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Abstract

A two-week batch experiment was conducted on three newly isolated Indonesian microalgal strains (Kb1-2 identified as *Chaetoceros* sp., Kb1-3 and Kb1-5) and *Tisochrysis lutea* to determine salinity effects upon the growth, proximate composition and ω-3, eicosapentaenoic acidand docosahexaenoic acid, (EPA and DHA) and ω-6 (arachidonic acid /ARA) fatty acids. Salinity within each strain growth of all microalgae tested. The highest cell densities were observed in Indonesian strains, Kb1-3 on day 8 at 25 psu and Kb1-5 on day 10 at 35 psu. Salinity significantly affected the lipid, protein and carbohydrate content in all microalgae cultured. The highest total lipid content was found in *T. lutea* cultured at 30 psu (28.3 %) followed by Kb1-2 cultured at 20 psu (25.0 %) and *T. lutea* at 35 psu (24.8 %). Kb1-3 produced highest protein when cultured at 20 and 25 psu, decreasing at higher salinities of 30 and 35 psu, 44.7 and 39.2 % to 31.5 and 32.6 %, respectively, similar to *T. lutea.* Kb1-5 had higher protein at both 25 and 35 psu but showed lower protein levels at 20 and 30 psu. Indonesian strains showed almost a similar content of carbohydrate across culture salinities similar to *T. lutea.* Although all Indonesian microalgae contained important ω-3 (EPA and DHA) and ω-6 (ARA) fatty acids, concentrations were low in comparison to *T. lutea.* All Indonesian microalgal strains also contained the dicarboxylic acid (DCA), phthalic acid, which was not present in *T. lutea*.

Keywords: Microalgae, nutrition, fatty acids, shrimp

Introduction

Local isolates of microalgae from different geographic locations have been investigated for use in various applications, including aquaculture, biofuel or bioproducts and bioremediation, with promising results (Brown et al., 1997; Renaud et al., 1999; Matsunaga et al., 1999; Nuñez et al., 2002; Yeesang and Cheirsilp, 2011; Talebi et al., 2011; Cai et al., 2013; Borowitzka, 2013; Ruangsomboon et al., 2013; Durvasula et al., 2015; Hende et al., 2016). In aquaculture, many cultured animals utilize live microalgae at certain life-history stages -- all growth stages of bivalve mollusks, and during larval stages of some fish and crustaceans -- underscoring the importance of microalgae in the aquaculture industry. In shrimp aquaculture in particular, the co-culture practice of microalgae and shrimp throughout or in some part of their growth cycle has resulted in significantly better growth and survival of the cultured species

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(Nuñes et al., 2011; Sanchez et al., 2012; Iba et al., 2014; ; Cao et al., 2014; Jamali et al., 2015).

The quality of microalgae as live food in aquaculture hatcheries is determined by several factors including: appropriate size, digestibility, robust growth under variable culture conditions, and above all, appropriate nutritional content (Brown, 2002). In general, microalgae can be considered good candidates for shrimp aquaculture if they contain protein higher than 25% of dry weight, 8-30% carbohydrate, and approximately 10% lipid (Tobias-Quinitio and Villegas, 1982). Usually, species with high fatty-acid content, particularly including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (ARA), are the most desirable for shrimp aquaculture (Coutteau, 1996; Nuñez et al., 2002). Furthermore, cholesterol also is considered to be an essential nutrient for shrimp; therefore, adequate cholesterol or metabolizable phytosterols is another desirable trait for microalgae to be used in shrimp aquaculture (Teshima et al., 1982; Lim, 1998; D'Souza and Loneragan, 1999).

Culture productivity and nutritional content of microalgae can vary based upon nutrients in culture medium and environmental conditions such as light intensity, temperature, and salinity (Renaud et al., 2002; de Castro Araujo et al., 2005; Schwenk et al., 2013). Salinity has been known to affect the lipid, protein and carbohydrate content of various species of microalgae, with different optimal ranges for different species (Renaud and Parry, 1994; de Castro Araujo et al., 2005; Khatoon et al., 2010; Zhila et al., 2011; Ruangsomboon et al., 2013). The present study was done to investigate the nutritional contents of newly isolated microalgae from Kendari Bay and the Wanggu River Estuary in South East Sulawesi, Indonesia under different salinity culture conditions. These two habitats are considered to be good potential sources of microalgae for use in regional white shrimp (*Litopenaeus vannamei*) hatcheries.

