Journal of Aquatic Biology & Fisheries | 2021 | **9** | pp. 142-151

ISSN 2321–340X

Comparative Nutritional Characterization of Marine Microalgae *Chaetoceros muelleri* and *Nannochloropsis oceanica* used as Live Feeds in Aquaculture

Jisha, K., Singh, I.S.B. and Valsamma, J.*

National Centre for Aquatic Animal Health, Cochin University of Science and Technology, Lakeside Campus, Fine Arts Avenue, Kochi 682016, Kerala, India
**Email: valsamma@cusat.ac.in*

Abstract

Microalgae enhance the nutritional value of conventional aquaculture feed based on the biochemical composition. Understanding of the nutritional composition of microalgae is essential for the effective utilization of biomass to meet the nutritional requirements of the target aquaculture organisms. The present study evaluates the nutritional composition of marine microalgae *Chaetoceros muelleri* and *Nannochloropsis oceanica* in terms of cell volume, pigments, carbohydrates, proteins, lipids, ash, moisture content, fatty acid profile, minerals and amino acids. The proximate composition, total PUFA, chlorophyll a, and total essential amino acids were higher in *N. oceanica* than *C. muelleri*. Potassium and iron (µg/g dry weight) were the major minerals in both the species. The major polyunsaturated fatty acids in *N. oceanica* were eicosapentaenoic acid, arachidonic acid and linoleic acid; and linoleic acid, hexadecatrienoate and decosahexaenoic acid in *C. muelleri*. Both the microalgal species were sources of essential amino acids -141.86 mg/g dry weigh in *N. oceanica* and 82.62 mg/g dry weigh in *C. muelleri* and proved as a good source for aquaculture nutrition. The detailed understanding of the biochemical composition of microalgae enables the best selection of microalgal species based on the specific nutritional need of cultured organism and also pave the way for further improvement of nutritional composition.

Keywords: Microalgae, *Chaetoceros muelleri*, *Nannochloropsis oceanica*, Nutritional composition, Live feed

1. Introduction

Aquaculture is a fast-growing sector of food industry (Maisashvili *et al*., 2015) with substantially increasing annual production over the past few decades, and the current trends point towards the need for increased aquaculture production to meet the future demand (Rodriguez *et al.*, 2013). Reliable microalgal production is an essential part of successful aquaculture production. Microalgae play a vital role in aquaculture as the primary food source for a large number of aquatic organisms (Vu *et al.*, 2016). Microalgae are fed to the cultured organisms either directly or indirectly depending on the species in culture, directly used as live feed for the larvae of crustaceans, fishes and molluscs or indirectly as feed for zooplankton such as artemia, rotifers, daphnia and copepods, which are in turn used as food to the target larval organisms (Sirakov *et al.*, 2015). So far, a large number of microalgal species have been screened for live feed application in aquaculture, but less than twenty gained potential application in aquaculture (Brown, 2002). To be used in aquaculture as food, microalgae should possess a number of essential characteristics, such as ease of cultivation, nontoxic, high nutritional value with appropriate cell size for injection, and an easily digestible cell wall to make nutrients accessible (Hemaiswarya *et al*., 2011). The use of microalgae as live feed in aquaculture has many beneficial effects to the cultured organisms such as, increased growth, disease resistance, physiological activity, protein and triglyceride content, fish digestibility, carcass quality, starvation tolerance and low nitrogen output to the environment (Becker, 2004; Fleurence *et al*., 2012).

The common genera of microalgae used as larval feed includes *Chaetoceros, Tetraselmis, Thalassiosira, Nannochloropsis* and *Isochrysis.* Mixed diet composed of different microalgal species provides better nutrition and better fish growth compared to unialgal diet (Spolaore *et al*., 2006). The nutritional value of microalgae varies significantly with the microalgal species and the culture conditions, which is determined by several factors such as microalgal shape, size, biochemical composition, cell wall structure and composition related to the digestibility and specific requirements of the cultured organism (Guedes and Malcata, 2012). Nutritional quality of microalgae mainly depends on the amino acid composition and the vitamin contents, in addition a microalgal diet must also contain high amount of polyunsaturated fatty acids, such as docosahexaenoic acid (DHA), arachidonic acid (AA) and eicosapentaenoic acid (EPA) to ensure good larval growth, survival and disease resistance (Morais *et al*., 2005). The contents of antioxidants, amino acid composition, sterols (Knauer *et al*., 1999), minerals (Fabregas and Herrero, 1986) and pigments of microalgae contribute to their nutritional quality in aquaculture.

The application of microalgae in aquaculture is rapidly increasing, depending on the nutritional requirements, several strains of microalgae are normally used in aquaculture. The evaluation of biochemical composition of microalgae, especially amino acid and fatty acid profiles enables the effectual screening of potent microalgal strains for cultured organisms. Apart from the strain, the quality and quantity of microalgal biochemical composition is greatly influenced by the environmental conditions (Renaud *et al*., 1994; Salama *et al*., 2013). Moreover, the

Received on: 25-01-2021; Revised on: 25-07-2021; Accepted on: 27-07-2021

potential strains should be easy to cultivate in the local culture conditions and passive for simple extraction of the biochemical compounds. Among the different microalgal species, *Nannochloropsis* spp. and *Chaetoceros* spp. are widely used in marine aquaculture as food sources as well as to maintain water quality (Riquelme and Herrera, 2003; Khatoon *et al*., 2007; Banerjee *et al*., 2011). There are limited works focused on the complete evaluation of biochemical composition of microalgae. A detailed understanding of the morphological and nutritional composition of microalgae enables the efficient and appropriate utilization of it as live feed either as single species or in combination according to the nutritional requirement of the target organism, it also pave the way for improvement of nutritional composition by optimization of culture medium and conditions or by genetic manipulation. Therefore, the present study was carried out for the detailed evaluation of biochemical composition and morphological characteristics of *C. muelleri* and *N. oceanica* used as live feeds in aquaculture.

2. Materials and Methods

2.1. Microalgal strains and culture conditions

The stock culture of marine diatom *Chaetoceros* sp. and marine eustigmatophyte *Nannochloropsis* sp. were obtained from the culture collection of Central Marine Fisheries Research Institute (CMFRI), Kochi, India. The cultures were identified as *Chaetoceros muelleri* (Gen Bank Accession No. KM202105) and *Nannochloropsis oceanica* (Gen Bank Accession No. KP057242) and the pure stock cultures of *C. muelleri* and *N. oceanica* were maintained in Erlenmeyer flasks using F/2 (Guillard, 1975) and Walne's (Walne, 1970) medium respectively (Table 1), prepared in sterile natural sea water. For experimental purpose, large scale cultivation was done in 10L Nalgene carboys in triplicate. The growth medium pH was adjusted to 8.00 and incubated at 25 ±2 °C for a photoperiod of 16L: 8D hours at a light intensity of 100 µmol photons/m^2/s. The cultures were agitated continuously by sparging with sterile air passed through 0.2µm air filters (Midisart, Sartorius, Germany) to prevent sticking as well to ensure proper exposure to light. Nutritional analyses were performed after the cells reached the late logarithmic phase of growth (12 days for *C. muelleri* and 15 days for *N. oceanica*).

