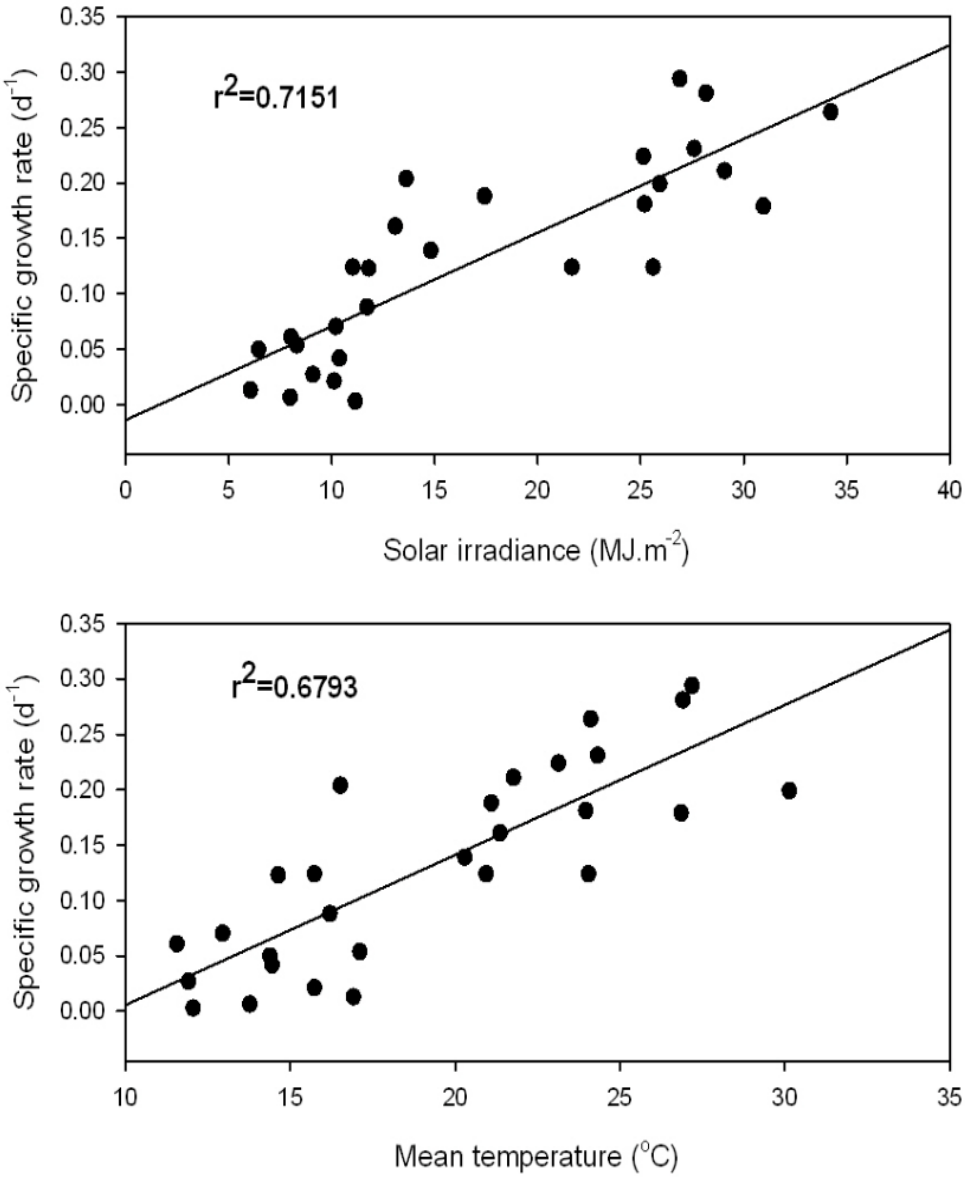


**Figure 4. Correlation of mean temperature and solar irradiance with the specific growth rate of *Amphora* sp. MUR 258 cultured in a 1m$^2$ outdoor raceway pond over one year period (December 2011-January 2013)**