Material and Methods

Algal Culture

Three distinct microalgal isolates from Kendari Bay, denoted as Kb1-2 (*Chaetoceros* sp.), Kb1-3, and Kb1-5, were studied, and *Tisochrysis lutea* (T-ISO; CCMP-1324) was used as a comparison species. All microalgal strains have been cultured and maintained the University of Rhode Island (URI) for more than a year. All four strains of microalgae were cultured in duplicate for 15 days in 1,000-mL Erlenmeyer culture flasks containing 600 mL of f/2 Guillard medium in artificial seawater at 28-29°C under 12:12 light: dark photoperiod at four different salinities: 20, 25, 30 and 35 practical salinity units (PSU) and under 160-170 µmol photons m^{-2} s^{-1} PAR. Procedures of obtaining microalgal cell weight and measuring growth were described elsewhere (Iba et al, Journal of Applied Phycology, in review).

Microalgal cultures were harvested after two weeks of growth by filtering the algae onto pre-combusted (100°C, 1 h), pre-weighed, 47-mm GF/F Whatman filters prior to analysis. Pre-combusted filters were stored in a vacuum desiccator over a $KMnO_4$ desiccant before being used for sample filtration**.** Respective volumes of cultured microalgae (Table 1) at each salinity were filtered onto pre-weighed, pre-combusted filters, and rinsed with 10 mL 0.65-M ammonium formate to eliminate salts. Filters were oven dried at 100±5°C for 1 h, placed overnight over $KMnO_4$ salts in a vacuum desiccator, and weighed to obtain dry weight of the algae. Dry weight of microalgae was calculated using the following equations:

$$\text{DW (mg)} = \text{Weight of filter with algae} - \text{weight of filter} \quad (1)$$

All filters contained microalgal samples were folded and kept at -20°C if not dried immediately. If not analyzed immediately, the dried microalgal samples were further kept at -20°C up to 2 weeks before being subjected to biochemical analysis procedures (Gonzalez-Araya et al., 2011; Mohameini et al., 2012).

Nutritional Analyses

Total lipid, protein and carbohydrate contents of microalgae were analyzed based upon

methods by Gonzalez-Araya et al (2012), Huang (2013) and Mohameini et al. (2012) from filtered and dried microalgal samples. Total lipid of the dried microalgae was extracted with chloroform–methanol-deionized water (2:1:0.8, v/v) according to the modified method of Bligh and Dyer (1959) as suggested by Mohameini et al (2013). In brief, the microfiber filter holding cells from 200 mL of culture was crushed in a mortar and pestle after adding 4 mL of liquid nitrogen. The sample was extracted twice with fresh solvent mixture and centrifuged at 1,000-2,000 x g for 10 minutes or until a compact pellet was formed. The supernatant from samples was placed in a 20-mL tube with a screw cap and left for 24 h in the dark at -5° C for phase separation. The methanol/water layer on the top was removed with a very fine Pasteur pipette, then 6-8 drops of toluene were added to the chloroform layer to remove any small amount of remaining water. The chloroform layer was transferred to a dry and pre-weighed vial. After the toluene/water on the surface of the chloroform layer in the vial was carefully removed, the chloroform was evaporated using rotary evaporator. After evaporating, the lipid sample was kept in a vacuum dessicator over KOH pellets over night and then the vial was weighed using a 5 digit balance. The lipid content was measured gravimetrically and was calculated using the following equation:

$$\text{Lipid (\%)} = \frac{\text{WL}}{\text{WDA}}\ x\ 100 \quad (2)$$

where, WL (mg) and WDA (mg) are the weights of the extracted lipid and the dry algae biomass, respectively (Huang et al, 2013; Mohameini et al, 2013).