2.2. Cell morphology characterization

2.2.1. Light microscopy

The cell size and morphological characteristics of *C.muelleri* and *N.oceanica* were observed under light microscope (Olympus CX41, Japan) and digital images were taken with micro publisher 3.3 RTV camera and images were analyzed by Q-imaging software. A measuring ocular calibrated to the different magnifications (10x, 40x and 100x magnitude oculars) was used to calculate cell sizes. At least 100 microalgal cells of each species were measured at stationary period. The cell surface area (A,) and cell volume of marine diatom *Chaetoceros muelleri* was calculated assuming an oval cylindrical cell shape using the following formulas.

Cell surface area (A, μm^2) = $2\pi rh+2\pi r^2$ (1)

Cell volume (V) = $\pi r^2 h$ (2)

where, A= cell surface area (μm^2); V= cell volume; π=3.14; r= average cell radius; h= average cell height.

For the spheroidal cells of *Nannochloropsis oceanica* the cell surface area and cell volume were calculated using the following formulas.

Cell surface area (A) = $4\pi r^2$ (3)

Cell Volume (V) = $4/3\pi r^3$ (4)

2.2.2 Scanning electron microscopy (SEM)

For the SEM analysis, 10 mL of microalgal cultures of both the species were harvested by centrifugation at 5000 rpm for 10 minutes and washed thrice in 1X PBS and pH was adjusted to 7.4. The cells were centrifuged at 5000 rpm for 10 minutes and the supernatant was decanted, to which 1 mL of 2.5% glutaraldehyde EM grade (Electron Microscopy Sciences, PA, USA) was added and incubated at 4°C for overnight. After incubation, the cells were washed thrice in 1XPBS and again incubated at 4°C for 4 hours with 1 mL of 2% osmium tetroxide (OsO_4) (Electron Microscopy Sciences, PA, USA). After incubation, the cells were harvested and washed thrice in 1X PBS. The obtained cells were dehydrated in a series of 10%, 25%, 50%, 75% and 100% acetone and dried in air (Grant, 2008). The cells were spread on SEM stubs dried in critical point drying apparatus, sputter coated with gold and viewed with analytical Scanning Electron Microscope (VEGA3 TESCAN) at Maharajas College, Kochi, India.

2.3. Nutritional characterization

2.3.1. Moisture content

The moisture content of the dried algal biomass was measured by drying a representative 2 g sample at 100±5°C for 20 hrs. or till constant weight was attained and the difference in the weights was calculated. The moisture content (%) was calculated using the following formula (Tokucsoglu and Uunal, 2003).

$$\text{Moisture content (\%)} = \frac{\text{weight before drying} - \text{weight after drying}}{\text{weight before drying}} \times 100 \quad (5)$$

2.3.2. Ash content

The ash content was determined by incineration of a representative 1 g of algal sample at 450°C for 5 hrs. in a pre-weighed silica crucible. The residue in the crucible was weighed and the difference in weights was calculated as the ash content (AOAC,1980; Zhu and Lee, 1997).

2.3.3. Estimation of total protein, carbohydrate and lipid

For the analysis of total lipid, protein and carbohydrate contents of *C.muelleri* and *N.oceanica*, 30L cultures of each species were harvested at the late logarithmic phase of growth. For the estimation of total lipid and carbohydrate, 50 mg of lyophilized biomass of *C.muelleri* and *N.oceanica* were first treated with 0.2N perchloric acid ($HClO_4$) and ground in chloroform: methanol (2:1 v/v) with glass powder to obtain lipid component (Lee et al., 1989). The lipids were extracted and quantified following the modified method of Bligh and Dyer (Bligh and Dyer, 1959), the lipid free pellets were treated with 2 mL of 2N sodium hydroxide (NaOH) at 95°C for 10 min,

and from this, aliquots (500 µL) were drawn and carbohydrates were estimated using the procedure described by Dubois *et al*. (1956) and the results obtained were expressed as % dry weight. For the estimation of protein, 50 mg of lyophilized biomass of *C.muelleri* and *N.oceanica* were treated with 5 mL of 10% trichloroacetic acid (TCA), vortexed for 30s and incubated for 30 minutes in a boiling water bath, cooled in an ice bath for 30 minutes and centrifuged at 5000xg for 5min. The pellets were dissolved in 1mL of 1N NaOH by boiling in a water bath for 30 minutes. An aliquot of 0.1mL was taken and made up to 1mL with distilled water and the protein concentration was measured using the procedure of Lowry *et al*. (1951).

2.3.4. Estimation of pigments

2.3.4.1. Chlorophyll

For the estimation of chlorophyll, 10 mg microalgal biomass of *C.muelleri* and *N.oceanica* were extracted with 4 mL of 80% methanol and absorbances were read at 663 nm in a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) against methanol blank. The amount of chlorophyll was calculated using the following equation (Mackinney,1941) and expressed as µg chlorophyll-a in gram dry weight.

Amount of Chl. a = Ab_{663} x 12.63 (correction factor) (6)

2.3.4.2. Carotenoids

Carotenoids include pigments like carotenes and xanthophylls. For the estimation of carotenes,10 mg microalgal biomass was extracted with 3 mL of 85% acetone and the absorbances were read at 450 nm in a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) against acetone blank. The concentration of carotenoids was calculated using the following equation (Jensen,1978) and expressed as µg in gram dry weight.

Concentration of carotenoids = Ab_{450}/2500 (extinction coefficient) (7)

2.3.4.3. Phycobiliproteins

For the estimation of phycobiliproteins,10 mg microalgal biomass was extracted with 3 mL of phosphate buffer (0.05M, pH 6.8) by repeated freeze-thawing and the absorbances were taken at 565, 615 and 652 nm respectively against phosphate blank and concentration of phycobilins were calculated using the following formula and expressed as µg in gram dry weight (Siegelman and Kycia, 1978).

Concentration of c-phycobilins (PB) =

$$Ab_{615}\text{-}0.474\ (Ab_{652})/5.34 \quad (8)$$

Concentration of c-allophycocyanin (APC) =

$$Ab_{652}\text{-}0.208\ (Ab_{615})/5.09 \quad (9)$$

Concentration of c-phycoerythrin (PE) =

$$\frac{Ab_{562}\text{-}2.41(PC) - 0.849\ (APC)}{9.6} \quad (10)$$

2.3.5. Analysis of fatty acids

Total lipid was extracted from 100 mg biomass using chloroform and methanol (2:1 v/v) and it was converted into fatty acid using 1% v/v H2SO4 in methanol and refluxing it for an hour at 70 °C methyl esters (FAME) by trans- esterification and were easily separated and analysed using gas chromatography with mass spectrometry (GC-MS) (Feng *et al*., 2011). Gas chromatographic analysis of FAME was performed on an Agilent 7890 A GC equipped with a mass detector (Agilent 5975 C mass spectrometer) and a fused silica capillary column (DB - 5MS column: ID 0.25mm; length 30 m; film thickness 0.25 mm). Samples were injected in split mode (split ratio 50:1). Helium was used as a carrier gas. The injector and detector temperatures were 250°C and 230°C respectively. The temperature was programmed as follows, initially at 40°C for 5 min; then 5°C/min to 280°C, hold for 10 min. The FAME was identified by co-chromatography with authentic commercially available FAME standards (Supelco™ 37 Component FAME Mix, Catalog No.: 47885-U, Supelco, Bellefonte, PA, USA). The fatty acids concentrations in the algal samples were quantified by comparing their peak area with that of the internal standard (C17:0).

