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Changes in Lipid Profiles of a Tropical Benthic Diatom in different Cultivation Temperature

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ABSTRACT

Growth temperature has a strong influence on lipids and fatty acids of all aquatic organisms including the benthic diatom. This study looks into the effects of 7 different cultivation temperatures on the lipid profile of tropical benthic diatom *Amphora subacutiuscula*. Temperatures of 5 °C not only significantly affect the quality and quantity of the lipids produced but also the biomass production of *Amphora subacutiuscula*. Temperature up to 10°C does not influence the distribution of fatty acid methyl ester. Higher temperatures up to 23°C benefits the diatom in terms of growth. At 23 °C, this locally isolated benthic diatom exhibits good quality and quantity of lipid as well as EPA. The results indicated that the *Amphora subacutiuscula* may serve as an alternative resource for fish oil.

Keywords: *Amphora subacutiuscula*, eicosapentaenoic acid, omega-3, fatty acid methyl ester, biomass

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INTRODUCTION

Rising ocean temperatures and others environmental factors are affecting the productivity of marine species as well as their distribution and species composition. As a result, the marine food chain is affected as well In order for marine organism including diatom to survive and compete for resources in the aquatic world, stress adaptation is the way out to address the impacts of climate change.

Among the various environmental factors, growth temperature is an important parameter that affects all biological reactions (Kleinschmidi and McMahon, 1970). Temperature stress

has a major effect on lipids and fatty acids profile as these are the energy source for stress adaptation in algae (Teoh *et al*., 2013). The relationship between nutritional profile and temperature is of great importance in getting the desirable lipids from the cultured diatom. In general, the high growth temperature has been associated to a significant increase in lipid content in several species (Han *et al*., 2013; Teoh *et al*., 2013); however, other studies have found that the response of microalgae chemical composition to growth temperature varies from species to species (Renaud *et al*., 2002).

Eicosapentaenoic acid (EPA) is an essential oil consumed by the senior citizen, diabetic and allergies patients for their well being in order to reduce inflammation and also to lower the cholesterol level (Cawood *et al*., 2010; Mullen *et al*., 2010 and Covington, 2004). In addition, regular intake of omega-3 fatty acids, mainly the EPA is scientifically proven to prevent coronary heart disease as well as hypertension (Simopoulos, 2002; Lebeau and Robert, 2003b; Ruxton *et al*., 2004, 2005; Pulz and Gross, 2004; Venegas-Calerón *et al*., 2010). Marine fish oils are the most well known EPA source. However, fish do not naturally produce EPA, but mainly accumulate it via bioaccumulation from the food chain (Hibbeln *et al*., 2006; Venegas-Calerón *et al*., 2010). Due to the seasonal and climatic variations that are radically altering the marine fish stocks, there is a need to find an alternative to fish resource for EPA.

Marine diatoms are one of the best natural producers of EPA compared to the terrestrial plants and fish (Simopoulos, 2002). Of all diatoms, benthic diatoms were chosen for this study as they grow in flocs under the heterotrophic mode of cultivation. This eliminates the expensive harvesting process in the downstream recovery associated with most microalgae products which can be up to 60% of the total production cost (Grima *et al*., 2003; Lim *et al*., 2012).

The cultivation of benthic diatoms in Malaysia is at infancy and most of the benthic diatom biofilm in the aquaculture industry are cultured by relying on the natural wild species to colonize on the available attachment of substrate provided to them (Lee, 1999; Lebeau and Robert, 2003a). The growths of the colonizing benthic diatom are therefore subjected extensively to the changes in the environment. There have been few studies reported the response of benthic diatom to temperature stress. Therefore, the aim of this study was to investigate the effect of different cultivation temperatures towards a set of biological reactions such as biomass, lipid contents and the fatty acid profiles of locally isolated benthic diatom *Amphora subacutiuscula*.

MATERIALS AND METHODS

Benthic diatom cultivation

The *Amphora subacutiuscula* strain was isolated from the intertidal area at Teluk Aling, Penang, Malaysia (5° 27' 46.33"N; 100° 12' 10.70"E). The benthic diatom stock was grown in 250 mL of fresh sterilized seawater, enriched with Conway medium Walne, 1974). The Conway medium (containing KNO_3 (216 mg L^{-1}) as simple nitrogen source, 75 mg L^{-1} of Na_2SiO_3, 5 g L^{-1} of glucose and complex nitrogen sources (yeast extract, YE and tryptone, TRYP at concentration of 2 g L^{-1} each). The axenic culture of *Amphora subacutiuscula* was grown heterotrophically in an incubated (water bath) orbital shaker (IKA-Werke GmbH, Staufen, Germany) at 100 rpm. The temperature was controlled by using a temperature controller before circulated to the water bath which had a temperature tolerance of 5 – 99 °C (LCB-22D Labtech, Korea). Experiments were conducted in seven cultivation temperatures (5°C, 10°C, 15°C, 20°C, 23°C, 30°C and 35°C) with the control at 23 °C. All treatments were performed in four replicates. After seven days of cultivation, the cell biomass, total lipid content, fatty acids methyl ester profile and EPA content of *A. subacutiuscula* were determined.