The total protein contents of microalgae cultures were determined using the Lowry method as described by Mohameini et al (2013). After filtered samples were crushed with a mortar and pestle in liquid nitrogen, 10 mL of the Biuret reagent was added to the extracted sample, and 5 mL to tubes containing 5 mL protein standard, before they were placed in a heating block at 100°C for 60 min. After the tubes were removed from the heating block, 0.5 mL of Folin-Phenol reagent was added immediately while mixing with a Vortex stirrer. The tubes were placed in a 10±5°C heating block for a further 20 min and finally allowed to equilibrate to room temperature for another 15 min before centrifuging at 1,000-2,000 x g for 5-10 min. Protein content of the supernatant was determined using spectrophotometry at 660 nm against bovine serum albumin (BSA) standard samples using the equation below:

$$\text{Protein content (\%)} = \frac{\text{Psample}}{\text{Wdw}} \text{ x } 100 \quad (3)$$

when, P_{sample} = milligrams of protein in samples against protein standard and W_{dw} = total dry weight of the microalgae (mg).

The carbohydrate contents of microalgal samples were determined using a method described by Mohameini et al (2012), also known as the modified Phenol-Sulfuric Acid method. In brief, after adding 5 mL 1M H_2SO_4, homogenized microalgal samples were incubated in a heating block at 100°C for 60 min. The samples were centrifuged at 1,000-2,000 x g for 5-10 minutes after having been incubated for ~30 min at room temperature. Two mL of supernatant were added to 1 mL of the phenol solution and mixed. After adding 5 mL concentrated H_2SO_4 and mixing again, the carbohydrate content was determined by spectrophotometry against a glucose standard curve at 485 nm. Carbohydrate content was calculated using the equation:

$$\text{Carbohydrate content (\%)} = \frac{\text{Csample}}{\text{Wdw}} \text{ x } 100 \quad (4)$$

when C_{std} = milligrams carbohydrate in sample based upon standard curve comparison and W_{dw} = total dry weight of microalgae (mg).

Total lipid extracts were collected to determine fatty acid compositions of the microalgae. Fatty acids within the lipid extracts were saponified after dilution in 2 mL of chloroform and addition of 1 mL of 3N NaOH before heating to 90°C for 1 h. After cooling to room temperature, samples were neutralized using 1.8 mL of 3.6 N HCl and reheated to 90°C for 10 minutes. Fatty acids from the membrane hydrolysate were extracted three times with 1 mL of a mixture of hexane:diethyl ether (1:1, v:v), and the organic layers were combined in

Erlenmeyer flask. The fatty-acid extract was dried overnight over anhydrous sodium sulfate with cover before being filtered with regular filter paper (Whatman Grade 5, diameter 25 µm) into round-bottom flask. The organic solvents were evaporated to dryness by rotary evaporator, and the fatty-acid hydrolysates were stored in a desiccator under argon at -80°C before further transesterification. The fatty acid extracts were derivatized to fatty acid methyl esters (FAME) using 5 mL boron tri-fluoride–methanol complex (BF3-2CH_3OH) at 60°C for 5 min. C-12 (Lauric Acid) was used as an internal standard at 50 µg for *T. lutea* and 150 µg for Indonesian strains. After cooling at room temperature, 1 mL of water and 1 mL of hexane were added to the mixture. To transfer the esters into the non-polar solvent thoroughly, the vials were shaken rigorously. The upper organic/hexane layer was removed into a Erlenmeyer flask. Those fatty acids methyl esters (FAME) were dried over anhydrous sodium sulfate overnight, filtered with filter paper (Whatman Grade 5, diameter 25 µm), and the solvent was evaporated by rotary evaporator. If the FAME samples were not analyzed immediately, they were stored in a dessicator under argon at -80°C. FAME mix (Supelco 37 Component FAME mix Sigma Aldrich USA) was used as a standard to identify ARA, EPA and DHA retention times by gas chromatography – mass spectrometry (GC-MS).

Analysis of FAME was conducted using GC-MS Agilent 6890 GC with 5973 MSD gas chromatography (Agilent Technologies/19091J-413, USA) at injector temperature of 250 °C. A HP-5 (0.32 mm x 30 m x 0.25 µm) film and capillary (30 m x 320 µm x 0.25 µm) fused silica column were installed in the GC with helium as the carrier gas. GC was also connected to mass Spec 15-800 m/z to ionize the samples at 1.91 scans/s. Quantification of ARA, EPA and DHA in each microalgal strain was based upon the relative abundance of the lauric acid internal FAME standard after identification by retention time using the NIST library database (NIST02.L). Other fatty acids were not quantified but identified by retention time using the same FAME standard and library.

