



Effects of nitrogen starvation on protein and carbohydrate contents of some marine microalgae and their efficiency as food for the rotifer *Brachionus plicatilis* (Müller)

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ABSTRACT

The utilization of microalgae biomass shows vast invaluable uses, in the biotechnology of aquaculture, and food science. However, microalgae exhibit swing in their chemical components created mainly by the culture different conditions. This study consider the impact of nitrogen starvation on protein and carbohydrate of *Nannochloropsis salina*, *Nannochloropsis oculata*, *Chlorella salina* and *Tetraselmis chuii*, and the subsequent effects of the previously mentioned microalgae on the fatty acid composition (FA) of the rotifer *Brachionus plicatilis*. Cultivation of microalgae were cultured with F/2 enriched seawater medium (control) and without nitrogen (N starved) in 8-day (exponential growth phase). The result indicated that *N. salina* is the best algal species, and have higher protein contents ($41.88 \pm 1.40 \mu\text{g/ml}$) cultured on N starved media against control, followed by *T. chuii*, otherwise, *T. chuii* have higher carbohydrate contents. The lipid classes and FA composition of *B. plicatilis* showed higher concentrations ($450.63 \mu\text{g.g}^{-1}$) with *N. salina* cultured on N starved media, in parallel, total (n-3) PUFA were recorded ($57.19 \mu\text{g.g}^{-1}$), while, total (n-6) PUFA amounted ($23.74 \mu\text{g.g}^{-1}$). On the other hand, DHA/EPA ratio was higher when rotifers were fed on *T. chuii*. The results confirmed that *N. salina* was the excellent producing protein which significantly could be used as a fish feed product. Moreover, the proximate analysis of rotifers was influenced by the type of microalgae they utilized.

INTRODUCTION

The chemical constituents of microalgae can be varied with culture age or the growth phase (Costard *et al.*, 2012) as well as the modification in culture conditions (Durmaz *et al.*, 2009; Carvalho *et al.*, 2009). Information on the chemical composition of microalgae may change due to differences of the methods of measurement used (Barbarino and Lourenço, 2005). Green algae such as *Chlorella* sp., *Tetraselmis* sp. and *Nannochloropsis* were commonly used as important live food microalgae in aquaculture, and therefore they have been commercially produced (Huerlimann *et al.*, 2010).

The use of live feed in aquaculture is a key precursor for the great success in nursery and larval stages of fish and crustacean; even though, the usage of such natural food source has high economical costs (**Lin et al., 2009**). Similarly, *Artemia* is expensive, sometimes scarce or may have doubtful quality (**Watanabe et al., 1983**). The study of **Fidalgo et al. (1998)**, showed important history of species metabolism through the effects of nitrogen sources on the chemical profile in different algal species. Recently, (**Fan et al., 2014**) revealed that nitrogen starvation alters biochemical composition, such as protein and carbohydrate, pigments and lipid content, fatty acids composition, as well as photosynthetic suitability of microalgae. The idea of using microalgae as a appropriate source of providing an alternative fish feed replacement is not something strange and it is being assumed important as unconventional feed ingredients in cost effectiveness of feed stuffs that has been increased obviously (**Thang et al., 2015**).

The rotifer, *Brachionus plicatilis*, is commonly used as a live food in large seed production of larvae of marine fish (**Lubzens, 1987**). Its suitable size and quality as live food as well as its stability in biochemical composition during the first larval feeding process has been frequently addressed (**Øie et al., 1997**). **Pérez-Legaspi et al. (2018)** studied the biochemical variations of three microalgae (*Nannochloropsis oculata*, *Dunaliella salina* and *Isochrysis* sp.) and their impact on the rotifer *Brachionus plicatilis* feeding. More recently, **Ashour et al. (2019)** recommended the application of lipid-free algal biomass in enhancing *Artemia* development and survival for improving aquaculture.

Lipids are among all the nutritional requirements that play an important role in larval growth and survival (**Ghonaime et al., 2020**). Eicosapentaenoic acid 20:5 (n-3) (EPA) and docosahexaenoic acid 22:6 (n-3) (DHA) are considered vital and essential acids due to their presence in the plasma membrane and they are highly abundant. Basically, marine fish larvae cannot synthesize them from the linoleic acid 18:3 (n-3). More specifically, DHA is present in higher concentrations in the neural and visual tissues. Therefore, a lack of this essential acid negatively affects several physiological and behavioral occasions (**Estévez et al., 1999**).