Table 1. Nutrient composition of different marine microalgal culture media used in the study

Constituents	F/2 (mg/L)	Walne's (mg/L)
$NaNO_3$	75	100
$NaH_2PO_4\ H_2O$	5	20
H_3BO_3	-	33.6
$CoCl_2 \cdot 6H_2O$	0.01	20
$CuSO_4\ 5H_2O$	0.0098	20
$MnCl_2\ 4H_2O$	0.18	0.36
$Na_2MoO_4 \cdot 2H_2O$	0.0063	-
$ZnSO_4 \cdot 7H_2O$	0.022	-
$FeCl_3 \cdot 6H_2O$	3.15	1.3
$Na_2EDTA \cdot 2H_2O$	4.36	45
$ZnCl_2$	-	21
$(NH_4)6Mo_7O_{24}.4H_2O$	-	9
Thiamine HCl	0.1	0.1
Cyanocobalamin	0.0005	0.005
Biotin	0.0005	-

2.3.6. Analyses of minerals

The analyses of minerals were carried out using inductively coupled plasma mass spectrometry (ICP-MS). The dried microalgal biomass (0.5g) was treated with 5 mL of concentrated HNO_3; 0.5 mL concentrated HCl and 1mL H_2O_2 and allowed to stand for 15 min for self-digestion, then kept for digestion in the microwave digestion system (MDS). After digestion, the contents were quantitatively transferred in to 10 mL tube and made up to 50 mL using extra pure water and filtered through Whatman No 1 filter paper. Metal analyses (Fe, K, Mg, Mn, Ca, Co, Cu and Zn) were carried out using Agilent 7700 ICP-MS. Working standards were prepared from stock solution (1ppm) by appropriate dilution so that all the standards were within the linear range of the element of interest. Mixed standards of all elements of interest were prepared at concentrations of 0.25 µg/L, 0.5 µg/L, 5 µg/L, 50 µg/L, 100 µg/L, 200 µg/L and 250 µg/L and analyzed using ICP-MS. The concentrations of metals were obtained from 3 different analyses and each was analysed in triplicate using ICP-MS (n=3) (Tokucsoglu and Uunal, 2003).

2.3.7. Analyses of amino acids

For the estimation of total amino acids, 100 mg dried

biomass was weighed into a glass vial, 10 mL of 6N HCl was added and the tubes were heat sealed after filling pure nitrogen gas. Hydrolysis was carried out in a hot air oven at 110°C for 24 hours. After the hydrolysis, the content was removed quantitatively and filtered, washed with distilled water and the HCl content in the extract was removed using rotary vacuum evaporator. The residue was made up to a definite volume with 0.05N HCl. The sample thus prepared was filtered again through a membrane filter of 0.45-micron size (Jayasree *et al*., 2012). For the detection and quantification of the extracted amino acids, pre-column derivatization with O-phthalaldehyde was carried out (Rodríguez *et al*., 1997) and the content was filtered through 0.22-micron syringe filter and 20 µL of sample was injected into the HPLC for analysis.

The sample was injected to Agilent 1260 Infinity Binary LC in superficially porous Agilent Poroshell HPH-C18 columns having a length of 100 mm and 4.6 mm internal diameter with a flow rate of 1.5 mL/minute. The oven temperature was maintained at 40°C. The chromatographic conditions were as follows. The solvent system consisted of two eluents. Solvent A, an aqueous buffer, was a solution of 10 mM Na_2HPO_4, 10 mM $Na_2B_4O_7$, and 5 mM NaN_3. The pH was adjusted to the desired value (8.2) using concentrated hydrochloric acid, and the solution was filtered through a 0.45 µm cellulose membrane filter. Solvent B consisted of acetonitrile: methanol: water (45:45:10). Aqueous solutions containing acetonitrile and methanol are the most common solvents used in the amino acid analysis in RP-HPLC (Bidlingmeyer *et al*., 1986; Hariharan *et al*., 1993). Ultraviolet spectrophotometric detection was carried out at 254 nm. Amino acid standard (Sigma Aldrich, USA) was also run by the same procedure and the identification of individual amino acids in the sample was achieved by comparison of retention times with those of standards.

3. Results and Discussion

3.1. Morphological characteristics

Light microscopic and scanning electron microscopic images of *C. muelleri* and *N. oceanica* are given in Fig. 1 and Fig. 2 respectively. The marine diatom *C. muelleri* had an oval cylindrical shape with orange-brown colour as shown in Fig. 1, each cell had four hair like projections one on each corner of the cell called setae. *N. oceanica* consisted of single spheroidal cells that were grass-green in colour mainly due to the presence of chlorophyll. The main characteristics of microalgae for aquaculture feed application is appropriate cell size for ingestion, for example, from 1 to 15µm for filter feeders and 10 to 100 µm for grazers and they must be readily digested (Brown, 2002). Cell sizes and cell volumes characteristics of *C. muelleri* and *N. oceanica* are given in Table 2. Cells of *C .muelleri* had an average length of 7.072±1.63 µm and 4.211±0.869 µm width with 122.05±33.08 μm^2 cell surface area and 46.51±12.06 μm^3 cell volume. Cells of *N. oceanica* had an average cell size of 2.87±0.48 µm with 26.60±8.06 μm^2 cell surface area and 13.35±5.9 μm^3 cell volume. The cell surface area to volume ratio of *C. muelleri* and *N. oceanica* were almost similar, 2.62±0.22/µm and 2.23±1.17/µm respectively. Microalgae used for larval nutrition are usually in the nanoplankton size range (2-20 µm) (Brown et al., 1997). Both *C. muelleri* (7.072±1.63µm) and *N. oceanica* (2.87±0.48µm) were coming under the suitable microalgal size range for aquaculture feed applications.

3.2. Nutritional composition

The proximate composition of *C.muelleri* and *N.oceanica* in terms of protein, lipid, carbohydrate, moisture and ash contents are presented in Table 3. Different classes of microalgae have different concentrations of protein, carbohydrate and lipid (Brown *et al*., 1997). Generally, microalgae contain 30-40% (w/w) protein, 5-15% (w/w) carbohydrates and 10-20% (w/w) lipids in the late-logarithmic phase of growth (Guedes and Malcata, 2012). In the present study, in both the species, protein was the major compound (36.51±2.85 % in *C. muelleri* and 49.31±6.88% in *N. oceanica*), followed by lipid (27.93±2.1% in *C. muelleri* and 31.33±1.15% in *N. oceanica*) and carbohydrate (3.16 ±0.23 in *C.muelleri* and 11.49±0.75 in *N.oceanica*). For aquaculture organisms, the optimum total protein requirements in microalgal diet varies from species to species, but may range from as low as 12% (Albentosa et al., 1996) to within the optimum range of 30-60% (Brown *et al*., 1989), the differences in