Statistical Analysis

The effects of salinity on lipid, protein and carbohydrate content in each strain of microalgae were analyzed using one-way analysis of variance (ANOVA). One-way ANOVA was also used to compare ARA, EPA and DHA across microalgal strains. Percentage data were transformed using square root transformation to meet ANOVA normality assumptions. Differences in growth was analyzed using repeated measure ANOVA. Significant differences in the data ($p<0.05$) were analyzed with pairwise multiple comparison of means (Tukey test). All statistical analyses were done in SAS Enterprise Guide 7.1.

Results

Microalgal growth

All microalgal strains in present study was harvested either during late log phase (*Chaetoceros* sp. and Kb1-3) or stationary phase (Kb1-5 and *T. lutea*). Results of microalgal experiment was covered in Iba et al. (2018). Cells weight, biomass and division rate of microalgae species used in this study are shown in Table 1.

Table 1. Cell weight in pg.cell^{-1}, division rate (k) and final biomass in 10^6 cells·mL^{-1} of Indonesian microalgal strains and *T. lutea*. Values are mean ± SE from 4 replicates for k and 8 replicates for final biomass.

Strain/ Variables	Kb1-2 (*Chaetoceros sp.*)	Kb1-3	Kb1-5	*Ti.lutea*	p-value
Cell weight	22.1	20.7	15.6	21.8	<.0001
Biomass		4.6 ± 2.6^{a}	2.5 ± 2.1^{b}	1.4 ± 0.7^{c}	<.0001
k	0.9 ± 0.04^{dc}	1.0 ± 0.07^{bcd}	1.4 ± 0.04^{a}	0.8 ± 0.03^{d}	<.0001

Proximate composition

Proximate compositions of microalgal strains tested in this study varied with salinity. Lipid, protein, and carbohydrate contents of each strain are shown in Fig. 1. Salinity affected the total lipid content in Indonesian microalgal strains (Kb13, p=0.0005 and Kb1-5, p=0.03) except *Chaetoceros* sp. Total lipid content in Kb1-3 was significantly higher at 25 and 35 psu (24.6 and 12.9%, respectively) compared to 30 and 25 psu (4.4 and 4.9%, respectively). Kb1-5 showed a higher total lipid content at 20, 25 and

30 psu but not at 35 psu. Also, salinity affected the total lipid content of *T. lutea* which was approximately 20% at 30 and 35 psu compared to ca. 10% at 20 and 25 psu.

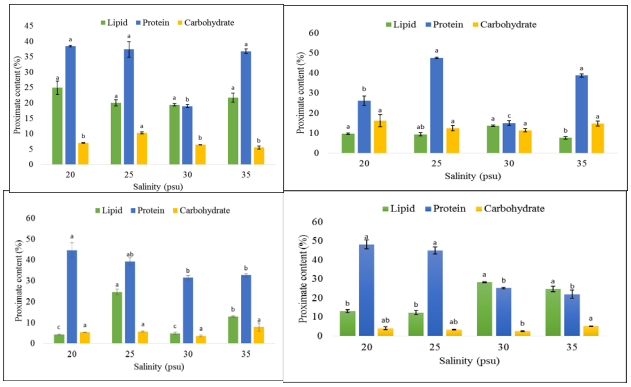

Figure 1. Proximate composition (% dry weight) of Indonesian microalgae (*Chateroceros* sp., Kb1-3, *T. lutea*, and Kb1-5 (clockwise) cultured at 20, 25, 30 and 35 psu. Bars indicate standard error (n=2). Bars with common letter for a particular component within a panel are not significantly different.