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BIOCHEMICAL ANALYSIS

Determination of biomass

The algal biomass were harvested through centrifugation (Kubota, 5000, Japan) at 2000xg for 2 mins and the dry weights of the cells were determined after freeze drying (Telstar, Cryodos-50, Spain) overnight until a constant weight was obtained. The dried biomass were then determined from the data collected.

Lipid extraction acid-catalyzed transesterification from *Amphora subacutiuscula*

The freeze dried *A. Subacutiuscula* cells were subjected to 2 steps extraction. Total lipid extraction and acid-catalyzed transesterification. The dried biomass was subjected to Branson 8510 sonication (Branson Ultrasonics Corporation, Danbury, USA) for five mins in a 25 ml screw cap culture tube. The lipids were extracted overnight in the water bath at 65°C in a mixture of methanol, chloroform and water at a ratio of 1:1:0.9 (vol/vol/vol). The mixture was then centrifuged at 2000xg for 1 min to separate into two obvious layers. The chloroform (Sigma Inc., St. Louis, Mo, USA) layer was transferred to a preweighed vial then evaporated to dryness under nitrogen gas and weighed to get the total lipid contents.

Analysis of fatty acid methyl ester

The lipids were then transesterified with 5% HCl in methanol (Sigma Inc., St. Louis, Mo, USA) at 85°C for 2 hrs and extracted with hexane (Sigma Inc., St. Louis, Mo, USA). The fatty acid methyl esters were then analyzed by GC (HP-Hewlett-Packard 5890 gas chromatography (Hewlett-Packard, Palo Aleo, USA) equipped with a mass spectrometer (5972 series). Analytical procedures for the GCMS were described in details accordingly to Toh *et al*. (2014). The methyl esters were identified by the MSD library and by comparison of retention times with their respective standard (Sigma Inc., St. Louis, Mo, USA). The fame standard had linear calibration curves through the origin ($R^2 = 0.9921$).

Statistical analyses

Data obtained from this study were analyzed using the one-way analysis of variance (ANOVA) from Minitab 16 Statistic Software. The Least Significant Different Test (LSD) was conducted on the biomass, total lipid and EPA content of *Amphora subacutiuscula* in order to compare the differences at 95% levels of significance ($p<0.05$).

RESULTS AND DISCUSSION

Biomass

The temperature had a significant effect ($P<0.05$) on the growth of *Amphora subacutiuscula* (Figure 1). The best growth with the maximum biomass of 0.36 g L^{-1} was observed at 23 °C. Best growth was observed at 23°C mainly because this temperature was the control group where the stock was cultured. As a result, the *A. subacutiuscula* adapted better at 23°C than other temperature with an increase in biomass. At the lowest tested temperature (5 °C), *A. subacutiuscula* growth was drastically inhibited, and no growth was observed in the culture. Higher growth temperature usually enhanced better growth as observed in *Nitzschia laevis* (Chen *et al*., 2008). However, temperature above 36 °C tends to inhibit the growth of many marine and estuarine algae (Scholz and Liebezeit, 2013).

In general, the diatom exhibited significantly better growth at higher temperature (≥ 23 °C) than at lower temperature (< 23 °C). This could be due to lower growth temperature potentially inhibits the enzyme function in the lipid formation pathway (Jiang and Chen,

2000). However, the optimum temperature for best growth is determined by which temperature the microalgae are exposed to in the natural environment. For example, At a temperature of 35 °C *Nitszchia closterium* and *Isochrysis* sp. were found growing very slowly, while *N. closterium* do not grow at all (Renaud *et al.*, 1995). This observation could be due to the increase in respiration of the tested species, at elevated temperature, which slows down the growth rate and potentially inhibited their growth as well.

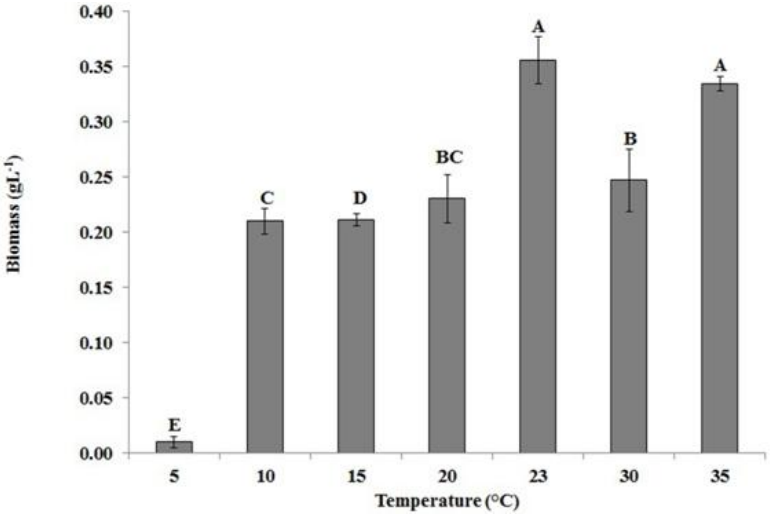

Figure 1: Biomass of *Amphora subacutiuscula* at different cultivation temperature. Different capital letters across bar chart indicate Mean of biomass was significantly different across the temperature tested (P<0.05).