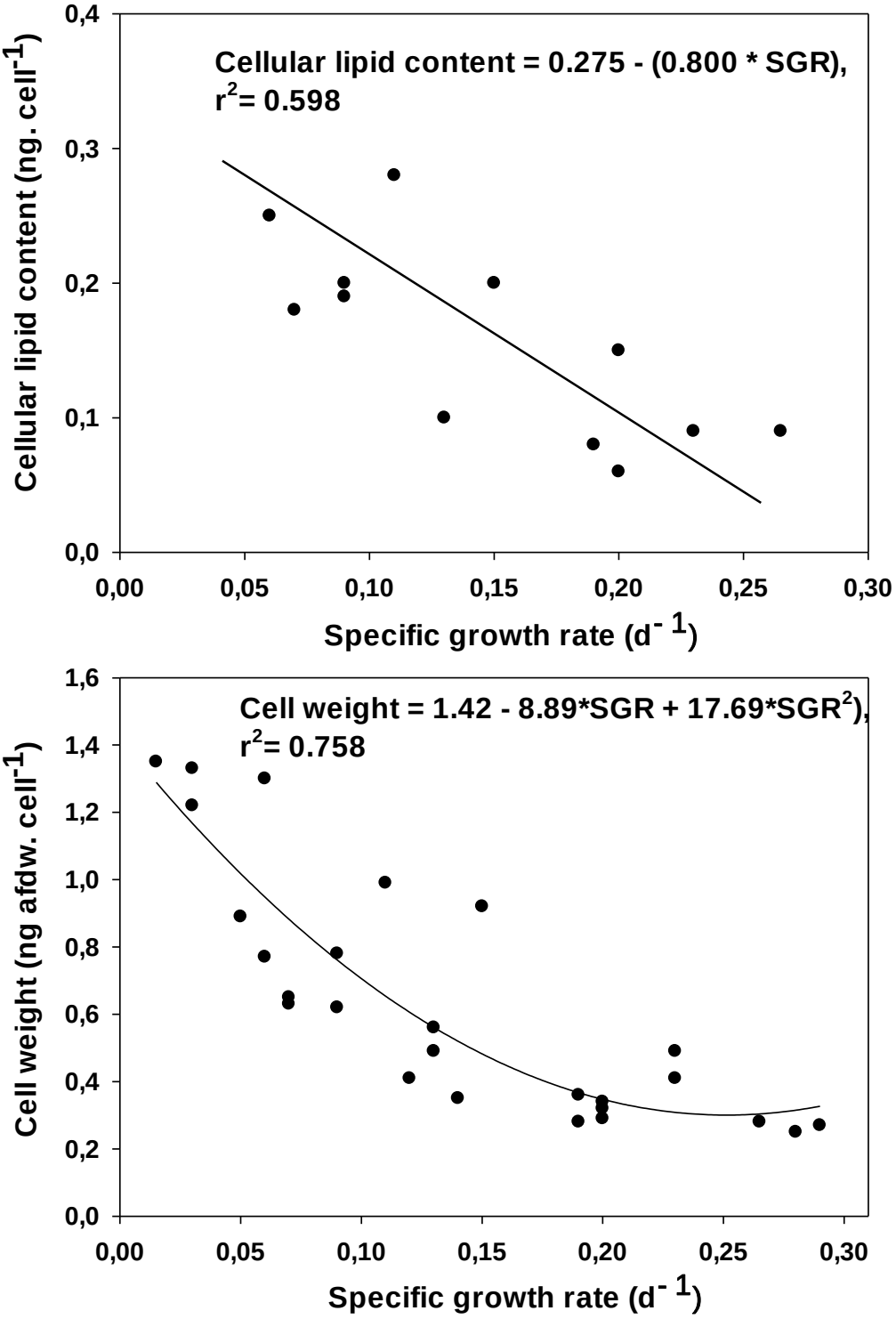


**Figure 5. Correlation of cellular lipid content (ng.cell$^{-1}$) and cell weight (ng afdw.cell$^{-1}$) with the Specific Growth Rate (SGR) of *Amphora* sp. MUR 258 cultured in a 1m$^2$ outdoor raceway pond over one year period (December 2011-January 2013).**