Protein content in all Indonesian strains was affected by culture salinity, similar to *T. lutea*. Similar protein contents were found in *Chaetoceros* sp. cultured at 20, 30 and 35 psu (>30%) but not at 25 psu that was 19% (p= 0.0007). Protein content was significantly higher when microalgae were cultured at low (20 and 25 psu) salinity in the Kb1-3 strain, and protein decreased at higher salinities (30 and 35 psu), similar to *T. lutea* (p= 0.03 and 0.001, respectively). Similar protein content was observed in Kb1-5 cultured at both low (20 psu) and high (35 psu) salinity, with decreasing content found at 25 and 30 psu (p= 0.0003)

Salinity levels affected carbohydrate content of only one Indonesian microalgal strain (*Chaetoceros* sp., p= 0.003) but not Kb1-3 and Kb1-5. Carbohydrate content above 10% was observed in Kb1-5; whereas, in Kb1-3 the content ranged from 4-8%. *Chaetoceros* sp. showed a higher level of carbohydrate at 25 psu, significantly different from all other salinities. Higher carbohydrate content was found at high salinity (35 psu) in *T. lutea* whereas at other salinities tested, carbohydrate content was similar (p=0.05).

Fatty Acids

Regardless salinity, all Indonesian microalgae contained essential ω-3 (EPA and DHA) and ω-6 (ARA) fatty acids, although they had low quantities of ARA (p=0.009), EPA (p=0.007) and DHA (p=0.001) compared to *T. lutea* (Table 1). Percentages of ARA and DHA in total lipid found in *T. lutea* ranged from 0.6-4.5%; whereas, DHA to total lipid was in the range of 0.6-4.1%. ARA, EPA and DHA were almost negligible in KB1-2 and Kb1-3 strains; whereas, in Kb1-5, these two essential fatty acids ranged from 0.001 to 0.1% of total lipid, respectively (Fig. 2).

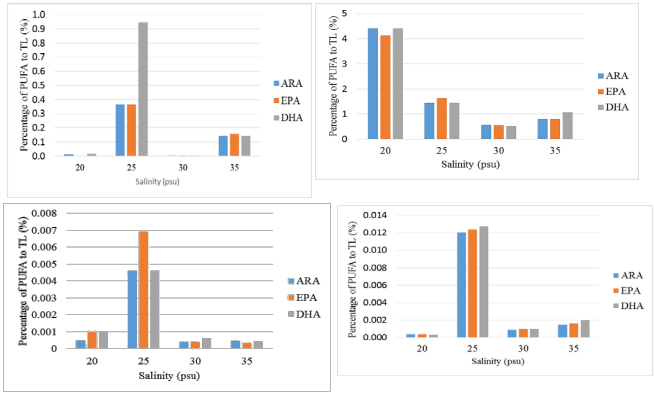

Figure 2. Percentage of PUFA (ARA. EPA and DHA) to total lipid (TL) of Indonesian microalgae (*Chateroceros* sp., Kb1-3, *T. lutea* and Kb1-5) cultured at 20, 25, 30 and 35 psu. Note that the y axes are not all on the same scale

Concentrations of fatty acids found in microalgal strains varied across microlagal strains as shown in Table 1. The presence or absence of certain fatty acids in each microalgal strain varied with culture salinity; for instance, the short-chain, saturated fatty acid, caproic acid, (6:0) was present in Kb1-5 only when cultured at 20 and 30 psu; whereas, capric acid (10:0) was found only at 35 psu in Kb1-2 and at 20 psu in *T. lutea*. The long-chain monounsaturated fatty acid nervonic acid (23:0) was present only in *T. lutea* at 25 and 30 psu. Similarly, thelong-chain, saturated fatty acid, lignoceric acid (24:0) was found only in *T. lutea* cultured at 30 and 35 psu. In general, Indonesian microalgal strains contained the short- and long-carbon chain fatty acids, both saturated such as caproic acid, caprylic acid (8:0), and tridecanoic acid (10:0), and monounsaturated such as

palmitoleic (16:1 ω7) and oleic acid (18:1 ω9) at all salinities. Long-chain, polyunsaturated fatty acids such as ARA, EPA and DHA were also present at all salinities in Indonesian microalgal strains.

The short-chain, saturated fatty acid, pelargonic acid (9:0), was found only in Kb1-5 cultured at 20 and 35 psu. Also, all Indonesian microalgal strains distinctively contained the dicarboxylic acid (DCA) phthalic acid, which was not present in *T. lutea*. In *T. lutea*, some short-carbon-chain and saturated fatty acids such as capric, palmitic, margaric, and stearic acids were only present at 20 psu. Moreover, *T. lutea* predominantly contained long-carbon-chain fatty acids, particularly at higher culture salinities tested (Table 1).