Microalgae have been used for mass production and enrichment of rotifers due to the content of essential nutrients such as polyunsaturated fatty acids, vitamins, amino acids and pigments that can be transferred to superior trophic levels. Diets such as microalgae, lipid or lipids plus proteins and carbohydrates have been employed (**Abugrara et al., 2019**). Enrichment of rotifers could be attained by feeding those microalgae rich with those EFA. *Isochrysis galbana* was found to contain authentic amounts of DHA and a low EPA content (**Fernandez-Reiriz et al., 1989**), whereas *Nannochloropsis gaditana* contains major amounts of EPA and 20:4n-6 (**Sukenik et al., 1993**).

This present study aimed to assess the protein and carbohydrate synthesis by four marine microalgae, *Nannochloropsis salina*, *Nannochloropsis oculata*, *Chlorella salina* and *Tetraselmis chuii* under two culture conditions (F/2 and N starved). Evaluation of the four potential microalgae strains as a diet for the rotifer *Brachionus plicatilis* and recognize the best algal species that improve their nutritional profile as protein, carbohydrate, and lipid contents.

MATERIALS AND METHODS

Four algal species were used in this study: *Nannochloropsis salina*, *Nannochloropsis oculata*, *Chlorella salina* and *Tetraselmis chuii*. These microalgae were obtained from the culture collection unit in the marine hatchery, National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt. Four algal strains cultured on Guillard F/2 (ESW) media (**Guillard, 1975**) as control and without nitrogen (N starved). This control media consisting of (per liter) 0.005g, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$; 0.03g $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$; 3.15g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$; 4.36g $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$; 0.18g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$; 0.02g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; 0.01g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$; 0.009g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; 0.006g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$; 200mg Thiamine HCl; 1mg Biotin and 1mg Cyanocobalamin. The cultures were maintained in 1000 ml Erlenmeyer flasks with 500 mL medium with controlled temperature ($25 \pm 1^\circ\text{C}$) providing 24 h fluorescent illumination (40-watt, white tube light). The cultures were shaken 2-3 times every day (incubation period of each culture must be determined) and all glassware and media were disinfected prior to inoculation and cultivation process.

***Brachionus plicatilis* cultivation and enrichment:**

The rotifer, *Brachionus plicatilis*, was cultivated at 28°C in 1m^3 conical glass fibre tanks at 25‰ salinity, and aerated to secure mixing and supply oxygen. The animals were initially scalled up on baker yeast emulsion mixed with the available algal strain in the hatchery. The cultures were run semi continuously at high and low dilution rates (32% and 12% of the culture volume was replaced per day, respectively) for one week to obtain fast growth of the rotifer culture till reached the desired density appropriate to start the enrichment experiment. The culture density was 250/mL at the time of harvest. The Rotifers were then washed and transferred to the enrichment containers separately and fed on the experimental algae (*C. salina*, *N. salina*, *N. oculata* and *T. chuii* in control and nitrogen starved conditions) for 24hrs enrichment (long term). Rotifer samples for chemical analysis were harvested on nylon net (70- μm mesh size)

Estimation of Protein and Carbohydrate

The method of protein extraction content was performed as described by **Lowry et al., (1951)** using Bovine Serum Albumin (BSA) as standard. Whereas **Dubois et al. (1956)** method was followed for the extraction and estimation of total carbohydrates by using D- glucose $\mu\text{g/ml}$ as standard.

Determination of total lipid and fatty acids

The first step to determine the fatty acids (FA) composition was to extract all lipids as described by **Folch et al. (1957)** and **Bligh and Dyer (1959)**. For the FA esterification, we added 2.5 mL of methanolic hydrochloric acid $\text{HCl}:\text{CH}_3\text{OH}$ (5%, v/v) for a 2.5 h at 85°C (**Sato and Murata, 1988**). The esterified fatty acids (FAME) derived were extracted with 1 mL of hexane (C_6H_{12}). The FA profile was estimated by GC-MS. The FAs present in the samples were identified by contrasting the obtained mass spectra with the mass spectral database. Data analysis was carried out using the equipment's software and showed as the percentage of the area according to the identification of the total FA.

Statistical analysis

One-way analysis of variance (ANOVA) was used to test the effects of lacking of nitrogen on the protein and carbohydrate synthesis of four algal strains. Duncan multiple comparison test (HSD) of the one-way ANOVA was used to match the mean differences

(Zar, 1999) by the Statistical Package for the Social Sciences (SPSS) (Version 12.0, SPSS, Chicago, IL). As such, the differences were considered to be significant at $p \leq 0.05$.

