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Optimum Temperature and Salinity Conditions for Growth, Lipid Contents, and Fatty Acids Composition of Centric Diatoms *Chaetoceros Calcitrans* and *Thalassiosira Weissflogii*

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ABSTRACT

Microalgae are very important organisms as primary producers and have a wide range of applications in areas such as aquaculture, pharmaceuticals, medicine, biofuels, and others. In this study, the effect of temperature and salinity on growth, biomass, proximate composition, and lipid production of *Chaetoceros calcitrans* (Paulsen) and *Thalassiosira weissflogii* (Grunow) were investigated. The best growth rate (SGR) and highest biomass production were observed at 30°C and 30 ppt for *C. calcitrans* and at 30°C and 25 ppt for *T. weissflogii*. At these optimum temperature and salinity combinations, the maximum cell density was accomplished by day 12 for *C. calcitrans* (6.74×10^6 cells ml^{-1}) and by day 10 for *T. weissflogii* (3.45×10^6 cells ml^{-1}). The proximate composition during this period was 38.25 ± 0.99% protein, 16.96 ± 0.90% lipid, and 9.39 ± 0.59% carbohydrate in *C. calcitrans*, compared to 13.49 ± 0.28% protein, 10.43% ± 0.25% lipid and 16.49 ± 0.47% carbohydrate in *T. weissflogii*. Furthermore, over 35% of lipids in *C. calcitrans* were palmitic acid (C16), while in *T. weissflogii*, over 24% of lipids were myristic acid (C14). Although *C.*

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calcitrans exhibited higher lipid content than *T. weissflogii*, both species displayed higher levels of saturated (SFA) and monounsaturated (MUFAs) fatty acids and low levels of polyunsaturated fatty acids (PUFAs). The findings illustrated that under their optimum temperature and salinity combinations, both species might produce significant sources of lipids, which can be utilised in various activities such as aquaculture, pharmaceuticals, medicine, biofuels and others.

Keywords: Algal biomass, cell density, microalgae, MUFA, myristic acid, palmitic acid, PUFA

INTRODUCTION

Microalgae are very important organisms in the natural environment as primary producers that support the stability and functioning of an ecosystem. Apart from serving as the base of the food pyramids, they also contain various chemicals that have a wide range of current and potential applications, such as in aquacultures, pharmaceuticals, medicine, biofuel industries and others (Becker, 2013; Mandal & Mallick, 2014; Koyande et al., 2019; Sathasivam et al., 2019). The high diversity of microalgae species that contain various types of compounds such as fatty acids, steroids, carotenoids, polysaccharides, amino acids, antioxidants, and others, making them highly significant and most important for such diverse applications (Sathasivam et al., 2019; Barkia et al., 2019; Levasseur et al., 2020). As compared to crops, microalgae can produce 10 to 20 times more lipids per unit area (Chaisutyakorn et al., 2018), apart from their ability to capture solar energy and fixed carbon dioxide 10 to 50 times higher than that of terrestrial plants (Li et al., 2008; Batista et al., 2015).

Diatoms emerged on our planet about 150 million years ago and are responsible for approximately 40% of marine primary productivity (Sims et al., 2006; Kooistra et al., 2007; Falkowski et al., 1998; Field et al., 1998). Diatoms contain many fatty acids and other related organic molecules (Ramachandran et al., 2009; Mandal & Mallick, 2014; Sathasivam et al., 2019). They are very promising in producing high quantities of lipids and polyunsaturated fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Vazhappilly & Chen, 1998; Harwood & Guschina, 2009). For better utilisations, optimum culture conditions that allow for maximum cultivations of the diatoms are very important. One successful approach is increasing growth rates and biomass by manipulating environmental standards (Mata et al., 2010). In their natural environment, the growth, biomass, and metabolisms can be highly influenced by temperature (Renaud et al., 2002; Wei et al., 2015) and salinity (Raghavan et al., 2008; Hemaiswarya et al., 2011; Garcia et al., 2012). Both temperature and salinity may affect the nutritional constituent of the microalgae (Renaud et al., 2002; Raghavan et al., 2008; Hemaiswarya et al., 2011). Most microalgae regulate their lipid production

as a physiological response to salinity stress, which, however, depends on the species' capacity to tolerate salinity (Sajjadi et al., 2018).