Discussion

The growth of Indonesian microalgal strains, particularly Kb1-3 and Kb1-5 strains, was higher than *T. lutea* although *T. lutea* showed a similar growth to Kb1-2, *Chaetoceros* sp. *Chaetoceros* sp. and Kb1-3 did not enter stationary phase at day 15 of culture, except for Kb1-3 at 25 psu. This growth pattern was different from Miller et al. (2014) who found that late stationary phase occurred at day 12 in cultured *Chaetoceros calcitrans*. Final, maximal cell density of *T. lutea* during stationary phase reported here was almost three times lower than that reported in a study by Huerlimann et al. (2010), but similar to that reported by Renaud et al. (1999) and almost six times lower than that reported by Fidalgo et al. (1998). The variation in final cell density may be attributable to different cells density at inoculation as well as culture conditions, such as light intensity, culture medium, temperature, and aeration (Fidalgo et al., 1998; Borowitzka, 2012; Gorgonio et al., 2013). Further studies are needed to determine if the Indonesian strains stopped dividing because of nutrient or light limitation.

Sufficient nutritional contents of microalgae determine efficacy as food for shrimp larvae in aquaculture hatcheries. Adequate levels of protein, lipid, and carbohydrate are particularly critical for normal growth and development of penaeid shrimp (Milamena, 1996; Nuñez, 2002). Our study showed that Indonesian microalgae, as well as *T. lutea,* during stationary phase contained protein higher than required by shrimp larvae (ca. 25%), with comparable lipid and carbohydrate contents (around 10 and 5%, respectively), as suggested by Tobias-Quinito and Villegas (1982).

Total lipid contents of two Indonesian microalgae (except Kb1-2) and *T. lutea* were affected by salinity. Kb1-3 contained lower lipid at 20 and 30 psu, and higher lipid contents were found at other salinity levels tested. Higher lipid was obtained at 20 and 25 psu as well, as at 30 psu, in Kb1-5, with 35 psu giving the lowest lipid content. This finding is in agreement with study by Khatoon et al (2014) and Ruangsomboon et al (2013) who found that *Tetraselmis* sp., *Nannochloropsis* sp., and a newly-isolated species of *Scenedesmus dimorphus* from Thailand responded differently to salinity in terms of lipid. *Nannochloropsis* sp. and *Tetraselmis* sp. showed significantly higher lipid contents at 30 psu compared to 20 and 40 psu salinities; whereas, *S. dimorphus* accumulated higher lipid at 5 psu salinity. Also, lipid content in the *Chaetoceros* sp. Analyzed in the present studycomparable to lipid in *Chaetoceros* sp. isolated from Innisfail, Queensland, Australia, reported by Renaud et al. (1999), that was 17% of dry weight.. Lipid content of *T. lutea* in the present study was higher compared to a study reported by Gorgonio et al. (2013), which was 10.54% during logarithmic phase of growth and similar with study by Huerlimann et al. (2010), which was 26.8% when cultured at seawater salinity and measured during the stationary phase of growth. Lipid content in microalgae was known to be affected by culture conditions, such as salinity, light intensity and temperature, as well as culture medium and growth phase (Renaud and Parry, 1994; Nalder et al, 2015).

Nalder et al. (2015) found that total lipid increased almost two fold from 6.7 ± 0.8 pg/cell during log phase to 13.2 ± 1.2 pg/cell during late stationary phase. Higher lipid production at certain salinities in microalgae is suggested as a coping mechanism under unfavorable conditions to reserve energy until favorable conditions resume (Kalita et al., 2011; Talebi et al., 2013).

Table 1. Fatty acids concentration in µg.mg^{-1} found in Indonesian microalgae strains and *T. lutea* at different salinity