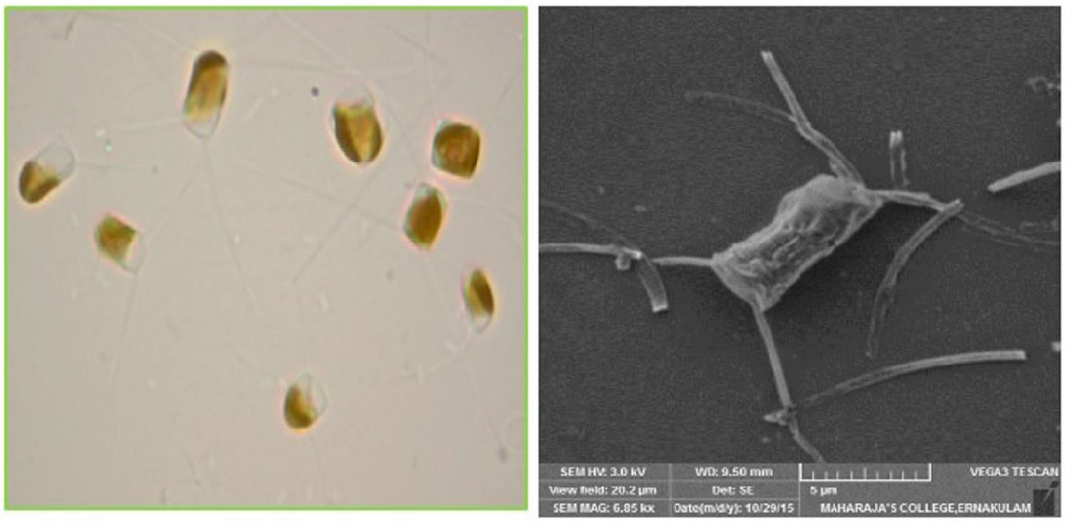

Fig. 1. Light microscopic and scanning electron microscopic (SEM) images of *Chaetoceros muelleri*

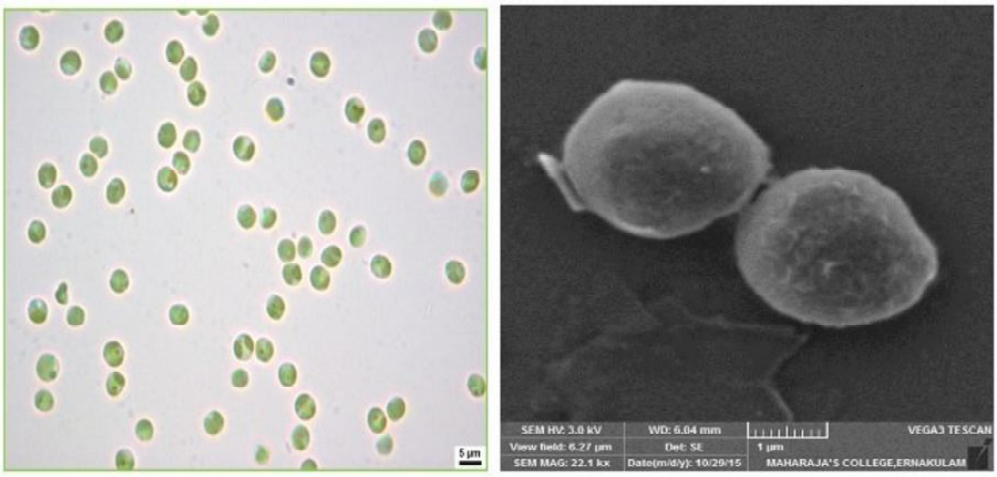

Fig. 2. Light microscopic and scanning electron microscopic (SEM) images of *Nannochloropsis oceanica*

protein concentrations can be due to the variations in culture conditions such as the level of illumination, availability of nutrients, and the growth phase of the algae (Lopez *et al*., 2010; Hempel *et al*., 2012). The pretreatment methods and subsequent measurement protocol can influence the protein contents in different microalgal strains, this may cause slight variation in protein contents among different microalgal strains (Lopez *et al*., 2010). Relatively higher concentrations of protein, lipid and carbohydrate were estimated in *N. oceanica* than *C. muelleri*.

The total lipid contents are relatively high for most microalgae ranging from 20-50% (Sun *et al*., 2018). The crude lipid includes all substances soluble in lipophilic organic solvents and is highly dependent upon the polarity and specificity. During the extraction of lipid from microalgae, the organic solvents used in the extraction may co-extract chlorophyll and other pigments to varying degrees depending on the solvents used and extraction protocols employed, this is a major challenge for the determination of reliable lipid content data in microalgae (Palmquist and Jenkins, 2003). Lipid content of various freshwater and marine microalgae is typically in the 1–38 % range [43], here the lipid content in *C. muelleri* was 27.93±2.1% and of *N. oceanica* was 31.33±1.15%.

Carbohydrates has an important role in the digestibility of the total algal biomass. The major form of carbohydrate in microalgae are starch, cellulose, sugars and other polysaccharides. The carbohydrate content in *C. muelleri* was 3.16 ±0.23 % and of *N. oceanica* was 11.49±0.75. Typically, 4-64% of carbohydrate is reported in microalgae (Becker, 2013; Volkman and Brown, 2006; Demirbas and Demirbas, 2011). Carbohydrate content of algae is highly variable not only depending upon species, growth phase, and culture conditions, but also due to differing analytical methods and the terms in which carbohydrate content is expressed. The differences in carbohydrate estimation and reporting methods used in the literature make it difficult to compare studies between and within species and under the same culture conditions.

The ash content was very low in *N.oceanica* (<10%). Generally, the ash content of marine microalgae is in the range of 4-20% with exception for silica rich diatoms, which may contain up to 43% ash (Volkman and Brown, 2006; Demirbas and Demirbas, 2011; Fuentes *et al*., 2001). In *C. muelleri*, the ash content was relatively moderate (10.51%). Moisture content was very high in *C. muelleri*, with a mean value of 38.67±1.28%. Generally, the moisture content <10% is recommended for microalgal quality (Fuentes et al., 2001). Here in *N. oeanica*, the moisture content was 10.96±1.05%. Ash, carbohydrate, lipid and protein accounted for an average 78.11% in *C. muelleri* and 94.57% in *N. oceanica*.

The major pigment concentrations of *C. muelleri* and *N. oceanica* are given in Table 4. Microalgae contain pigments like, chlorophylls, carotenoids and phycobiliproteins, most of them are important in aquaculture (Roy and Pal 2015). In the dry algal biomass, about 3-5% is constituted by lipid soluble pigments such as chlorophylls and carotenoids (Venkataraman and Becker, 1985). In the present study, chlorophyll-a is the dominant pigment group for both the microalgal species (2955±0.311µg/g dry weight in *C. muelleri* and 3010±0.681 µg/g dry weight in *N. oceanica*). Five different type of chlorophylls are mainly present such as, a, b, c, d and e and the amount usually in the range of 0.5-1.5 % of dry weight (Becker,1994). Carotenoid concentration was at 0.1036±0.005311µg/g dry weight in *C. muelleri* and 0.1073±0.0003 µg/g dry weight in *N. oceanica*. The nutritional and therapeutic value of certain carotenoids is

Table 1. The morphological characteristics of *Chaetoceros muelleri* and *Nannochloropsis oceanica* (n=100)

Species	Cell size (µm)	Average cell surface area (µm^2)	Average cell volume (µm^3)	Cell surface area volume ratio (SA/V) (µ/m)
C. muelleri	7.072±1.63(length) 4.211±0.869 (width)	122.05±33.08	46.51±12.06	2.62±0.22
N. oceanica	2.87±0.48 (diameter)	26.60±8.06	13.35±5.9	2.23±1.17

Table 3. Biochemical composition of *Chaetoceros muelleri* and *Nannochloropsis oceanica* (n=3)

Species	*C. muelleri*	*N. oceanica*
Total protein (%)	36.51±2.85	49.31±6.88
Total carbohydrate (%)	3.16 ±0.23	11.49±0.75
Total lipid (%)	27.93±2.1	31.33±1.15
Ash (%)	10.51	2.44
Moisture (%)	38.67±1.28	10.96±1.05