In aquaculture, two important characteristics to evaluate the potential of microalgae species as feeds are their biochemical composition and the rate of biomass increase (De Castro Araújo & Garcia, 2005). The main biochemical constituents of diatoms are lipids, proteins, and carbohydrates, which are stored in their cells and are directly based on their biomass production (Kwan et al., 2021). Apart from the application as live feeds, a recent study showed the high potential of diatoms as aquaculture feed ingredients as an alternative to traditional fish meal ingredients (Nagappan et al., 2021). Since demand for fish meals has increased by 300% in the last ten years (Nagappan et al., 2021), alternatives are, therefore, highly critical for aquaculture industries. It requires rapid mass production of the algae, which needs a great understanding of their basic cultural requirements. Overall, *Chaetoceros* spp and *Thalassiosira* spp possess the largest distribution areas amongst the marine algae and thus are high potential for mass production and development into aquaculture feeds (Aydýn et al., 2009). However, there is still a scarcity of data on their development characteristics in different environments. Currently, there are limited studies on the influence of temperature and salinity on these marine-centric diatoms. This research aimed to determine the optimal conditions for the best growth and biomass production of *C. calcitrans* and *T. weissflogii* under different temperature and salinity combinations. Apart from that, the protein, carbohydrate, and lipid contents and the fatty acid profiles of both species were also analysed to clarify further the effect of temperature and salinity on the species.

MATERIALS AND METHOD

Microalgal Culture Conditions

Chaetoceros calcitrans, and *Thalassiosira weissflogii* were obtained from the microalgae culture facility of the International Institute of Aquaculture and Aquatic Sciences (I-AQUAS), Universiti Putra Malaysia, Malaysia. Stock cultures of the microalgae strain were regularly maintained in liquid cultures and agar plates. *Chaetoceros calcitrans*, and *T. weissflogii* were grown under controlled conditions in Conway medium solution with the addition of metasilicate (Bennett, 2020). The chemical composition of the Conway medium used in the culture medium is presented in Table 1. About 1 ml of stock solution A, 0.1 ml of stock solution C, 1 ml of stock solution D, and 1 ml of stock solution E were transferred into a volumetric flask and brought to volume. The laboratory glass wares, seawater, and other apparatus were sterilised by autoclave at 121°C for 15 min (LABSTAC vertical autoclave AV223 85L, United Kingdom).

Table 1

Chemical composition of Conway Medium used for the culture of microalgae Chaetoceros calcitrans *and* Thalassiosira weissflogii

Stock solution	Chemicals	Quantity (gL^{-1})
A	**Main mineral solution**	
1	$NaNO_3$	100.00
2	Disodium EDTA $C_{10}H_{16}N_2O_8$	45.00
3	H_3BO_3	33.00
4	$NaH_2PO4.\ 4H_2O$	20.00
5	$FeCl_3.6H_2O$	1.30
6	$MnCl_2.4H_2O$	0.36
7	Trace metal solution (Sol. B)	1.0 ml
8	distilled water	1000 ml
B	**Trace metal solution**	
1	$ZnCl_2$	2.10
2	$COCl_2.6H_2O$	2.00
3	$(NH_4)6MO_7O_{24}.4H_2O$	0.90
4	$CuSO_4.5H_2O$	2.00
5	distilled water	1000 ml
C	**Vitamin solution**	
1	Thiamine B1	0.20
2	Cyanocobalamin B12	0.01
3	distilled water	100 ml
D	**Silicate solution**	
1	Na_2SiO_3	20.00
2	distilled water	1000 ml
E	**Nitrate solution**	
1	KNO_3	100.00
2	distilled water	1000 ml

Experimental Design

Batch cultures of *C. calcitrans* and *T. weissflogii* were incubated for four days under similar culture room conditions where the temperature was maintained at 27 ± 3°C and pH 8 ± 0.2, with continuous aeration. Experiments were carried out simultaneously in 20 L cylindrical containers for 16 days under 12:12 light-dark conditions to evaluate the effect of different temperatures and salinities by using cool white light lamps (fluorescent

lamps 150 µmol photons $m^{-2}s^{-1}$). A factorial experimental design was conducted with two temperature settings (20°C and 30°C) and four salinities (20 ppt, 25 ppt, 30 ppt, and 35 ppt). The temperature range was selected by considering the wide geographical distribution of the diatom species and the average temperature of tropical waters of around 30°C (Krichnavaruk et al., 2005).

Specific Growth Rate and Biomass

A 5 ml aliquot was collected from each culture container every two days and fixed with Lugol's Iodine solution, from which 1 ml aliquot was taken for cell counting, using a Sedgwick-Rafter counting chamber under a compound microscope (Leica DM1000 LED, Germany) (Liang et al., 2013). Another 30 ml aliquot was collected from each culture container to estimate microalgae biomass. It was done using the previous method described by Ebrahimi and Salarzadeh (2016), with some modifications. After the filtration of culture through GF/F Whatman filter paper. The pre-weighed filter paper with algal biomass was oven-dried at 55°C and then re-weighed. A pre-weighed filter paper soaked in distilled water and dried was used as a blank. The dry weight (DW) of algae biomass was determined gravimetrically, and the growths were expressed in terms of dry weight (g DW L^{-1}). The specific growth rate (SGR) was defined as the increase in biomass per unit of time and calculated using Equation 1:

$$\mu\ (day^{-1}) = \ln (N_1 / N_o)/ t_1 - t_o \qquad [1]$$

where N_o is the initial microalgae biomass at time (t_o), and N_1 is the biomass at the time of harvest (t_1) (Adenan et al., 2013).