***Contamination.*** No contamination by foreign organisms was observed during the first 3 months of *Amphora* sp. cultivation, but after this contamination by other microalgae (*D. salina, Tetraselmis* sp. and *Navicula* sp) was observed **(Error! Reference source not found.f)**. Very minor contamination by protozoans was also observed in the winter months. In parallel with the *Amphora* sp. culture, *D. salina* and *Tetraselmis* sp were being grown in adjacent ponds. Therefore, cross contamination is likely to have occurred. Contamination by *Dunaliella* was first recorded on 1/Mar/2012, followed by the *Tetraselmis* sp on 16/Mar/2012. The highest *D. salina* cell density was $6x10^4$ cells.mL$^{-1}$ (about 5% of the *Amphora* population) recorded on 7/May/2012. From 15/June/2012 onwards, *Dunaliella* cell density decreased to below $1x10^4$ cells.mL$^{-1}$ before it completely disappeared.By the end of August.*Tetraselmis* sp reached its highest cell density at $16.8x10^4$ cells.mL$^{-1}$ (28% of the*Amphora* population) on 25/Jun/2012 (winter). This was the time when the salinity dropped to below 9% due to heavy rainfall periods. From 30/Aug/2012 onwards, the *Tetraselmis* sp concentration decreased to below $5x10^4$ cells.mL$^{-1}$ before completely disappearing in mid of October (culture salinity between 11-13%). Another contaminant, *Navicula* sp, started to appear at the end of July (23/Jul/2012) and reached its highest cell density of $51x10^4$ cells.mL$^{-1}$ on 20/9/2012. Interestingly, with the presence of the latter, the growth of the former contaminants continued to decrease before completely disappearing by mid October 2012. However, as summer approached, *Navicula* sp cell density gradually decreased whereas the *Amphora* sp population continued to increase and again dominating the culture**.** By the end of the culture period, *Navicula* sp cell density was about $35x10^4$ cells.mL$^{-1}$ (22% of the *Amphora* sp. population).

**Discussion**

Long-term reliable culture with minimum management requirements is critical for successful commercial microalgae mass production. Thus, as part of the process of evaluating and developing new algal strains long-term culture trials are necessary to examine the reliability of culture over a long period even though they are expensive and very time-consuming (Moheimani and Borowitzka 2006). The present study clearly showed that the newly isolated hypersaline *Amphora* sp. MUR 258 can be cultured reliably outdoors for a period of at least 13 months (December 2011 to January 2013). To the best of our knowledge, this is the first study on the long-term culture of a hypersaline diatom under outdoor conditions.

To grow in open ponds outdoors, microalgae must be able to tolerate a wide range of environmental conditions, especially changes in salinity, irradiance and temperature, which

vary on a daily and seasonal basis (Richmond 1986). *Amphora* sp. MUR 258 is considered as euryhaline and halophilic alga due to its ability to tolerate wide range of high salt concentration. Some species of diatoms can also be found in hypersaline environments including *Amphora coffeaeformis*, *Nitzschia* and *Navicula* species (DasSarma and Arora 2001). However, no information is available regarding their growth performance over a wide range of hypersaline conditions.

In terms of salinity tolerance, the culture of *Amphora* sp. MUR 258 in outdoors could grow up to 15% NaCl. In a separate study indoors (unpublished data), *Amphora* sp. showed no growth at salinity 14% NaCl. This suggests that long-term exposure to high salinity has led to adaptation allowing the *Amphora* to grow at much higher salinity. In addition, outdoor conditions are a complex situation in which many factors interact simultaneously at a time. The interaction between various factors including temperature, salinity, and irradiance are changing simultaneously from time to time and how this interaction affected the growth of the *Amphora*sp are still not well understood. Therefore, more studies are required to better understand adaptation mechanisms of *Amphora* sp MUR 258 in response to salinity changes as well as the interaction between salinity and other limits to growth factors. .