Total lipid content

As shown in Figure 2, exposure to different temperatures (5 – 35 °C) resulted in a significant (P<0.05) change in *A. subacutiuscula* cell composition.

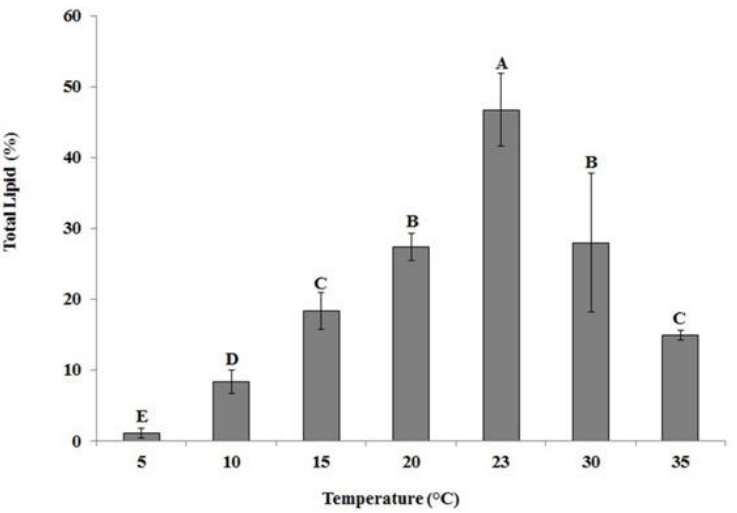

Figure 2: Total lipid composition of *Amphora subacutiuscula* at different cultivation temperatures. Different capital letters above the bars indicate significantly different values (P<0.05).

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A sharp increment in total lipid was observed in *A. subacutiuscula* when the growth temperature was shifted from 10 °C (8.4 %) to 23 °C (46.7 %). Whereas further increase in temperature from 23 °C to 35 °C, the total lipid content decreased by 31.8 %.

Temperature stress plays an importance role in lipid accumulation. In order to obtain the highest lipid content of *C.* cf. *wighamii*, temperature between 20 °C and 25 °C was recommended by Castro Araújo and Garcia (2005). Renaud *et al.* (1995) reported that optimum range in growth temperature always enhance lipid content in many species of microalgae, and the content is lower at temperatures other than this range. However, different algae regulate lipid composition differently in order to maintain the structural integrity (Wagenen, 2012). For example, *Chaetoceros* sp. showed higher lipid content at temperature of 25 °C. In contrast, optimum growth temperature range between 27 to 30 °C was reported for other species such as *Rhodomonas* sp., *Cryptomonas* sp., and *Isochrysis* sp. (Renaud *et al.*, 2002). *Cynidium caldarium* that cultured at 20 °C showed greater in lipid content by more than two fold than that of those cells grown at 55 °C (Kleinschmidt and McMahon, 1970). These finding clearly illustrated that tolerance to temperature changes are species specific (Oliveira *et al.*, 1999). Data in this study suggest that 23 °C would be the best cultivation temperature of *A. subacutiuscula* due to higher biomass and total lipid content that can be obtained at this temperature.

Fatty acid profile

Temperatures of growth medium significantly affected the quality and quantity of the fatty acid profile of *A. subacutiuscula* especially when it was cultured at 5 °C. When *A. subacutiuscula* was cultured under the unfavored condition such as at a very low temperature (5 °C), the fatty acid profile of *Amphora subacutiuscula* was significantly different compared to that of others temperatures. The C16:0, C18:0, C16:1 and C14:0 were found to be significantly higher than others (Figure 3). About 85 % of the total fatty acid in the cell was composed of saturated fatty acid (Figures 4).