Analysis of Protein and Carbohydrate Content

The algal biomass was harvested by centrifugation at 4000 rpm for 15 min (ALC Multispeed, PK 121R, Korea). Harvested biomasses were freeze-dried (BAXIT BXT-FD-10N, China), recorded dry weights, and microalgal cells were stored at -70°C before analysis. The protein and carbohydrate contents were determined following the methods of the Association of Official Analysis Chemists (AOAC, 2016; Renaud et al., 2002).

Lipid Extraction, Esterification, and Fatty Acids Analysis

The *C. calcitrans* and *T. weissflogii* from the best growth condition amongst the different temperatures and salinity combinations were selected to analyse total lipid and fatty acid composition. The samples were harvested by centrifugation (ALC Multispeed,

PK 121R, Korea) at 7000 rpm for 5 min (Japar et al., 2017) and preserved in an -80°C freezer and finally freeze-dried into powder form (Mini Lyotrap, LTE Scientific, UK). The total lipid and fatty acid composition were then analysed in triplicates, following the Soxhlet method described by Prartono et al. (2013).

The fatty acid composition was determined using the method outlined by Miller et al. (2012) and Nalder (2014). Fatty acids were identified and quantified using Gas Chromatography with Flame Ionization Detection (GC-FID) with an external 38-Component Fatty Acid Methyl Esters (FAME) standard (Supelco 37 Component FAME Mix, St Louis, Missouri, USA). About 15 mg of lipid samples were added to 0.5 ml, 0.5M sodium methoxide (CH_3NaO) solution and were heated at 65°C for 5 min. After that, 1.5 ml of methylating agent ($NH_4Cl/MeOH/H_2SO_4$; 2/60/3 w/v/v) was added and allowed to react for another 3 min at the same temperature (65°C). A Hewlett Packard Series II GC equipped with an FID and a DB- 225 capillary column (15 m x 0.25 mm, film thickness 0.25 μm) was used to determine the fatty acid methyl esters (FAMEs) produced. The FAMEs were extracted using hexane (Miller et al., 2012; Nalder, 2014).

Statistical Analysis

All experiments were conducted in triplicates, and the results were expressed as the mean values ± standard deviation. Data were analysed at a significant level of $P < 0.05$. Two-way analysis of variance (ANOVA), followed by Turkey's tests, was obtained from SPSS version 23 (SPSS Inc., Chicago, IL, USA).

RESULTS

Growth and Biomass

The specific growth rate of *C. calcitrans* and *T. weissflogii* varied greatly under various temperature and salinity conditions, as shown in Table 2. The best growth for *C. calcitrans* was observed in 30°C temperature and 30 ppt salinity, with the SGR value of 0.262 ± 0.001 day^{-1}. The lowest growth rate was observed at 20°C and 25 ppt (0.246 ± 0.002 day^{-1}) ($P<0.05$). For *T. weissflogii,* the best growth was recorded at 30°C temperature and 25 ppt salinity, with an SGR value of 0.245 ± 0.081 day^{-1}. At all temperatures and salinity levels, the SGR of *T. weissflogii* was generally lower as compared to *C. calcitrans*. However, there was no significant difference in SGR at all salinity concentrations and temperatures between the two species 168 ($P>0.05$).

Table 2

Specific growth rates (SGR, day^{-1}) of Chaetoceros calcitrans *and* Thalassiosira weissflogii *cultured under two temperatures and four different salinity concentrations*

		Salinity			
	Temp.	20 ppt	25 ppt	30 ppt	35 ppt
Chaetoceros calcitrans	20°C	0.250 ± 0.004	0.246 ± 0.004	0.254 ± 0.002	0.257 ± 0.004
	30°C	0.257 ± 0.002	0.248 ± 0.002	0.262 ± 0.001	0.257 ± 0.0002
Thalassiosira weissflogii	20°C	0.221 ± 0.007	0.226 ± 0.007	0.222 ± 0.007	0.222 ± 0.008
	30°C	0.241 ± 0.075	0.245 ± 0.081	0.239 ± 0.002	0.225 ± 0.002

The biomass of *C. calcitrans* throughout the culture period is presented in Figure 1. Generally, the biomass showed increasing trends and reached maximum biomass at day 12 for most treatments, except for 20°C/25 ppt and 30°C/35 ppt temperature/salinity combinations, with maximum biomass at day 10. Afterwards, the biomass decreased, and the experiment was terminated on day 16. Overall, the highest biomass was recorded in 30°C and 30 ppt treatment (0.657 ± 0.014 g DW L^{-1}), which was significantly higher than all other treatments ($P<0.05$). The result also indicated that the culture temperature of 20°C showed a low biomass increment during the earlier days of the culture experiment ($P<0.05$).