*Amphora* sp. MUR 258 is also considered to be a mesophilic strain able to tolerate the wide range of temperatures prevailing outdoors conditions throughout summer and winter. Its optimum temperature for growth is relatively high and an increase in water temperature up to 46$^{o}$C on hot sunny days during summer did not cause any harmful effects to the culture. *Amphora* sp achieved its maximum cell density, specific growth rate and biomass productivity in summer. Other diatoms are also known to thrive at high temperature. For instance, *Chaetoceros muelleri* has been grown over a wide range of temperature up to 35$^{o}$C on a thermal gradient plate (optimum at 30˚C) (McGinnis et al. 1997). Similarly, *Coscinodiscus granii* also achieved the maximum growth rate and cell yield at 30$^{o}$C (Fukao et al. 2012). However, in the present study, it was found that *Amphora* sp MUR 258 can grow well at much higher temperatures up to 46$^{o}$C indicating its suitability for outdoor culture in regions of high irradiance such as the Pilbara region of Western Australia. During winter, the maximum cell density of *Amphora* sp was over three times less than that in summer. It was due to the low irradiance and low temperature that contribute to the low cell density and biomass productivity of the culture. The irradiance received by the algae during winter was only about 10-30% than that of irradiance during summer. Similarly, the

temperature was also dropped to far below the optimum temperature of the alga which seems to prefer warm temperature. Therefore, multiple stressors of low irradiance, low temperature and heavy rainfall occurred simustaneously at the same time retarded the growth of the alga. According to Borowitzka (2018), multiple stressors of environmental factors that disturb homeostasis and stress as the response to the stressors acting at the same time or repeatedly over a short time period is more stressful than a single stressor acting at any one time as greater resources are required for acclimation. However, as the Winter disappear, the cells gradually restored their homeostatis as reflected by the gradual increase in the cell density,

Long-term data on productivity of microalgae cultured in open ponds are very limited (Borowitzka and Moheimani 2013b). Among them are the productivity of *Pleurochrysis carterae* cultured in $1m^2$ raceway ponds in Perth, Australia over 12 months period ranged from 16 to 33.5 g Dry weight.$m^{-2}$.$d^{-1}$ (Moheimani and Borowitzka 2006). *Tetraselmis* sp cultured in a raceway pond in Japan for 12 months period achieved the productivity range of 5-40 g dry weight.$m^{-2}$.$d^{-1}$(Matsumoto et al. 1995). An annual average productivity of 15 g dry weight.$m^{-2}$.$d^{-1}$ of *Scenedesmus obliquus* was achieved in a raceway pond in Bangkok, Thailand (Payer et al. 1978). In the present study, the productivity ranged from 3-24 g AFDW.$m^{-2}$.$d^{-1}$ (annual average of 7 g ash free dry weight.$m^{-2}$.$d^{-1}$). It is to be noted that that in here we aimed to study the reliability of *Amphora's* long-term cultivation under real outdoors conditions with very minimum management. No $CO_2$ was added to the culture for the duration of the cultivation. If $CO_2$ was added, the productivity could potentially be increased as previous studies indicated (Moheimani and Borowitzka 2011; Moheimani 2015). High growth and productivity of *Amphora* sp. can be consistently maintained throughout the year if the alga is grown under optimum conditions (note that the average biomass and lipid productivity during summer were about 18.3 g ash-free dry weight.$m^{-2}$.$d^{-1}$ and 4.5 g ash-free dry.$m^{-2}$.$d^{-1}$, respectively). Since the specific growth rate of *Amphora* sp was positively correlated with both temperature and solar irradiance, it is therefore important to consider of culturing this alga in places with high average solar irradiance and consistent daily air temperature. There are some locations in Australia with these conditions (i.e Karratha in North of Western Australia) (Moheimani 2013; Boruff et al. 2015). If we cultured this alga in Karratha, the biomass productivity could possibly be increased to over 20 g ash-free dry weight.$m^{-2}$.$d^{-1}$ considering that the solar irradiance and daily air temperature in this area are higher and relatively more stable throughout the year compared to Perth. Furthermore, regular harvesting every 1-2 days with removal rate of 50% could be maximizing the

productivity considering that the doubling time of the alga is about two days and the higher productivity is achieved at that renewal rate in summer.