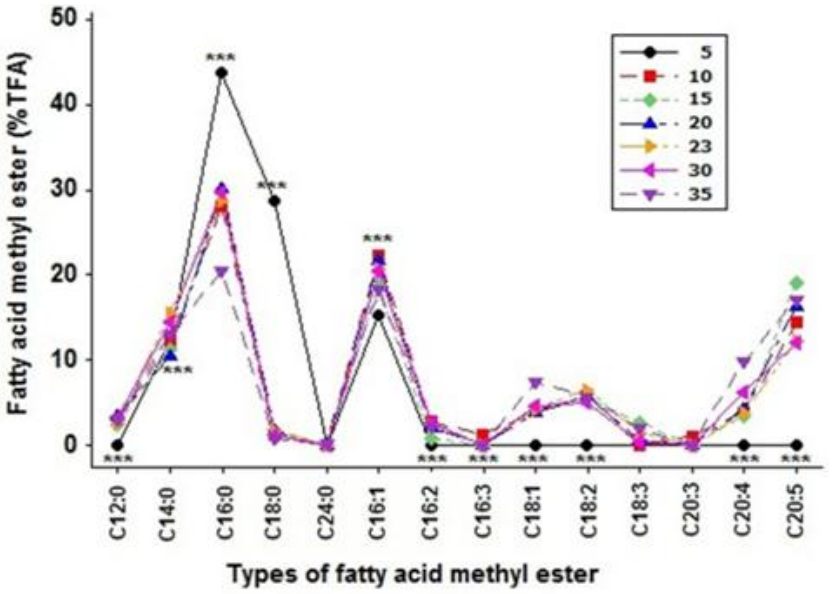

Figure 3: Fatty acid methyl ester profile of *Amphora subacutiuscula* at different cultivation temperature. Symbols indicate mean fatty acid methyl ester (%TFA) was significantly different for the treatments tested ($P<0.05$).

A similar result was reported by Converti *et al*. (2009), that an increase in temperature led to a decrease in SFA 16:0 of *C. vulgaris* from 66 % (25 °C) to 47 % (38 °C). A drastic decrease in total C16 and C18 fatty acids was observed in *A. subacutiuscula* when the temperature was increased from 5 °C (28 %) to 35 °C (0.9 %). No DUFA and PUFA were produced when *A. subacutiuscula* was cultured at a temperature of 5 °C (Figure 3 and Figure 4).

At temperature higher than 5 °C, *Amphora subacutiuscula* produced more diverse fatty acid with the C16:0 was the highest at between the temperatures of 10 °C to 30 °C, with the lowest was recorded at a temperature of 35 °C. As shown in Figure 3, the fatty acid profiles produced by *A. subacutituscula* were almost similar for temperature 10 °C onwards with C16:1 was highest right after C16:0 followed by C20:5, C14:0, C20:4, C18:1, C18:2, C12:0, C20:3, C18:0 and C24:0.

At the same time, as shown in Figure 4 (PCFA), the quantity and diversity fatty acid of *Amphora subacutiuscula* were significantly correlated with the tested temperature. *A. subacutiuscula* which was cultured at higher temperature (>20 °C) produced higher quality or more diverse fatty acid (C12:0, C14:0, C16:1, C16:2, C18:1, C18:2, C18:3, C20:4, C20:5 and C24:0) (the first PCFA axis). In addition, *A. subacutiuscula* was negatively associated with C16:0 and C18:0 when it was cultured at temperature higher than 5 °C. Whereas at 10 °C, more trienoic fatty acid (C16:3 and C20:3) were produced by *A. subacutiuscula*. At the same time, C16:1 was the highest when *A. subacutiuscula* was grown at 10 °C, and its composition, decreased to a range of 15 % to 22 % (% TFA) at higher temperatures.

Changes in temperature significantly affected ($P<0.05$) the composition of fatty acid methyl ester profiles (SFA, MUFA, DUFA, and PUFA) in *Amphora subacutiuscula* (Figure 5). The highest SFA was recorded in the cells grown at 5 °C (84.5 %) and decrease to the lowest (37.8 %) as the temperature increase from 5 °C to 35 °C. High SFA contents at lowest temperature of 5 °C could be linked to the adaptation of *Amphora subacutiuscula* to low temperature. MUFA was the second highest after SFA, these findings are in agreement with previous work (Teoh *et al*., 2013), where the SFA (C16: and C18:0) was the major fatty acid detected when diatom was cultured at low temperature.

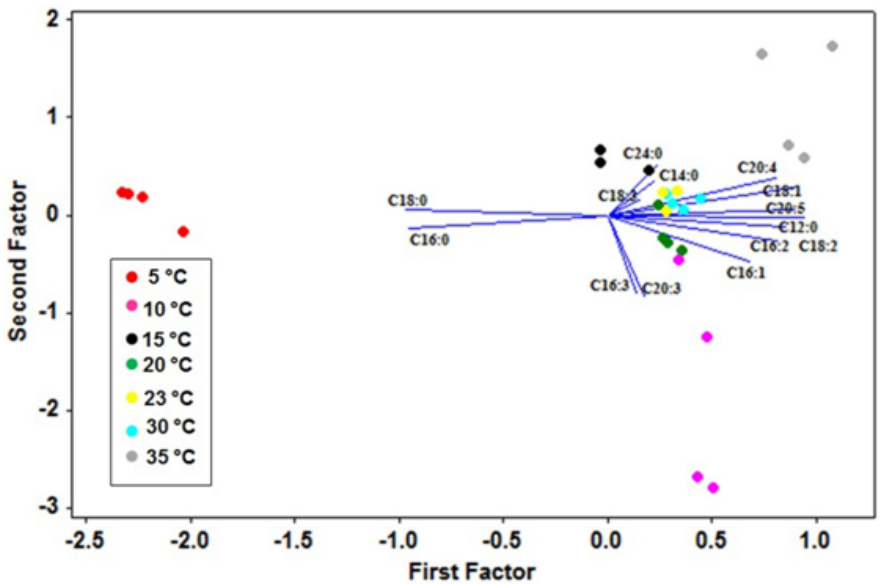

Figure 4: Principal component factor analysis, PCFA of correlation matrix for fatty acid methyl ester of *Amphora subacutiuscula* under different cultivation temperatures.