The biomass of *T. weissflogii* throughout the culture period is presented in Figure 2. The biomass also showed increasing trends in general and reached maximum output at different times throughout the experiment. For the 20°C treatment, the maximum biomass was recorded on day 10 for the 35 ppt salinity treatment, while the others reached maximum output on day 12. For the 30°C culture temperature, the maximum biomass was recorded as early as day 8 for the 35 ppt treatment, day 10 for the 20 ppt and 25 ppt treatments, and day 12 for the 30 ppt treatment. *Thalassiosira weissflogii* also showed a low biomass increment during the earlier phase of the 20°C temperature treatment. Overall, the highest biomass was recorded at 30°C and 25 ppt treatment with a biomass value of 0.503 ± 0.035 g DW L^{-1}, which was significantly higher compared to 30 ppt and 35 ppt treatments at similar temperatures ($P<0.05$).

The cell density of *C. calcitrans* throughout the experimental culture period is presented in Figure 3. The growth was significantly higher in the 30°C treatment than in the 20°C treatment, particularly after day 6 ($P<0.05$). Nevertheless, at both temperatures, the cell density peaked at day 10 and then decreased, except for treatment at 30°C and 30 ppt. The maximum cell density was recorded in the 30°C and 30 ppt treatments on day 12, with a cell density of 6.74 x 10^6 cells ml^{-1}. It was therefore considered as the optimum temperature and salinity for *C. calcitrans*. The cell density of *T. weissflogii* is presented in Figure 4. Likewise, the growth was significantly higher in the 30°C temperature than in the 20°C treatment, particularly after day 6 of culture ($p<0.05$). In the 20°C treatment, the cell

density decreased after day 10, while in the 30°C, the cell density decreased after day 12, except for the 30°C and 25 ppt treatments. The maximum cell density was recorded at 30°C and 25ppt on day 10, with a cell density of 3.45 x 10^6 cells ml^{-1}.

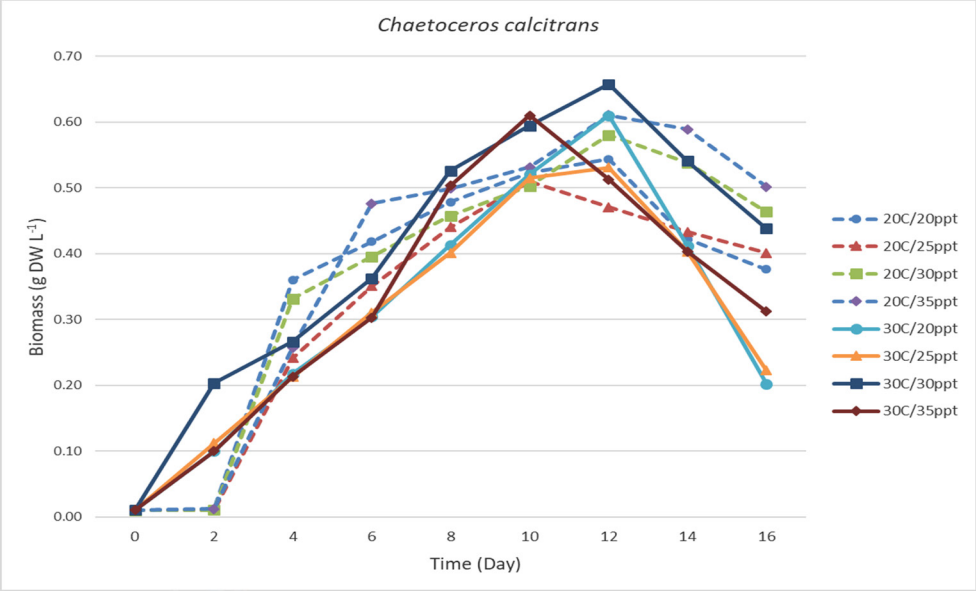

Figure 1. Biomass (g DW L^{-1}) of *Chaetoceros calcitrans* at different temperatures and salinities throughout the culture period