RESULTS

In this study, the effects of nitrogen starvation (N starved) in comparison of control (F/2) on cellular development, protein and carbohydrate contents were examined (Figure 1, 2). The simple comparison of the protein quantities between four algal strains represents the *N. salina* cultured on N starved media as the species with the highest protein accumulation ($41.88 \pm 1.40 \mu\text{g/ml}$) as compared with control ($35.13 \pm 1.06 \mu\text{g/ml}$), followed by *T. chuii* ($35.08 \pm 4.84 \mu\text{g/ml}$). In contrast, *N. salina* have lower carbohydrate content cultured on N starved media ($7.42 \pm 0.03 \mu\text{g/ml}$) as compared with control ($6.91 \pm 0.28 \mu\text{g/ml}$), Figure (1). On the contrary, *T. chuii* cultured on N starved media have higher carbohydrate contents ($30.38 \pm 0.87 \mu\text{g/ml}$) compared with control (Figure 2).

Regarding the FAs composition of *B. plicatilis* enriched with four algal strains cultured on N starved media were observed in Table (1). Higher total FA concentrations ($450.63 \mu\text{g.g}^{-1}$) were recorded in rotifer enriched with *N. salina*, in parallel, total (n-3) PUFA were recorded ($57.19 \mu\text{g.g}^{-1}$). While, total (n-6) PUFA amounted ($23.74 \mu\text{g.g}^{-1}$) was recorded in *B. plicatilis* enriched with *N. salina* cultured on N starved media (Table 1). On the other hand, DHA/EPA ratio was higher when rotifers were fed on *T. chuii* cultured on N starved media (Table. 1).

As represented in Figure (3), protein, carbohydrate and lipid of *B. plicatilis* were affected by *N. salina* cultured on control and N starved media. The results showed that rotifer fed on *N. salina* cultured on N starved media showed the highest protein, carbohydrate and lipid concentrations (67.82 ± 0.28 , 13.9 ± 0.97 , $17.8 \pm 0.35\%$), respectively as matched with control.

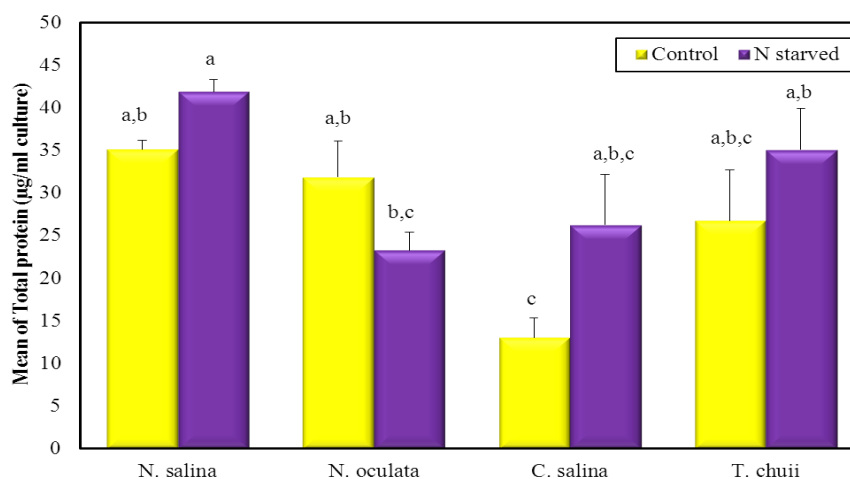


Fig. 1. Total protein contents of four marine microalgae strains cultured on N starved media as compared with control at exponential growth phase (day 8). *Different letters between the lines indicate significant difference at 5% by Duncan