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Higher SFA at low temperature may act as an adaptive mechanism for enhancing the chance of survival in the cold (Teoh *et al*., 2013). The percentage was found to be relatively constant at around 15 – 20 % in the range of temperature tested. The highest PUFA was found in 35 °C (28.9 %) whereas DUFA were found at 23 °C (8.9 %). No DUFA and PUFA was found in *A. subacutiuscula* cells which grown in the temperature of 5 °C but are relatively stable at a temperature of 10 °C to 35 °C.

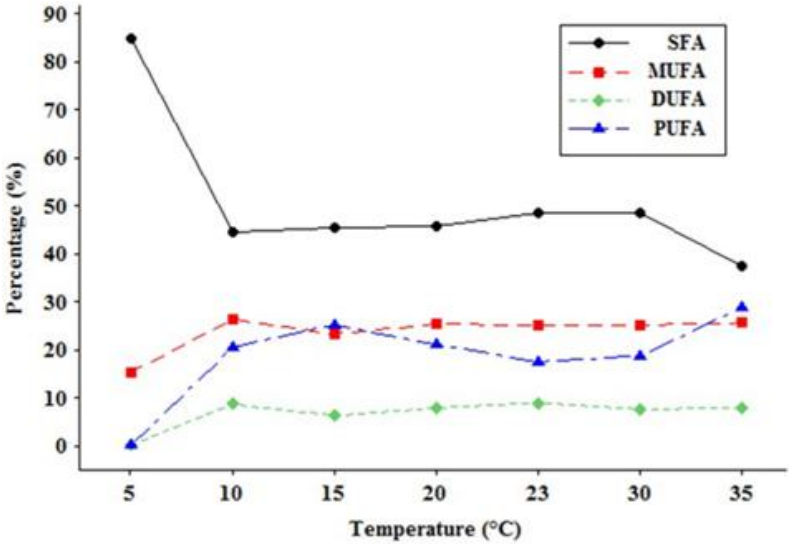

Figure 5: Fatty acid profiles of *Amphora subacutiuscula* in response to different cultivation temperatures.

Temperature has a major effect on the quality and quantity of fatty acid produced by microalgae (Sato and Murata, 1981; Thompson *et. al*., 1992). As reported by Renaud *et al*. (1995), *Nitszchia frustulum* responded to a low growing temperature (10 °C) by decreasing the production of SFAs and increasing the production of PUFAs. However, it should be the highlight here that the effect of temperature on PUFA production may not be always the same as mentioned above (Wen and Chen, 2003). A sharp decrement of SFA (C16:0) was shown by *Amphora subacutituscula* when it was cultivated at higher growth temperature. A similar observation was made by Converti *et al*. (2009), where an apparent decrease in C16:0 FAMEs when *N. oculata* was cultured at higher growth temperature.

Eicosapentaenoic acid content

EPA content of *A. subacutiuscula* was significantly affected by growth temperature (Figure 6). At lower temperature of 5 °C, only a small increment in biomass was observed in *A. subacutiuscula* cells and therefore no EPA was detected. The highest EPA content was observed in cells maintained at 23°C and 10 °C while the lowest was recorded at 15 °C. Temperature higher than 23 °C is not recommended for EPA production; low temperature leads to high EPA production as reported in *N. laevis* and *Phaeodactylum tricornutum* (Wen and Chen, 2001; Jiang and Gao, 2004). The relatively high EPA content at low temperature might be explained by the fact that the algae need to produce more EPA to maintain proper cell membrane fluidity (Chen and Chen, 2006). Besides that, the low temperature also leads to a higher level of intracellular molecular oxygen and hence improved activities of the desaturase and elongases for fatty acid biosynthesis (Jiang and Chen, 2000).

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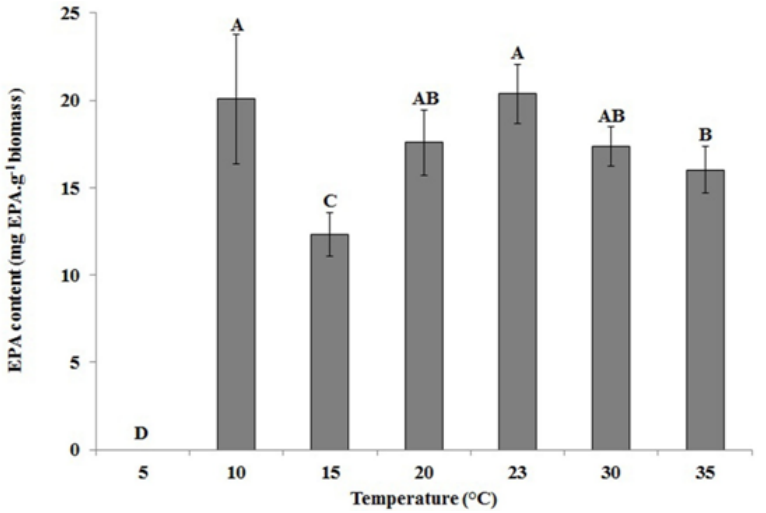

Figure 6: EPA content of *Amphora subacutiuscula* at different culture temperatures. Different capital letters above the bars indicate significantly different values (P<0.05).