Fatty Acids	Fatty acids concentration within strain per culture salinity (psu)																	
	Kb1-2				Kb1-3				Kb1-5				*T. lutea*					
	20	25	30	35	20	25	30	35	20	25	30	35	20	25	30	35		
6:0					0.03	-	-	-	0.23	-	0.07	-						
8:0	-	0.11	-	0.06					0.18	-	0.10	1.69	-	130.72	-	-		
9:0									0.40	-	-	1.27						
10:0	-	-	-	0.16									181.82	-	-	-		
11:0	-	-	0.12	0.06	0.22	0.35	0.08	0.13	0.44	0.10	0.12	1.69						
13:0	4.83	5.26	3.14	3.84	-	0.88	3.24	0.64	23.87	10.20	3.97	59.32	90.91	-	-	-		
15:0	1.16	0.53	0.25	0.32	0.06	0.14	0.25	0.08	1.59	3.06	0..40	5.08						
16:0	6.77	11.84	4.30	0.96	1.00	2.12	8.62	2.56	43.77	38.27	7.94	9.32	984.82	-	-	136.65		
16:1 ω7	0.19	0.12	0.25	0.64	-	0.21	0.04	0.06	1.79	0.46	0.40	1.02	37.88	-	-	-		
17:0	-	-	0.02	-	-	-	0.07	0.04	0.36	0.51	-	1.44	45.45	-	304.26	-		
18:0	3.87	3.95	4.92	1.60	0.56	-	5.34	0.13	19.89	17.86	3.77	61.02	393.94	-	60.85	62.11		
18:1 ω9	0.14	0.12	0.18	0.03	-	-	0.20	0.08	0.28	1.79	0.05	-	45.45	-	-	-		
t18:1 ω9	-	-	0.06	-	-	-	0.04	-	0.48	0.51	0.12	0.85						
18:2 ω6	-	-	-	0.16	0.04	0.71	0.08	-	0.20	0.82	-	0.59	-	196.08	405.68	310.56		
t18:2 ω6	-	-	0.04	0.32	0.05	-	0.08	0.08	0.80	1.02	0.20	0.85						
18:3 ω3													-	-	12.68	-		
20:0	0.05	0.09	-	0.02	-	-	0.08	0.03	0.36	2.04	0.10	1.40	45.45	98.04	-	-		
20:1 ω9	0.19	0.26	-	0.06	0.04	0.39	0.07	-	0.60	3.06	0.14	2.03	60.61	65.36	81.14	186.34		
20:2 ω6	0.19	0.26	-	0.05	-	-	0.07	-	0.80	7.65	0.09	1.69						
20:4 ω6	0.05	0.26	0.05	0.06	0.02	0.12	0.04	0.04	0.18	5.10	0.06	0.85	48.48	13.07	16.73	18.63		
20:5 ω3	0.10	0.39	0.05	0.05	0.02	0.12	0.04	0.04	-	5.10	0.06	0.93	45.45	14.71	16.23	18.63		
21:0	0.02	0.12	-	0.02	-	0.13	0.04	-	-	1.02	-	0.85	-	261.44	-	248.45		
22:0									0.44	4.08	-	1.69	-	-	-	24.84		
22:6 ω3	0.10	0.26	0.07	0.06	0.02	0.13	0.04	0.05	0.20	13.27	0.10	0.85	48.48	13.07	15.21	24.84		
23:0													-	22.88	45.64	-		
24:0													-	-	20.28	124.22		
ArC$_5$DCA	3.38	9.21	4.30	0.48	0.28	1.42	2.96	-	36.60	45.92	6.94	110.17						

Salinity affected protein content in Indonesian strains of microalgae and *T. lutea.* Generally, higher protein content was observed at lower salinity in each strain tested, although some variation occurred in *Chaetoceros* sp., and Kb1-5. Protein contents in Indonesian microalgal strains presented here were comparable to protein contents reported by Renaud and Parry (1994) for *Nannochloropsis oculata* and *Nitzschia frustulum* cultured at similar salinity levels as this study and harvested at late log phase. Furthermore, Renaud et al. (1999) reported similar protein content in *Chaetoceros* sp. from Queenlsand, also harvested at late log phase, to protein content of *Chaetoceros* sp reported here. Unlike the recent study by Khatoon et al. (2014), who reported that *Nannochloropsis* sp. and *Tetraselmis* sp contained higher protein content (ca. 45% of dry weight) at 30 psu when cultured under natural sunlight and harvested at logarithmic phase of growth, all Indonesian strains and *T. lutea* in this present study had lower protein contents at 30 psu. The protein content of *T. lutea* at 30 and 35 psu presented here at stationary phase is comparable to protein concentration of *Isochrysis galbana* reported by Fidalgo et al. (1998).