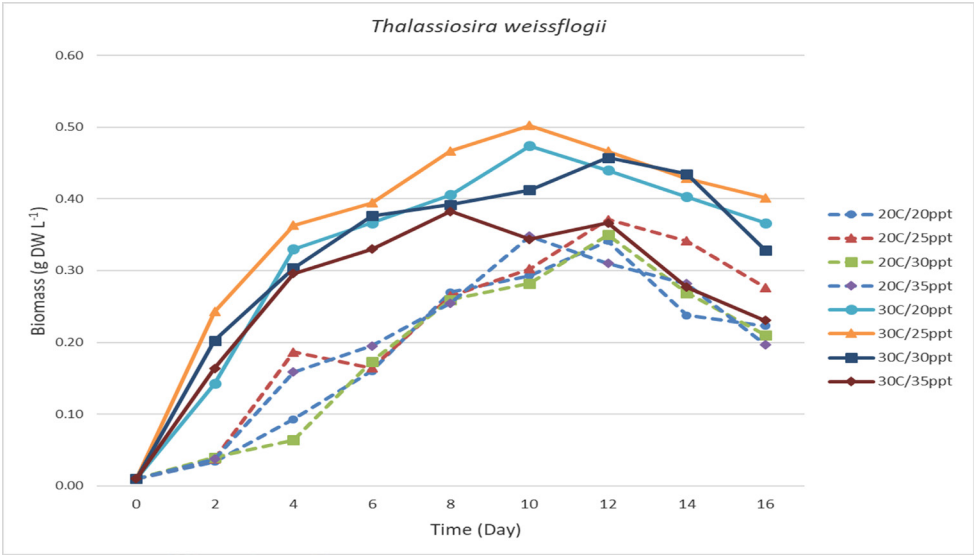

Figure 2. Biomass (g DW L^{-1}) of *Thalassiosira weissflogii* at different temperatures and salinities throughout the culture period

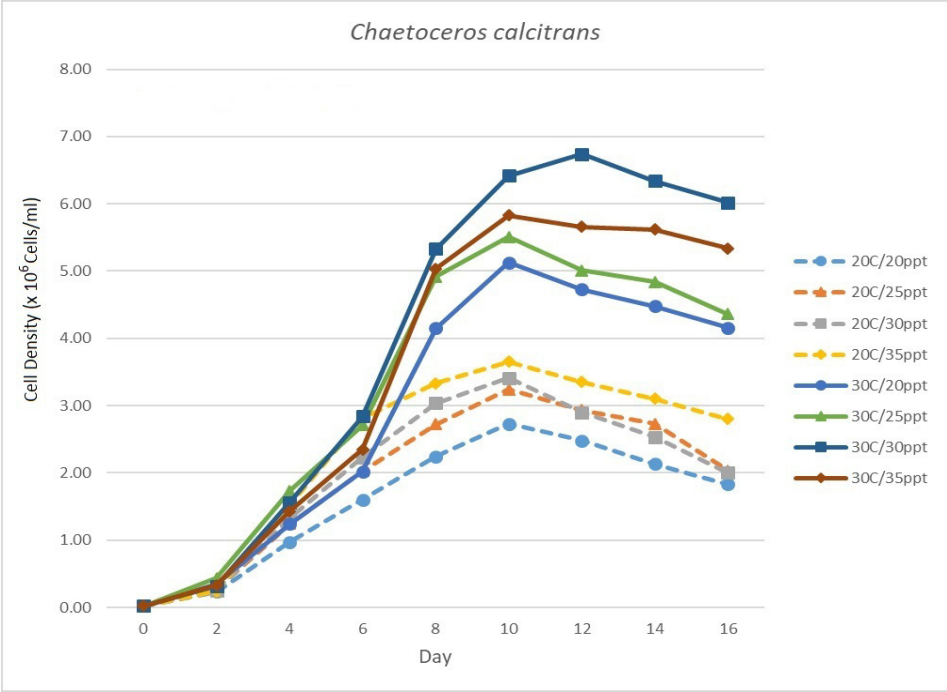

Figure 3. The cell density (cells ml^{-1}) of *Chaetoceros calcitrans* at different temperatures and salinities throughout the culture period

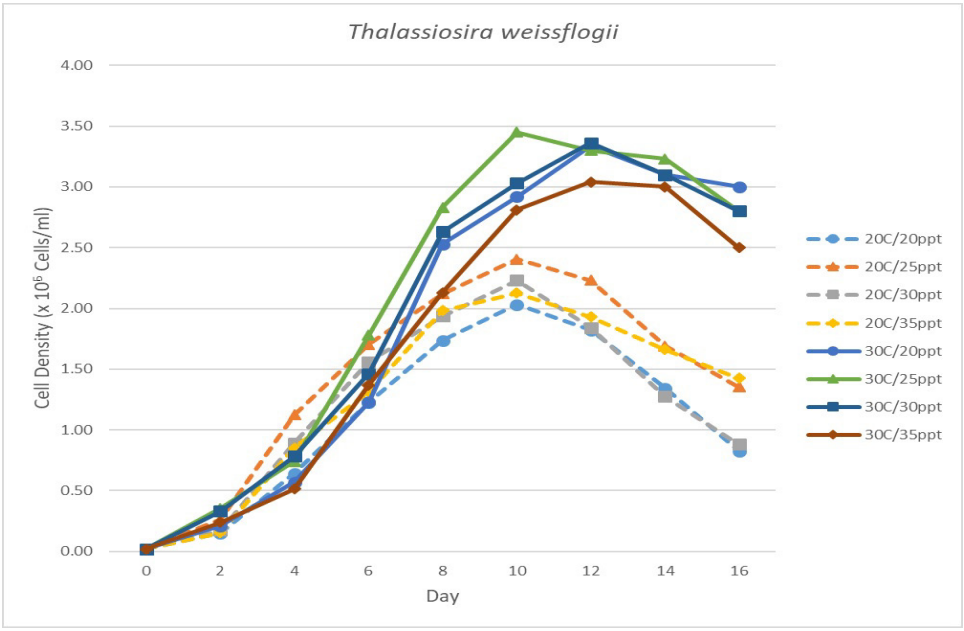

Figure 4. The cell density (cells ml^{-1}) of *Thalassiosira weissflogii* at different temperatures and salinities throughout the culture period