Diatoms adapt to temperature stress by producing more EPA especially when there was a sudden drops in temperature (Khozin-Goldberg *et al*., 2000). Moreover, higher temperatures always enhance microalgal to grow instead of producing EPA, such as in the culture of *Porphyridium cruentum* (Springer *et al*., 1994), *Nannochloropsis* sp. (Sukenik, 1991) and *Pythium irregulare* (Stinson *et al*., 1991). However in this study, the optimal temperature for growth of *A. subacutiuscula* resulted in biomass with highest lipid and EPA production. Similar finding was reported by Ohta *et al*. (1993) where the optimal temperature for growth and EPA production was found to be the same. The living system has evolved responded networks, homeoviscous or homeophasic adaptations, to maintain appropriate membraneous liquidity or phase relationship. The necessary structural adaptation can be brought about in a variety of ways.

Table 1: Proportion of eicosapentaenoic Acid (%Total Fatty Acid) of benthic diatom

Benthic diatom strain	EPA (20:5 n-3) (%TFA)	References
Amphora subacutiuscula	11.9-19.0	This study
Amphora coffeaformis	1.39	Renaud *et. al*., 1999
Navicula pelliculosa	9.4	Tan and Johns, 1996
Navicula saprophila	16	Kitano *et. al*., 1997
Nitzschia closterium	15.2	Renaud *et. al*., 1994
Nitzschia laevis	19.1	Wen and Chen, 2000

The comparison of the EPA content of *Amphora subacutiuscula* with published data is shown in Table 1. As shown in Table 1, the proportion of EPA recorded in this study is considered to conform to proportion of EPA or even higher than in other benthic diatom species. The highest proportion of EPA was observed in *Nitzschia laevis* (Wen and Chen, 2000) and *Amphora subacutiuscula* (this study) followed by *Navicula saprophila* (Kitano *et. al*., 1997), *Nitzschia closterium* (Renaud *et. al*., 1994), *Navicula pelliculosa* (Tan and Johns, 1996) and *Amphora coffeaformis* (Renaud *et. al*., 1999). The tropical *A. subacutiuscula* strain isolated from local environment had a high satisfactory accumulation of the essential fatty acid 20:5 (n-3), which also share a common trait with others diatoms, eustigmatophytes, cryptomonads, rhodophytes and some prymnesiophytes that have been used successfully as aquaculture feed (Brown *et. al*., 1997).

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CONCLUSION

The bethic diatom *Amphora subacutiuscula* exhibited significantly better growth at higher temperature (≥ 23 °C) than at lower temperature (< 23 °C). Besides resulted in highest biomass, growth temperature of 23 °C also enhances *Amphora subacutiuscula* to produce more lipid and EPA. Changes in temperature significantly affected the composition of fatty acid methyl ester profiles, with the highest SFA was recorded in the cells of *Amphora subacutiuscula* when it was cultured at a temperature of 5 °C. Quality and diversity fatty acid of *Amphora subacutiuscula* were significantly correlated with the tested temperature especially when *Amphora subacutiuscula* was cultured at higher temperature (>20 °C). In conclusion, optimal temperature of 23 °C is recommended to culture *Amphora subacutiuscula* for highest biomass while higher EPA content can be achieved by shifting the *Amphora subacutiuscula* cell to a lower temperature of 10 °C.

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REFERENCES

Brown, M.R., Jeffrey, S.W., Volkman, J.K. and Dunstan, G.A. (1997). "Nutritional properties of microalgae for mariculture". *Aquaculture*, 151, pp. 315– 331.

Castro, A.S. and Garcia, V.M.T. (2005), "Growth and biochemical composition of the diatom *Chaetoceros* cf. *wighamii* brightwell under different temperature, salinity and carbon dioxide levels. I. Protein, carbohydrates and lipids", *Aquaculture*, 246, pp. 405-412.

Cawood, A.L., Ding, R., Napper, F.L., Young, R.H., Williams, J.A., Ward, M.J., Gudmundsen, O., Vige, R., Payne, S.P. and Ye, S. (2010), "Eicosapentaenoic acid (EPA) from highly concentrated n-3 fatty acid ethyl esters in incorporated into advanced atherosclerotic plaques and higher plaque EPA is associated with decreased plaque inflammation and increase stability", *Artherosclerosis*, 212, pp. 252-259.

Chen, G.Q. and Chen, F. (2006), "Growing phototrophic cell without light", *Biotechnology Letter*, 28, pp. 607-616.