Carbohydrate contents in both Indonesian strains and *T. lutea* were also affected by the culture salinities. Among Indonesian strains, range of carbohydrate content was comparable to local strains from Australia as reported by Renaud et al (1999), but slightly lower than strains isolated from the South China Sea (Khatoon et al. 2014). Carbohydrate content of *T. lutea* presented here was comparable to *Isochrysis galbana* reported by Fidalgo et al. (1998). Nevertheless, our study and other studies summarized by Mata et al. (2010), suggested that the differences in biochemical composition in microalgae were not only determined by culture medium and conditions such as salinity but also by the differences in strain that may be attributed to genetic differences. Therefore this finding is a promising result for exploring other local microalgal strains because there will be a chance to find several good strains for various purposes.

Our study showed that Indonesian microalgae had low concentrations of ARA, EPA and DHA compared to *T. lutea,* but they contained almost complete profiles of fatty acids. This may be a factor that contributed to reasonable weight gain of shrimp larvae up to mysis stage when fed on Indonesian strains of microalgae (Iba et al, Journal of World Aquaculture Society, in review). These weight gains were comparable to a study by Piña et al (2006), although the survival of our shrimp was considerably lower. Survival rates of shrimp larvae fed Indonesian strains were similar to that of shrimp fed *C. neogracile and Te. chui,* with the exception of Kb1-2 (Iba et al, Journal of World Aquaculture Society, in review).

Shrimp larvae from the genus *Penaeus* and *Litopaneus* require long-chain, polyunsaturated fatty acids, particularly those of C20 and C22: ω-3 and ω-6 compounds during metamorphosis to be able to survive this critical period and continue to grow (Castell, 1982; Coutteau et al, 1996; D'Souza and Loneragan, 1999). The level of these compounds in microalgae varies based upon culture conditions such as salinity, although the results were contradictory as suggested by several studies. Several microalgal strains cultured at high salinity such as *Dunaliella* sp., *Nannochloropsis* sp., and *Nannochloropsis frustulum* showed low contents of polyunsaturated fatty acids (Xu and Beardall 1997; Hu and Gao 2006). Zhilla et al (2011) suggested that salinity and growth phase may affect fatty-acid composition of microalgae in which polyunsaturated fatty acids were lower during the early phase of growth under high salinity but increased during late logarithmic and stationary phases. PUFA in *T. lutea* presented here was lower compared to findings of Ben-Amotz et al. (1985), who found that *Isochrysis* sp. cultured at high salinity

contained high polyunsaturated fatty-acid contents, but comparable to Renaud and Parry (1994) who conversely found that these fatty acids in *Isochrysis* sp. decreased under high salinity culture conditions.

Another finding in the present study is the presence of Phthalic Acid in all Indonesian microalgal strains. Phthalic acid is a dicarboxylic acid that is usually found as an aerosol in the atmosphere (Mochida et al 2003) or as a product of diisobutyl phthalate (DiBP) hydrolysis catalised by the enzyme esterase (Ding et al, 2015). DiBP is one form of plasticizer phthalate esters (PAEs) that have become a public concern because of their effects on environmental contamination and toxicity in mammals (Ding et al, 2015). The C8 and C9 DCAs including phthalic acid have been proposed as oxidation products of unsaturated fatty acids such as oleic acid (Kawamura and Gagosian, 1987; Stephanou and Stratigakis, 1993). The presence of phthalic acid in all Indonesian microalgae may be an indicator that these strains were either able to hydrolyze DiBP by using specific enzymes or oxidation of unsaturated fatty acids. Conversely, presence of this compound could indicate sample contamination by plastic components used in sample processing (e.g., pipit tips, tubes). Further study in this area will be an interesting topic to be explored in the future.

The present study showed that the proximate compositions of Indonesian microalgae were roughly comparable to *T. lutea* and that these isolates may have potential to be used as larval shrimp food. All essential fatty acids required by shrimp larvae, ARA, EPA and DHA, were present in Indonesian strains although at low levels, which suggests that the use of these strains in white shrimp hatcheries may be best in conjunction with other species, such as *T. lutea,* that contain higher levels of essential PUFA. Further study on the effect of other environmental factors, such as light intensity, temperature, and culture medium may reveal the best culture condition for Indonesian strains to yield optimal PUFA.

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