One of the major challenge of algal cultivation in outdoor open system is maintaining monoalgal culture (Borowitzka and Moheimani 2013a). Contamination by other algae, bacteria, protozoa, zooplankton and fungi is unavoidable and may reduce the yield/productivity. In the worst case scenario, contaminants may take over the culture and become the dominant species or cause culture collapse (Richmond 1986). The main reason for the absence of the contaminants in the first three months of the cultivation was that the culture conditions during summer (high temperature and irradiance) appear to be optimum for *Amphora*sp.MUR 258. Contamination by *Dunaliella* sp and *Tetraselmis* was unavoidable because these two species were cultured at the neighbouring ponds whereas, another contaminant, *Navicula* sp seems to favour winter conditions. Interestingly, in the presence of the latter, the growth of *Dunaliella* sp and *Tetraselmis* decreased gradually before they were completely eliminated by the end of October 2012. The *Navicula* sp seems to out-compete the other species in terms of low temperature tolerance and ability to optimize use of low light. It is also possible that the *Navicula* sp might release inhibitory substances/toxins that inhibit the growth of other microalgae. Some microalgae are known to produce inhibitory substances including *Pleurochrysis carterae* (Moheimani and Borowitzka 2006), *Nitzschia palea* (Jorgensen 1956) and, *Skeletonema costatum* (Imada et al. 1991).

Some strategies were applied to control the contaminants. Maintaining the salinity in the pond at around salinity optimum for the *Amphora*sp (10±1% NaCl) but sub-optimal for the growth of the *Dunaliella* and *Tetraselmis* was successful to inhibit/supress the growth these contaminants. Also, with a fast growing alga like *Amphora* sp, regular harvesting washed out the contaminants from the pond. However, the presence of a cold-loving contaminant (*Navicula* sp) that appeared to have similar salinity optimum with *Amphora* sp changed the whole story. *Navicula* sp became the dominant species for about two months before the *Amphora* sp recovered by the end of October 2012.

Although contamination by other algae is a big problem, it can also be a good starting point for future studies. The ability of a contaminant to dominate an algal culture show its superiority over the target species and if the superior characteristics are combined with other desirable characteristics for commercial applications (i.e high lipid content and PUFAs), the

contaminant could be considered as a potential candidate for future development. Furthermore, understanding the contaminants will help dealing with their reoccurrence.

Lipids are the main storage product in diatoms due to the fact that oil droplets are present in the cells (Lewin and Guillard 1963). Diatoms also use the polysaccharide chrysolaminarin as a sometimes significant storage product (instead of starch) (Myklestad 1977). Diatom lipid accumulation is affected by numerous factors including salinity (Khatoon et al. 2010), silicon deficiency (Roessler 1990), nitrogen depletion (Collyer and Fogg 1955; Badour and Gergis 1965), drying or desiccation (Evans 1958), culture age (Lombardi and Wangersky 1995; Popovich et al. 2012). In the present study, it was found that the *Amphora* sp. lipid content per cell was higher in winter than summer. On the other hand, very low specific growth rate and biomass yield contributed to the low lipid productivity of *Amphora* in winter. Higher lipid accumulation within the cells is resulted from the continued accumulation of the lipids as the storage products while cell division was inhibited during winter when the temperatures dropped far below the optimal temperature range for growth. As pointed out by Ramachandra et al. (2009), storage lipids are used for long-term survival under unfavourable environmental conditions.

In conclusion, *Amphora* sp. MUR 258 showed an ability to grow in outdoors over one year period (13 months). The highest cell density, specific growth rate and biomass and lipid productivity were achieved in summer. Biomass and lipid productivities can be further increased since the growing conditions of the alga in this study have not been optimized. Therefore, further studies aiming to maximize the productivities are needed including $CO_2$ addition, determination of optimum depth and harvesting rate at different seasons as well as interaction of limits to growth factors.

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