Chen, G.Q., Jiang, Y. and Chen, F. (2008), "Variation of lipid class composition in *Nitzschia laevis* as a response to growth temperature change", *Food Chemistry*, 109, pp. 88-94.

Converti, A., Casazza, A.A., Ortiz, E.Y., Perego, P. and Borghi, M.D. (2009), "Effect of temperature and nitrogen concentration on growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production", *Chem. Engineering and Processing*, 48, pp. 1146-1151.

Covington, M.B. (2004), "Omega-3 fatty acids", *Am. Fam. Physician*, Vol. 70 No.1, pp. 133-140.

Grima, E.M., Belarbi, E.H., Fernandez, F.G.A., Medina, A.R. and Chisti, Y. (2003), "Recovery of microalgae biomass and metabolites: process options and economics", *Biotechnol. Adv.*, 20, pp. 491-515.

Han, F.F., Wang, W.L., Li, Y.G., Shen, G.M., Wan, M.X. and Wang, J. (2013), "Changes of biomass, lipid content and fatty acids composition under a light-dark cyclic culture of *Chlorella pyrenoidosa* in response to different temperature", *Biores. Technol.*, 132, pp. 182-189.

Hibbeln, J.R., Nieminen, L.R., Blasbalg, T.L., Riggs, J.A. and Lands, W.E. (2006), "Healthy intakes of n-3 and n-6 fatty acids: estimations considering worldwide diversity", *Am J. Clin. Nutr.*, 83, pp. 93- 1483.

Jiang, H.M. and Gao, K.S. (2004), "Effect of lowering temperature during culture on the production of polyunsaturated fatty acids in the marine diatom *Phaeodactylum triconutum* (Bacillariophyceae)", *J. Phycol.*, 40, pp. 651-654.

Jiang, Y. and Chen, F. (2000), "Effects of temperature and temperature shift on docosahexaenoic acid production by the marine microalga *Crypthecodinium cohnii*", *J. Am. Oil Chem. Soc.*, 77, pp. 7-613.

Khozin-Goldberg, I., Yu, H.Z., Adlerstein, D., Didi-Cohen, S., Heimer, Y.M. and Cohen, Z. (2000), "TRiacylglycerols of the red microalgae *Porphyridium cruentum* can contribute to the biosynthesis of eukaryotic galactolipids", *Lipids*, 35, pp. 881-889.

Kitano, M., Matsukawa, R. and Karube, I. (1997). "Changes in eicosapentaenoic acid content of *Navicula saprophila*, *Rhodomonas salina* and *Nitzschia* sp. under mixotrophic conditions". *J. Appl. Phycol.* 9, pp. 559-563.

Kleinschmidi, M.G. and McMahon, V.A. (1970), "Effect of growth temperature on lipid composition of *Cyanidium caldarium*", *Plant Physiol.*, 46, pp. 286-289.

Lebeau, T. and Robert, J.M. (2003a), "Diatom cultivation and biotechnically relevant products. Part II: Current and putative products", *Appl. Microbiol. Biotechnol.*, 60, pp. 624-632.

Lebeau, T. and Robert, J.M. (2003b), "Diatom cultivation and biotechnically relevant products. Part I: Cultivation at various scales", *Appl. Microbiol. Biotechnol.*, 60, pp. 612-623.

Lee, R.E. (1999), "Heterokontophyta, Bacillariophyceae", in Lee, R.E. (Ed.), *Phycology*. Cambridge University Press, Cambridge, pp. 415-458.

Lim, J.K., Derek, C.J.C., Jalak, S.A., Toh, P.Y., Mat Yasin, N.H., Ng, B.W. and Ahmad, A.L. (2012), "Rapid magnetophoretic separation of microalgae", *Small*, 11, pp.1683-1692.

Mullen, A., Loscher, C.E. and Roche, H.M. (2010), "Anti-inflammatory effects of EPA and DHA are dependent upon time and dose-response elements associated with LPS stimulation in THP-1-derived macrophages", *J. Nutrition. Biochem.*, Vol. 21 No. 5, pp. 444-450.

Ohta, S., Chang, T., Aozasa, O., Ikegami, N. and Miyata, H. (1993), "Alterations in fatty acid composition of marine red alga *Porphyridium purpureum* by environmental factors", *Bot. Mar.*, 36, pp. 7-103.

Oliveira, M.A.C.L., Monteiro, M.P.C., Robbs, P.G. and Leite, S.G.F. (1999), "Growth and chemical composition of *Spirulina maxima* and *Spirulina platensis* biomass at different temperature", *Aquacult. Int.*, 7, pp. 261-275.

Pulz, O. and Gross, W. (2004), "Valuable products from biotechnology of microalgae", *Applied Microbiology and Biotechnology*, 65, pp. 635-648.

Renaud, S.M., Parry, D.L., Thinh, L.V. (1994). "Microalgae for use in tropical aquaculture: I. Gross chemical and fatty acid composition of twelve species of microalgae from the North Territory, Australia". *J Appl* Phycol. 6, pp. 45-337.