Table 4. Pigments (µg/g dry weight) in *Chaetoceros muelleri* and *Nannochloropsis oceanica* (n=3)

Species	*C.muelleri*	*N.oceanica*
Chlorophyll-a	2955±0.311	3010±0.681
Carotenoids	0.1036±0.005	0.1073±0.0003
Phycobilins	0.1932±0.0012	0.5175±0.027
Allophycocyanin	0.5644±0.017	0.6923±0.015
Phycoerythrin	0.1732±0.008	0.3997±0.033

due to their ability to act as provitamin A, that can be converted into vitamin A (Gouveia and Empis, 2003; Gonzalez *et al*., 2005). In addition, carotenoids have anti-inflammatory properties because of their quenching action on reactive oxygen species. The average carotenoid concentration of algae is 0.1-2 % of the dry weight though exceptions are there (Becker, 1994). C-phycocyanin, C-phycoerythrin and allophycocyanin are water soluble pigments coming under phycobiliproteins. *C. muelleri* has 0.1932±0.0012 µg C-phycobilins, 0.5644±0.017 µg C-allophycocyanin and 0.1732±0.008 µg C-phycoerythrin per gram dry weight. *N. oceanica* contained 0.5175±0.027 µg C-phycobilins, 0.6923±0.015 µg C- allophycocyanin and 0.3997±0.033 µg C-phycoerythrin per gram dry weight. The major commercial producers of phycobiliproteins (phycoerythrin and phycocyanin) are cyanobacterium *Arthrospira* and rhodophycean member *Porphyridium* (Viskari and Colyer, 2003; Roman *et al*., 2002).

Gas chromatography/mass spectroscopy (GC/MS) analysis results revealed the FAME profiles of *C. muelleri* and *N. oceanica* (Table 5). Microalgal fatty acid compositions were first studied in the 1940s (Millner,1948). In humans and animals, fatty acids are essential components of the diet and are important feed additives in aquaculture also (Borowitzka,1988). The major fatty acids identified in *C. muelleri* were lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, palmitoleic acid, eicosadienoic acid, linoleic acid, hexadecatrienoate, and docosahexaenoic acid. The fatty acid profile of *N. ocenica* consisted of lauric acid, myristic acid, palmitic acid, caprylic acid, capric acid, stearic acid, oleic acid, palmitoleic acid, eicosapentaenoic acid, arachidonic acid and linoleic acid. The major essential poly unsaturated fatty acids (PUFA) for larval nutrition of various finfish and shellfish includes eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (Brown, 2002). For crustaceans and marine fish larvae, EPA and DHA are the essential PUFA (Akiyama,1992), which is essential for the development of the nervous system and synthesis of prostaglandins and growth precursors (Harel *et al*., 2000). The major PUFAs present in *C. muelleri* were linoleic acid, decosahexaenoic acid, hexadecatrienoate and eicosadienoic acid, altogether consisted of 30.39%, total monounsaturated fatty acid (MUFA) was 30.19% and total saturated fatty acids (SFA) was 39.42%. The major PUFAs present in *N. ocenica* were eicosapentaenoic acid, linoleic acid, and arachidonic acid, and consisted of 33.36%, total monounsaturated fatty acid (MUFA) was 32.33% and total saturated fatty acids (SFA) was 33.95%. The fatty acid contents of microalgae vary with taxonomic group and it may vary even within the same class. The EPA content of most microalgal species is in the range of 7 to 34%, the DHA content is relatively high in prymnesiophytes and cryptomonads (0.2 to 11%) and relatively high amount of AA (up to 4%) is present in eustigmatophytes and diatoms. The chlorophytes have generally poor nutritional quality with small amounts of EPA (up to 3.2%) in some species and are deficient of C20 and C22 PUFAs, so generally chlorophytes are not efficient as single species-diet (Brown *et al*., 1997). The fatty acid profile of *C. muelleri* and *N. oceanica* revealed that they are important PUFA source for aquaculture organisms.

Table 5. Fatty acid profile (% of total FAME) of *Chaetoceros muelleri* and *Nannochloropsis oceanica*

Fatty acids	*C.muelleri*	*N.oceanica*
Lauric acid	2.51	0.86
Myristic Acid	18.79	6.37
Palmitic Acid	11.58	24.22
Caprylic Acid	-	0.21
Capric Acid	-	0.24
Stearic Acid	6.54	2.05
Total SFA	39.42	33.95
Oleic Acid	21.63	14.15
Palmitoleic Acid	8.56	18.18
Total MUFA	30.19	32.33
11,14 Eicosadienoic Acid	3	-
5,8,11,14,17-Eicosapentaenoic Acid	-	20.59
Arachidonic Acid	-	4.22
Linoleic Acid	16.82	8.55
6,9,12 Hexadecatrienoate	4.52	-
4,7,10,13, 16,19-decosahexaenoic Acid	6.05	-
Total PUFA	30.39	33.36

The elemental composition of *C. muelleri* and *N. oceanica* are given in Table 6. The major minerals present in *C. muelleri* were potassium (2793 µg**),** iron (335.42 µg),

Table 6. Elemental composition of *Chaetoceros muelleri* and *Nannochloropsis oceanica*

Minerals	*C. muelleri* (µg/g dry weight)	*N. oceanica* (µg/g dry weight)
Iron	335.42	244.72
Potassium	2793	3731
Copper	6.11	1.72
Magnesium	1.36	1.01
Manganese	45.89	73.32
Zinc	15.77	37.14
Cobalt	-	-
Calcium	294.9	73.73

Table 7. Amino acid profiles of *Chaetoceros muelleri* and *Nannochloropsis oceanica*

Amino acids	*C. muelleri* (mg/g dry weight)	*N. oceanica* (mg/g dry weight)
Histidine	5.824577	7.519046
Threonine	10.04286	15.33741
Valine	11.65174	19.04923
Methionine	7.985685	7.331325
Phenylalanine	13.3722	19.07554
Isoleucine	12.7338	17.23601
Leucine	18.57429	33.90156
Lysine	2.429694	22.41032
Total EAA	82.614846	141.86044
Proline	22.67854	7.372127
Glycine	0.309621	14.15517
Aspartic acid	24.36203	27.7096
Glutamic acid	22.61801	43.78388
Serine	14.07362	12.53144
Arginine	13.00335	17.65821
Alanine	15.33466	24.7357
Tyrosine	7.599629	11.25145
Cysteine	1.323293	1.634122
Total NEAA	121.302753	160.8317
Total	203.176	302.6921