Protein and Carbohydrate Composition

Under the optimal temperature and salinity conditions, *C. calcitrans* recorded 38.25 ± 0.99% of protein and 9.39 ± 0.59% of carbohydrates, whilst *T. weissflogii* recorded 13.49 ± 0.28% of protein and 16.49 ± 0.47% of carbohydrates. The protein content was significantly higher in *C. calcitrans* compared with *T. weissflogii* ($P<0.05$). On the other hand, the carbohydrate contents were significantly higher in *T. weissflogii* compared with *C. calcitrans* ($P<0.05$).

Lipid and Fatty Acid Composition

Table 3 demonstrates the total lipid content, and fatty acid compositions of *C. calcitrans* and *T. weissflogii* recorded from their optimal temperature and salinity conditions. The lipid content of *C. calcitrans* was significantly higher than *T. weissflogii*, with total percentages of 16.96 ± 0.90% and 10.43 ± 0.25%, respectively ($P<0.05$). Both species recorded higher composition of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) as compared to polyunsaturated fatty acids (PUFA). In *T. weissflogii*, the total SFA was about 76.10%, the highest fatty acid component, followed by MUFA at 13.79% and PUFA at 10.11%. *Chaetoceros calcitrans* showed a similar trend with an SFA content of 52.32%, followed by MUFA at 27.45% and PUFA at 20.23%. The most abundant fatty acids methyl esters detected from *C. calcitrans* were Palmitic (C 16) 35.63%, Palmitoleic (C 16:1) 22.0%, EPA (C 20:5n3) 13.58%, Myristic (C 14) 7.10%, and Pentadecanoic (C15) 5.08%. On the other hand, *T. weissflogii* mainly contained Myristic (C14) 24.3%, Palmitic (C16) 23.10%, Lauric (C 12:0) 20.21%, Palmitoleic (C 16:1) 7.32%, EPA (C 20:5n3) 6.75%, and Oleic (C 18:1n9c) 5.44%.

Table 3

Lipid content and fatty acid composition (n = 3) of Chaetoceros calcitrans *and* Thalassiosira weissflogii *during their optimal growth at 30°C / 30 ppt and 30°C / 25 ppt, respectively*

N. D.* = not detected		*C. calcitrans*	*T. weissflogii*
Lipid Contents (LC)		16.96 ± 0.90%	10.43% ± 0.25 %
C 4	Butyric	0.06 ± 0.03	0.24 ± 0.08
C 6	Caproic	0.04 ± 0.05	0.23 ± 0.02
C 8	Caprylic	0.12 ± 0.01	2.40 ± 0.13
C 10	Capric	0.12 ± 0.01	2.13 ± 0.03
C 11	Undecanoic	N.D.	N.D.
C 12	Lauric	1.20 ± 0.22	20.10 ± 1.61
C 13	Tridecanoic	0.10 ± 0.01	N.D.
C 14	Myristic	7.10 ± 0.30	24.3 ± 3.93

Table 3 *(Continue)*

C 15	Pentadecanoic	5.08 ± 0.67	0.43 ± 0.11
C 16	Palmitic	35.63 ± 1.13	23.10 ± 3.60
C 17	Heptadecanoic	0.64 ± 0.03	N.D.
C 18	Stearic	1.38 ± 0.09	3.17 ± 31
C 20	Arachidic	0.32 ± 0.05	N.D.
C 21	Henicosanoic	N.D.	N.D.
C 22	Behenic	0.53 ± 0.16	N.D.
C 23	Tricosanoic	N.D.	N.D.
C 24	Lignoceric	N.D.	N.D.
Σ Saturated Fatty Acid (SFA %)		52.32 ± 2.59	76.10 ± 2.00
C14:1	Myristoleic	0.10 ± 0.04	0.19 ± 0.08
C 15:1	Cis-10-Pentadecenoic	0.34 ± 0.002	0.84 ± 0.30
C 16:1	Palmitoleic	22.0 ± 0.83	7.32 ± 2.84
C 17:1	Cis-10-Heptadecanoic	0.90 ± 0.01	N.D.
C 18:1n9t	Elaidic (Trans)	1.62 ± 0.07	N.D.
C 18:1n9c	Oleic	1.77 ± 0.11	5.44 ± 2.21
C 20:1n9	Cis-11-Eicosenoic	N.D.	N.D.
C 22:1n9	Erucic	0.72 ± 0.04	N.D.
C 24:1	Nervonic	N.D.	N.D.
Σ Monounsaturated Fatty Acid (MUFAs %)		27.45 ± 0.67	13.79 ± 0.72
C 18:2n6	Linolelaidic (Trans)	N.D.	N.D.
C 18:2n6c	Linoleic (Cis)	0.90 ± 0.08	N.D.
C 18:3n6	g-Linolenic	0.52 ± 0.33	0.20 ± 0.31
C 18:3n3	a-Linolenic	0.54 ± 0.04	N.D.
C 20:2	Cis-11,14-Eicosadienoic	N.D.	N.D.
C 20:3n6	Cis-8,11,14-Eicosatrienoic	N.D.	N.D.
C 20:3n3	Cis-11,14,17-Eicosatrienoic	1.52 ± 0.52	N.D.
C 20:4n6	Arachidonic	N.D.	N.D.
C 20:5n3	Cis-5,8,11,14,17-eicosapentaenoic (EPA)	13.58 ± 0.95	6.75 ± 0.67