Renaud, S.M., Thinh, L.V., Lambrinidis, G. and Parry, D.L. (2002), "Effect of temperature on grow, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures", *Aquaculture*, 211, pp. 195-214.

Renaud, S.M., Thinh, L.V., Parry, D.L., (1999). "The gross composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in marineculture". *Aquaculture*. 170, pp. 147-159.

Renaud, S.M., Zhou, H.C., Parry, D.L., Thinh, L.V. and Woo, K.C. (1995), "Effect of temperature on the growth, total lipid content and fatty avid composition of recently isolated tropical microalgae *Isochrysis* sp., *Nitzschia closterium*, *nitzschia paleacea*, and commercial species *Isochrysis* sp. (clone T.ISO)", *J. Appl. Phycol.*, 7, pp. 595-602.

Ruxton, C.H.S., Calder, P.C., Reed, S.C. and Simpson, M.J.A. (2005), "The impact of long-chain n-3 polyunsaturated fatty acids on human health", *Nutr. Res. Rev.*, 18, pp. 113-129.

Ruxton, C.H.S., Reed, S.C., Simpson, M.J.A. and Millington, K.J. (2004), "The health benefits of omega-3 polyunsaturated fatty acids: a review of evidence", *J. Hum. Nutr. Diet.*, 17, pp. 449-459.

Sato, N. and Murata, N. (1981), "Studies on the temperature shift induced desaturation of fatty acids in monogalactosyl diacylglycerol in the blue-green alga (Cyanobacterium), *Anabaena variabilis*", *Plant Cell Physiol.*, 22, pp. 50 – 1043.

Scholz, B. and Liebezeit, G. (2013), "Compatible solutes and fatty acid composition of five marine intertidal microphytobenthic Wadden Sea diatoms exposed to different temperature regimes", *Diatom Res.*, 28, pp. 337-358.

Simopoulos, A.P. (2002), "Omega-3 fatty acids in wild plants, nuts and seeds", *Asia Pacific J. Clin. Nutr.*, 11, pp. 163-173.

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Springer, M., Franke, H. and Pulz, O. (1994), "Increase of the content of polyunsaturated fatty acids in *Porphyridium cruentum* by low temperature stress and acetate supply", *J. Plant Physiol.*, 143, pp. 7-534.

Stinson, E.E., Kwoczak, R. and Kurantz, M. (1991), "Effect of culture condition on production of eicosapentaenoic acid by *Pythium irregulare*", *J. Ind. Microbiol.*, 8, pp. 8-171.

Sukenik, A. (1991), "Ecophysiological considerations in the optimization of eicosapentaenoic acid production by *Nannochloropsis* sp. (Eustigmatophyceae)", *Bioresour. Technol.*, 35, pp. 263-269.

Tan, C.K. and Johns, M.R. (1996). "Screening of diatoms for heterotrophic eicosapentaenoic acid production". *J. Appl. Phycol*. 8, pp. 59-64.

Teoh, M.L., Phang, S.M. and Chu, W.L. (2013), "Response of Antartic, temperate and tropical microalgae to temperature stress", *J. Appl. Phycol.*, 25, pp. 285-297.

Thompson, P.A., Guo, M.X. and Harrison, P.J. (1992), "Effects of variation in temperature: I. On the biochemical composition of 8 species of marine-phytoplankton", *J. Phycol.*, 28, pp. 481– 488.

Toh, P.Y., Ng, B.W., Chong, C.H., Ahmad, A.L., Yang, J.W., Chan, D.J.C. and Lim, J.K. (2014), "Magnetophoretic separation of microalgae: the role of nanoparticles and polymer binder in harvesting biofuel", *RSC Advances*, 4, pp. 4114-4121.

Venegas-Calerón, M., Sayanova, O. and Napier, J.A. (2010), "An alternative to fish oil: Metabolic engineering of oil-seed crops to produce omega-3 long chain polyunsaturated fatty acids", *Progress in Lipid Research*, 49, pp. 108-119.

Wagenen, J.V., Miller, T.W., Hobbs, S., Hook, P., Crowe, B. and Huesemann, M. (2012), "Effect of light and temperature on fatty acid production in *nannochloropsis salina*:, *Energies*, 5, pp. 731-740.

Walne, P. R. (1974), "Culture of bivalve mollusks", 50 years experience at Conway. Fishing News (Books) Ltd., pp. 1-173.

Wen, Z.Y. and Chen, F. (2000). "Production potential of eicosapentaenoic acid by the diatom *Nitzschia laevis*". *Biotechnol. Lett*. 22, pp. 727–733.

Wen, Z.Y. and Chen, F. (2001), "Optimisation of nitrogen sources for heterotrophic production of eicosapentaenoic acid by the diatom *Nitzschia laevis*", *Enzyme Microb. Technol.*, 29, pp. 341–347.

Wen, Z.Y. and Chen, F. (2003), "Heterotrophic production of eicosapentaenoic acid by microalgae", *Biotechnology Advance*, 21, pp. 273–294.

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