calcium (294.9 µg), manganese (45.89 µg), zinc (15.77 µg), copper (6.11 µg) and magnesium (1.36 µg) in gram dry weight of microalgal biomass and the composition of major minerals in *N. oceanica* were potassium (3731µg**),** iron (244.72 µg), calcium (73.73µg), manganese (73.32µg), zinc (37.14µg), copper (1.72µg) and magnesium (1.01 µg) in gram dry weight of microalgal biomass. Minerals such as calcium, phosphorus, magnesium, potassium, copper, iron, zinc, manganese, selenium, and iodine have been reported as essential in the diet of fish. The recommended minerals in the diet of lobster and penaeid shrimp are calcium, copper, magnesium, phosphorus, potassium, selenium, and zinc (Davis and Gatlin, 1996). In marine organisms, minerals are the structural components of exoskeleton and endoskeleton, and required for the synthesis of proteins, phospholipids and nucleic acids. Minerals are the important component of metalloprotein such as copper in hemocyanin; iron in hemoglobin and zinc in carboxypeptidase and act as cofactors/activators of different enzymes. Minerals have important function in acid base balance, osmoregulation and in the production of membrane lipids in marine organisms (Davis and Gatlin, 1996). Potassium was the major element in *C.muelleri* and *N.oceanica*. Potassium is taken up in to the microalgal cells by both passively and actively (Markou et al., 2014), microalgal potassium content varies from 1.2% to 1.5% (Tokucsoglu and Uunal, 2003), and in some microalgae up to 7.5% (Grobbelaar, 2004). Cobalt was found to be below detectable level in both the species. Cobalt is an essential microelements, low concentration of cobalt is optimum for microalgal growth and pigment content, at high concentration it has an inhibitory effect (Sheekh *et al*., 2003). Calcium and iron contents were high in *C.muelleri* when compared to *N.oceanica*. Calcium is an important element for microalgal growth, an important cell wall constituent which act as second messenger and affect cell division (Sheekh *et al*., 2003). Calcium is taken up by the cells actively and passively and the content varies from 0.2 to 1.4% ((Tokucsoglu and Uunal, 2003). Iron is an essential microelement, involved in nitrogen assimilation, electron transfer, oxygen metabolism, DNA, RNA and chlorophyll synthesis (Straus, 2004; Naito *et al*., 2005). The elemental composition of numerous macroalgal (seaweeds) species are well characterised when compared to microalgal species, because of the limited available data, the major efforts toward commercialization of microalgae have generally focused on its organic constituents (e.g., essential PUFA, high-value pigments, and energy feedstock) than their inorganic elemental content (Tibbetts *et al*., 2015). This is not entirely unexpected because, the inorganic elemental composition of macroalgae is generally higher (22–64 %) when compared to microalgae (4-20 %) (McDermid and Stuercke, 2003; Volkman and Brown, 2006).

The amino acid composition of *C. muelleri* and *N. oceanica* are given in Table 7. *N. oceanica* contained higher levels (mg/g dry weight of biomass) of most essential amino acids (EAA) (82.61 mg in *C. muelleri* and 141.86 mg in *N. oceanica*) and non-essential amino acids (NEAA) (121.30 mg in *C. muelleri* and 160.83 mg in *N. oceanica*) than *C. muelleri*. The composition of major essential amino acids (EAA) in *C. muelleri* were histidine (5.82 mg), threonine (10.04 mg), valine (11.65 mg), methionine (7.98 mg), phenylalanine (13.37), isoleucine (12.73 mg), leucine (18.57 mg) and lysine (2.43 mg). The composition of major essential amino acids (EAA) in *N. oceanica* were histidine (7.52 mg), threonine (15.34 mg), valine (19.05 mg), methionine (7.33 mg), phenylalanine (19.07 mg), isoleucine (17.24 mg), leucine (33.90 mg), lysine (22.41 mg). The amino acid pattern of almost all algae compared favorably with that of other food proteins, with minor deficiencies in sulfur containing amino acids methionine and cysteine (Becker, 2004). The nutritional value of protein is considered to be high if the composition of essential amino acid is close to that of feeding animal. For instance, the EAAs lysine, methionine, and tryptophan are typically limiting and often deficient in plant-based protein meals commonly used in animal nutrition (e.g., soybean, canola, sunflower, and flax). Reported levels (% of DW) for these products are 1.2–2.2 % (lysine), 0.6–1.5 % (methionine), and 0.4-0.7 % (tryptophan) (National Research Council, 2011), while the algal biomass of *C. muelleri* contained lysine at 0.24 % and methionine 0.79 % and *N. oceanica* contained 2.2% lysine and 0.73 % methionine. The algal biomass could be desirable as a supplement in animal feeds and aquaculture feeds to provide EAAs and reduce the costs associated with crystalline amino acid supplementation.

4. Conclusion

To develop microalgae-based food products, a deep understanding of the nutritional composition and digestibility of microalgal species is essential. The nutritional composition of microalgal cells is greatly influenced by various factors such as species of microalgae, culture conditions, growth medium composition and growth phase. For the complete and appropriate exploitation of microalgae in the aquaculture feed sector,

a detailed evaluation of the nutritional composition, size and toxic properties of microalgal species available in the culture collections and isolation and characterisation of potential species from nature is required. The present study provides detailed information on the proximate composition, the fatty acid and amino acid profiles, mineral contents and the morphological characteristics of two marine microalgae *C. muelleri* and *N. oceanica* that could be exploited for the appropriate use of these microalgae in aquaculture as single species live feed or in combination. For the complete evaluation of nutritional quality of microalgae, vitamins composition, bioactivity and bioavailability of compounds also should be taken into consideration and by optimizing the culture conditions and growth medium composition, it is possible to significantly improve the digestibility and nutritional quality of microalgal biomass. Availability of microalgal biomass with improved nutritional composition produce more dependable aquafeed and this can ensure the sustainable growing of aquaculture industry to meet the current and future demands.

Acknowledgements

This study was funded by Kerala Biotechnology Commission, Kerala State Council for Science Technology and Environment (KSCSTE), Government of Kerala, India and M/s Oriental Aquamarine Biotech. India (Pvt.) Ltd, Coimbatore, India, under the Industry Linked Biotechnology Research Scheme (File No. 17 /IBRS / KBC/2009/KSCSTE). The first author is grateful to University Grants Commission (UGC), Government of India, for providing BSR fellowship and to Dr. K. Asok Kumar, Central Institute of Fisheries Technology, Kochi, India, for his support in nutritional analysis.

5. References

Akiyama, D. 1992. Future considerations for shrimp nutrition and the aquaculture feed industry. Special session on shrimp farming. The World Aquaculture Society. Meetings. Baton Rouge, Louisiana, USA, pp 198–211

Albentosa, M., Labarta, U., Reiriz, M.J.F. & Camacho, A.P. 1996. Fatty acid composition of *Ruditapes decussatus* spat fed on different microalgal diets. Comparative Biochemistry and Physiology, 133 A: 113-119

AOAC (Association of Official Analytical Chemists). 1980. *Official Methods of Analysis*, 13th ed. Association of Official Agricultural Chemists. Washington, DC

Banerjee, S., Hew, W.E., Khatoon, H., Shariff, M. & Yusoff, F.M. 2011. Growth and proximate composition of tropical marine *Chaetoceros calcitrans* and *Nannochloropsis occulata* cultured outdoors and under laboratory conditions. African Journal of Biotechnology, 10 (8): 1375-1383;

Becker, E.W. 1994. Microalgae: Biotechnology and Microbiology. Cambridge University Press, Cambridge, pp 1-291

Becker, E.W. 2013. Microalgae for human and animal nutrition. In: Richmond, A., Hu, Q. (eds) *Handbook of microalgal culture: applied phycology and biotechnology*, 2nd edn, Wiley-Blackwell, Oxford. pp 461–503

Becker, W. 2004. Microalgae for Aquaculture: The Nutritional Value of Microalgae for Aquaculture. In: Richmond, A. (ed) Handbook of Microalgal Culture: Biotechnology and Applied Phycology, Oxford, pp. 380-391.