Table 3 *(Continue)*

C 22:2	Cis-13, 16-Docosadienoic	N.D.	0.67 ± 0.01
C 22:6n3	Cis-4,7,10,13,16,19-Docosahexaenoic (DHA)	3.17 ± 0.77	2.49 ± 0.03
Σ Polyunsaturated Fatty Acid (PUFAs %)		20.23 ± 1.36	10.11 ± 1.36

DISCUSSIONS

Diatoms such as *C. calcitrans* and *T. weissflogii* have widely been used as feed for live food organisms such as *Artemia* and rotifers. They were also used directly as feeds for the early larval stages of fish, molluscs, and crustaceans. The temperature and salinity of cultivation are two important environmental factors affecting microalgae growth and performance, particularly in tropical areas. However, many microalgae grew across a wide range of temperature and salinity values, demonstrating high adaptability to these two important parameters (Cho et al., 2007). This study showed that both microalgae species increased in growth rates with increasing temperature, with the highest SGR of 0.262 ± 0.001 and 0.245 ± 0.081 day^{-1}, respectively, in their optimum culture conditions. These findings agreed with the study by Yusoff et al. (2013), who reported higher growths of diatoms and green algae when cultured at higher temperatures. Other studies reported that *C. calcitrans* could tolerate high ambient temperatures of more than 30°C (Banerjee et al., 2011; Lai et al., 2012). Microalgae growth is influenced by temperature partly due to variations in cell metabolic processes and the activity of essential enzymes (Chaisutyakorn et al., 2018). However, their growth rate may drop to some extent due to increased respirations at higher temperatures (Fogg & Thake, 1987).

Furthermore, this study showed that the highest growth rates were achieved at different salinity levels. The *C. calcitrans* recorded the highest SGR at 30 ppt, while *T. weissflogii* showed the highest SGR at 25 ppt, considered intermediate salinity relative to the range of salinities tested. These results were also in accordance with the previous report by Adenan et al. (2013) on *C. calcitrans,* whereby the optimum salinity for the best growth was about 30 ppt. Salinity is considered a major factor in the life cycle of plants, as it can slow down important metabolic activities such as photosynthesis (Liska et al., 2004). Based on the ability to regulate and tolerate salinity variations, microalgae can be categorised as halophilic or halotolerant (Rao et al., 2007). For instance, *Thalassiosira pseudonana* and *T. curviseriata* have been described as euryhaline and eurythermal in local Korean aquatic ecosystems (Popovich & Gayoso, 1999; Baek et al., 2011).

In terms of cell density and biomass, both species showed a similar pattern where the best values were recorded at higher temperatures (30°C) and again at 30 ppt for *C. calcitrans* and 25 ppt for *T. weissflogii*. These results were in accordance with the previous report on *C. calcitrans,* where the optimum salinity for growth was about 30 ppt (Adenan et al., 2013), and the report on *T. weisfloggi* at optimum salinity of 25 ppt (García et al., 2012). In addition, Baek et al. (2011) reported that the density of *T. pseudonana* was higher with increasing temperatures from 10 to 30°C. According to Sheehan (1998), increasing temperatures may induce cell multiplication, most likely as a result of changes in cell metabolic activity in reaction to environmental stress. In this study, the abundance and biomass of both species were increased gradually, with a sharp increased around day 6 to day 8 and reached maximum values around day 10 to day 12. Adenan et al. (2013) reported that active duplication in microalgae usually begins on day 5 or day 7 of the development phase, and cultivation generally can last from 2 to 3 weeks, depending on the species.