Bidlingmeyer, B.A., Cohen, S.A. & Tarvin, T.L. 1984. Rapid analysis of amino acids using pre-column derivatization. Journal of chromatography. B: Biomedical sciences and applications, 336: 93-104

Bligh, E.G. & Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37 (8): 911-917; https://doi.org/10.1139/o59-099

Borowitzka, M.A. 1988. Microalgae as sources of pharmaceuticals and other biologically active compounds. *Journal of Applied Phycology*, 4: 267–279

Brown, M.R. 2002. Nutritional value of microalgae for aquaculture. In: Suarez, L.E.C., Marie, D.R., Salazar, M.T., Cortes, M.G.G., Simoes, N. (eds) Avancesen Nutricion Acuicola VI. Memorias del VI Simposium Internacional de Nutricion Acuicola. 3 al 6 de Septiembre del. Cancun, Quintana Roo, Mexico.

Brown, M.R., Jeffrey, S.W. & Garland, C.D. 1989. Nutritional aspects of microalgae used in mariculture: a literature review. CSIRO Marine Laboratories Report No. 205, CSIRO Hobart, Australia.

Brown, M.R., Jeffrey, S.W., Volkman, J.K. & Dunstan, G.A. 1997. Nutritional properties of microalgae for mariculture. Aquaculture, 151: 315-331

Davis, D.A. & Gatlin, D.M. 1996. Dietary mineral requirements of fish and marine crustaceans. Revies in Fisheries Science, 4(1): 75-99

Demirbas, A. & Demirbas, M.F. 2011. Importance of algae oil as a source of biodiesel. Energy Conversion and Management, 52:163–170;doi: 10.1016/j.enconman.2010.06.055

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. 1956. Colorimetric method for the determination of sugars and related substances. Analytical Chemistry, 28: 350-356; https://doi.org/10.1021/ac60111a017

Fabregas, J. & Herrero, C. 1986. Marine microalgae as a potential source of minerals in fish diets. Aquaculture, 51: 237-243; doi:10.1016/0044-8486(86)90315-7

Feng, D.,Chen Z., Xue S. & Zhang W. 2011. Increased lipid production of the marine oleaginous microalgae Isochrysis zhangjiangensis (Chrysophyta) by nitrogen supplement. Bioresouce Technology, 102:6710-6716

Fleurence, J., Morançais, M., Dumay, J., Decottignies, P., Turpin, V., Munier, M., Bueno, N.G. & Jaouen, P. 2012. What are the prospects for using seaweed in human nutrition and for marine animals raised through aquaculture? Trends in Food Science & Technology, 27:57-61; https://doi.org/10.1016/j.tifs.2012.03.004

Fuentes, M.M.R., Perez, A.N., Camacho, F.G., Miras, J.J.R. & Guerrero, J.L.G. 2001. Biomass nutrient profiles of the microalga *Nannochloropsis*. Journal of Agricultural and Food Chemistry, 49: 2966-2972; https://doi.org/10.1021/jf0010376

Gonzalez, M.G., Moreno, J., Manzano, J.C., Florencio, F.J. & Guerrero, M.G. 2005. Production of *Dunaliella salina* biomass rich in 9-*cis*-â-carotene and lutein in a closed tubular photobioreactor. Journal of Biotechnology, 115: 81–90; https://doi.org/10.1016/j.j biotec.2004.07.010

Gouveia, L. & Empis, J. 2003. Relative stabilities of microalgal carotenoids in microalgal extracts, biomass and fish feed: effect of storage conditions. Innovative Food Science & Emerging Technologies, 4:227–233; https://doi.org/10.1016/S1466-8564(03)00002-X

Grant, E. 2008. *Hand book, phycological methods: developmental and cytological methods*. Cambridge university press, Cambridge, 425 pp.

Grobbelaar, J.U. 2004. Algal Nutrition – Mineral Nutrition. In: Richmond. A. (ed) *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, Blackwell Publishing Ltd, Oxford, pp 97–115

Guedes, A.C. & Malcata, F. 2012. Nutritional value and uses of microalgae in aquaculture. In: Muchlisin, Z.A. (ed) *Aquaculture*, pp. 59–78.

Guillard, R.R.L. 1975. Culturing of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (eds) Culture of marine invertebrate animals, Plenum Press, NY, pp 26-60.

Harel, M., Lund, E., Gavasso, S., Herbert, R. & Place, A.R. 2000. Modulation of arachidonate and decosahexaenoate in *Morone* sp. larval tissues and the effect on growth and survival. Lipids, 35 (11): 1269–1280; doi: 10.1007/s11745-000-0643-6

Hariharan, M., Naga, S. & Noord, T.V. 1993. Systematic approach to the development of plasma amino acid analysis by high-performance liquid chromatography with ultraviolet detection with precolumn derivatization using phenyl isothiocyanate. Journal of chromatography. B: Biomedical sciences and applications, 621:15-22

Hemaiswarya, S., Raja, R., Kumar, R.R., Ganesan, V. & Anbazhagan, C. 2011. Microalgae: a sustainable feed source for aquaculture. World Journal of Microbiology and Biotechnology, 27: 1737-1746; https://doi.org/10.1007/s11274-010-0632-z

Hempel, N., Petrick, I. & Behrendt, F. 2012. Biomass productivity and productivity of fatty acids and amino acids of microalgae strains as key characteristics of suitability for biodiesel production. Journal of Applied Phycology, 24: 1407-1418

Jayasree, N.B., Aneesh, T.P., Prabhakar, V. & Anandan, R. 2012. GC-MS, HPLC and AAS analysis of fatty acids, amino acids and minerals in red algae *Amphiroa anceps*. International Journal of Pharmacy and Pharmaceutical Sciences, 4:187-190

Jensen, A. 1978. Chlorophylls and carotenoids. In: Hellebust, J.A., Craige, S. (eds) *Handbook of phycological methods*. Cambridge University Press, Cambridge, pp 60-70.

Khatoon, H., Yusoff, F.M., Banerjee, S., Shariff, M. & Mohamed, S. 2007. Use of periphytic cyanobacterium and mixed diatoms coated substrate for improving water quality, survival and growth of *Penaeus monodon* Fabricius post larvae. Aquaculture, 271:196-205; https://doi.org/10.1016/j.aquaculture.2007.06.036

Knauer, J., Barrett, S.M., Volkman, J.K. & Southgate, P.C. 1999. Assimilation of dietary phytosterols by Pacific oyster *Crassostrea gigas* spat. Aquaculture Nutrition, 5: 257-266; doi: 10.1046/j.1365-2095.1999.00122.x

Lee, Y.K., Tan, H.M., Low, C.S. 1989. Effect of salinity of medium on cellular fatty acid composition of marine alga *Porphyridium cruentum* (Rhodophyceae). Journal of Applied Phycology 1(1): 19-23; https://doi.org/10.1007/BF00003531

Lopez, C.V., Garcia, M.C., Fernandez, F.G., Bustos, C.S., Chisti, Y. & Sevilla, J.M. 2010. Protein measurements of microalgal and cyanobacterial biomass. Bioresource Technology, 101: 7587-7591; https://doi.org/10.1016/j.biortech.2010.04.077

Lowry, O.H, Rosebrough, N.J., Farr, A. & Randall, R.J. 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry, 193: 265-275

Mackinney, G. 1941. Absorption of light by chlorophyll solution. Journal of Biological Chemistry, 140: 315-322

Maisashvili, A., Bryant, H., Richardson, J., Anderson, D., Wickersham, T. & Drewery, M. 2015. The values of whole algae and lipid extracted algae meal for aquaculture. Algal Research, 9:133-142; doi:10.1016/j.algal.2015.03.006

Markou, G., Vandamme, D. & Muylaert, K. 2014. Microalgal and cyanobacterial cultivation: The supply of nutrients. Water Research, 65: 186-202; https://doi.Org/10.1016/j.watres. 2014.07.025

McDermid, K.J. & Stuercke, B. 2003. Nutritional composition of edible Hawaiian seaweeds. Journal of Applied Phycology, 15: 513–524; https://doi.org/10.1023/B:JAPH.000000 4345.31686.7f

Millner, H.W. 1948. The fatty acids of *Chlorella*. Journal of Biochemistry, 176: 813–817

Morais, S., Koven, W., Ronnestad, I., Dinis, M.T. & Conceicao, L.E.C. 2005. Dietary protein: lipid ratio and lipid nature affect fatty acid absorption and metabolism in a teleost larva. British Journal of Nutrition, 93: 813–820; doi:10.1079/BJN20051378

Naito, K., Matsui, M. & Imai, I. 2005. Ability of marine eukaryotic red tide microalgae to utilize insoluble iron. Harmful Algae 4 (6): 1021-1032; https://doi.org/10.1016/j.hal. 2005.02.003

National Research Council. 2011. Nutrient requirements of fish and shrimp. National Academies Press, Washington, DC, 376

Palmquist, D.L. & Jenkins, T.C. 2003. Challenges with fats and fatty acid methods. Journal of animal science, 81: 3250–3254; doi:10.2527/2003.81123250x

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 Northern Territory, Australia. Journal of Applied Phycology, 6:337-345; https://doi.org/10.1007/BF02181948

Riquelme, C.E. & Herrera, R.E.A. 2003. Microalgae and bacteria interaction in the aquatic environment and their potential use in aquaculture. Revista Chilena de Historia Natural, 76:725-736

Rodriguez, J.C., García, M.C.C., Lopez, C.V.G., Sevilla, J.M.F., Gomez, A.C. & Grima, E.M. 2013. A low-cost culture medium for the production of *Nannochloropsis gaditana* biomass optimized for aquaculture. Bioresource Technology, 144: 57-66; doi: 10.1016/j.biortech.2013.06.083.

Rodríguez, J.R.B., Reina, G.G. & Rodríguez, J.J.S. 1997. Determination of Free Amino Acids in Microalgae by High-performance Liquid Chromatography Using Pre-Column Fluorescence Derivatization. Biomedical Chromatography, 11: 335-336

Roman, R.B., Pez, J.M.A., Fernandez, F.G.A. & Grima, E.M. 2002. Recovery of pure B-phycoerythrin from the microalga *Porphyridium cruentum*. Journal of Biotechnology, 93: 73–85; https://doi.org/10.1016/S0168-1656(01)00385-6

Roy, S.S. & Pal, R. 2015. Microalgae in Aquaculture: A Review with Special References to Nutritional Value and Fish Dietetics. Proceedings of the Zoological Society, 68 (1):1–8; https://doi.org/10.1007/s12595-013-0089-9

Salama, E.S., Kim, H.C., Shanab, R.A.A., Ji, M.K., Oh, Y.K., Kim, S.H. & Jeon, B.H. 2013. Biomass, lipid content, and fatty acid composition of freshwater *Chlamydomonas mexicana* and *Scenedesmus obliquus* grown under salt stress. Bioprocess and Biosystem Engineering, 36: 827-833; doi:10.1007/s00449-013-0919-1

Sheekh, M.M.E., Naggar, A.H., Osman, M.E.H. & Mazaly, E. 2003. Effect of cobalt on growth, pigments and the photosynthetic electron transport in *Monoraphidium minutum* and *Nitzchia perminuta*. Brazilian Journal of Plant Physiology, 15: 159-166; https://doi. org/10.1590/S1677-04202003000300005

Siegelman, H.W. & Kycia, J.H. 1978. Algal biliproteins. In: Hellebust, J.A., Craige, S. (eds) *Handbook of phycological methods, Physiological and biochemical methods*, Cambridge University Press, Cambridge. pp 72-77.

Sirakov, I., Velichkova, K., Stoyanova, S. & Staykov, Y. 2015.The importance of microalgae for aquaculture industry. Review. International Journal of Fisheries and Aquatic Studies, 2, 4: 81-84.

Spolaore, P., Cassan, C.J., Duran, E. & Isambert, A. 2006. Commercial Applications of Microalgae. Journal of Bioscience and Bioengineering 101 (2): 87-96; https://doi.org/10.1263/jbb.101.87

Straus, N. 2004. In: Bryant, D. (ed) *The Molecular Biology of Cyanobacteria*. Springer, Netherlands, pp 731-750

Sun, X.M., Ren, L.J., Zhao, Q.Y., Ji, X.J. & Huang, H. 2018. Microalgae for the production of lipid and carotenoids: a review with focus on stress regulation and adaptation. Biotechnology for Biofuels 11:1-16; https://doi.org/10.1186/s13068-018-1275-9

Tibbetts, S.M., Milley, J.E. & Lall, S.P. 2015. Chemical composition and nutritional properties of freshwater and marine microalgal biomass cultured in photobioreactors. Journal of Applied Phycology, 27:1109–1119; https://doi.org/10.1007/s10811-014-0428-x

Tokucsoglu, O. & Uunal, M. 2003. Biomass nutrient profiles of three microalgae: *Spirulina platensis, Chlorella vulgaris,* and *Isochrysis galbana*. Journal of Food Sciience, 68(4): 1144-1148; https://doi.org/10.1111/j.1365-2621.2003.tb09615.x

Tokucsoglu, O. & Uunal, M. 2003. Biomass nutrient profiles of three microalgae: *Spirulina platensis, Chlorella vulgaris,* and *Isochrysis galbana*. Journal of Food Science, 68(4): 1144-1148; doi: 10.1111/j.1365-2621.2003.tb09615.x

Venkataraman, L.V. & Becker, E.W. 1985. Biotechnology and utilization of algae-The Indian experience. Mysore: Central Food Technological Research Institute. 257.

Viskari, P.J. & Colyer, C.L. 2003. Rapid extraction of phycobiliproteins from cultured cyanobacteria samples. Analytical Biochemistry, 319: 263–271; https://doi.org/10.1016/ S0003-2697(03)00294-X

Volkman, J.K. & Brown, M.R. 2006. Nutritional value of microalgae and applications. In: Subba Rao, D.V. (ed) *Algal cultures, analogues of blooms and applications*, CABI, pp 407–457

Vu, M.T.T., Douette, C., Rayner, T.A., Thoisen, C., Nielsen, S.L. & Hansen, B.W. 2016. Optimization of photosynthesis, growth, and biochemical composition of the microalga *Rhodomonas salina*-an established diet for live feed copepods in aquaculture. Journal of Applied Phycology, 28:1485–1500; doi:10.1007/s10811-015-0722-2

Walne, P.R. 1970. Studies on food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea*, *Crassostrea*, *Mercenaria* and *Mytilus.* Fish Invest Lond Ser 226:1-62.

Zhu, C.J. & Lee, Y.K. 1997. Determination of biomass dry weight of marine microalgae. Journal of Applied Phycology, 9 (2): 189-194; https://doi.org/10.1023/A:1007914806640