Several studies have linked the variations of microalgal chemical composition in response to changes in temperatures and salinity among microalgae species (Renaud et al., 2002; Ebrahimi & Salarzadeh, 2016). Banerjee et al. (2011) cultured *C. calcitrans* at an average temperature of 30°C and recorded a much higher protein of 41.60 ± 4.20% compared with this study of only around 13%. The lipid and carbohydrates were similar in this study (26.80 ± 5.20 lipid and 8.70 ± 1.20% carbohydrate). They also reported that protein and carbohydrate were not significantly different when cultured at a different temperature, but there was significantly higher lipid production at 30°C compared with the 20°C average culture temperature (Banerjee et al., 2011). The *T. weissflogii* in this study showed higher carbohydrate content but lower lipid and protein content. Unfortunately, no previous study was conducted on temperatures and the proximate composition of this algae species. However, a study on the effect of salinity showed maximum protein and carbohydrate production at lower salinities (Garcia et al., 2012). This study also recorded that the lipid content of *C. calcitrans* was significantly higher than *T. weissflogii*. It agreed with Lin et al. (2018), who also reported that *Chaetoceros muelleri* showed higher lipid production than *T. weissflogii*. Indeed, the percentage of lipids obtained from this study was quite similar to the reported lipid content for *Chaetoceros* by Renaud et al. (2002) and Bhattacharjya et al. (2020). For *Thalassiosira* sp., Bhattacharjya et al. (2020) and Ohse et al. (2015) reported a much higher percentage of lipids than the value reported for *T. weisflogii* recorded in this study. The analysis of the FAMEs for *C. calcitrans* and *T. weissflogii* showed higher SFA and MUFA content than PUFA at the optimal culture temperature (30°C).

According to Renaud et al. (2002), higher temperatures tend to favour SFA synthesis due to changes in the fluidity of cell membrane phospholipid layers in many marine microalgae species. Ohse et al. (2015) also reported a higher proportion of MUFAs to PUFAs

in *Thalassiosira pseudonana* cultured at 25°C. Previous studies indicated that above the optimal temperature, the lipid content of microalgae tends to decrease due to stress in photosystem II activity (Sheng et al., 2011; Mathur et al., 2014). It has also been reported that environmental stress affects lipid composition, lipid content, and, ultimately, species biomass and growth (Sajjadi et al., 2018). In terms of fatty acid compositions in membrane lipids, microalgal cells adapt to exist at various temperatures by modifying membrane fluidity (Olofsson et al., 2012). This study indeed showed that the lipid content of microalgae was inversely proportional to growth rate, similar to the study by Prartono et al. (2013). It has been proven that when the growth rate is slow, the energy required for growth is diverted to lipid production as food storage (Sajjadi et al., 2018).

Miller et al. (2012) reported higher total production of the PUFA in *C. calcitrans* cultivated at 20°C culture temperature. Despite the lower production of PUFAs recorded in this study, *C. calcitrans* is considered one of the microalgae with high nutritional value for aquatic culture systems (Raghavan et al., 2008; Nalder, 2014). As shown in this study, the predominant fatty acids in *C. calcitrans* were palmitic and palmitoleic and myristic. On the other hand, myristic, palmitic and lauric contributed the highest percentages of fatty acids in *T. weissflogii*. These results were consistent with other studies whereby the myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1n-7), DHA (C 22:6n3), and EPA (C20: 5n3) were the most frequent fatty acids found in diatoms (Volkman et al., 1989; Nalder, 2014; Yi et al., 2017), which are important for the manufacturing of animal feed additives. The relative content of MUFAs was remarkably high in palmitoleic acid (C16:1) and oleic acid (C18:1n9c) in both species. This finding supports previous research that confirmed an increase in the relative proportion of palmitic acid and oleic acid of *Desmodesmus abundans* under high salinity (Xia et al., 2014). This study suggested that temperature and salinity may affect lipid production in the species studied. According to Sajjadi et al. (2018), high salinity stress affects lipids primarily on the membrane fluidity and permeability, but optimal salinity stress can boost lipid synthesis. Nevertheless, apart from temperature and salinity, there are other factors affecting the lipid content and fatty acids, such as life cycle, growth phase, culture medium composition, carbon content, nitrogen and phosphorus deprivation, pH, light intensity and others (Zhukova & Aizdaicher, 2001; Chaisutyakorn et al., 2018; Sajjadi et al., 2018). More studies are therefore needed for these two important microalgae species in tropical waters.

CONCLUSION

The highest growth of both *C. calcitrans* and *T. weissflogii* occurred at a similar temperature of 30°C but in different salinity regimes of 30 ppt and 25 ppt, respectively. These are therefore suggested as optimum temperature and salinity for the cultivation of the two species, particularly in tropical areas. In terms of quality, *C. calcitrans* exhibited

higher lipid content than *T. weissflogii*. However, both species displayed high levels of saturated and monounsaturated fatty acids and low levels of polyunsaturated fatty acids. Lipid analysis of *C. calcitrans* indicated that over 35% of lipids were (C16) palmitic acid, while in *T. weissflogii*, over 24% of lipids were (C14) myristic acid.

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