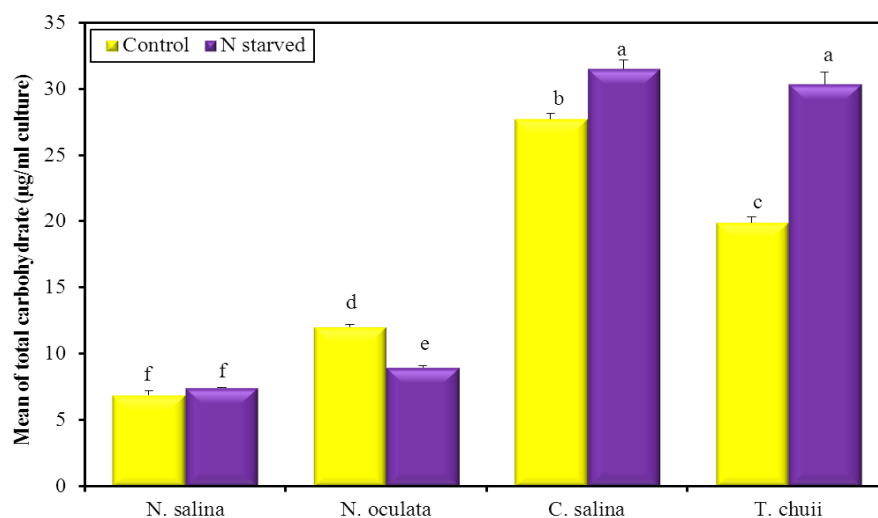


Fig. 2. Total carbohydrate contents of four marine microalgae strains cultured on N starved media as compared with control at exponential growth phase (day 8). *Different letters between the lines indicate significant difference at 5% by Duncan

Table 1. Fatty acids composition of *Brachionus plicatilis* fed on different algal strains cultured on N starved media.

Fatty acids	Algal species			
	<i>N. salina</i>	<i>N. oculata</i>	<i>C. salina</i>	<i>T. chuii</i>
Total fatty acids (µg.g-1)	450.63	299.02	180.45	297.77
SFA	129.21	63.62	67.74	402.58
MUFA	240.49	181.34	71.21	126.29
C 18:2 (n-6)	8.31	5.25	4.13	29.72
C 18:3 (n-6)	3.43	2.52	0.97	2.30
C 20:2 (n-6)	6.84	2.38	0.00	30.8
C 20:4 (ARA) (n-6)	5.16	2.49	2.63	0.00
Total (n-6) PUFA	23.74	12.64	7.73	62.82
C 20:3 (n-3)	6.67	3.48	4.37	3.12
C 20:5 EPA (n-3)	18.57	9.44	5.75	2.96
C 22:6 (DHA) (n-3)	31.95	28.5	23.65	0.18
Total (n-3) PUFA	57.19	41.42	33.77	6.26
n-3:n-6	2.40	3.30	4.40	0.10
DHA:EPA	1.72	3.00	4.10	6.10

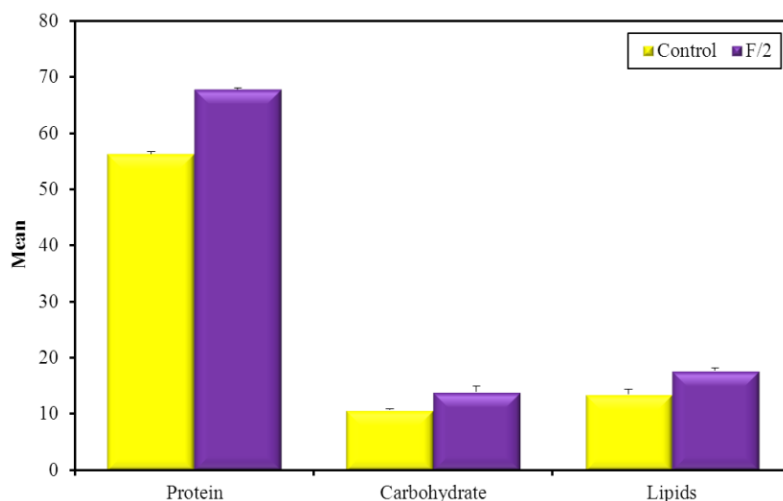


Fig. 3. Protein, carbohydrate and lipid percentages of *Brachionus plicatilis* enriched with *N. salina* cultured on N starved media.

DISCUSSION

Changes in culture media affect the biochemical constituents of *N. salina*, *N. oculata*, *C. salina* and *T. chuii*. The effect of N deprivation on protein and carbohydrate contents of those selected algae was investigated. The study revealed that the highest protein was observed in *N. salina* cultured on N starved media as compared with control, otherwise, carbohydrate contents was decreased. In contrast to our study, the finding of **Zhu et al. (2015)**, as nitrogen may be a vital for the most imperative precursor for protein and carbohydrate synthesis, cause a depletion in their amounts under nitrogen starvation. Increasing in carbon availability which leads to carbon flux change from the protein yield to carbohydrate or lipid synthesis was related to nitrogen depletion (**El-Kassas, 2013; Zhu et al., 2015**). Simultaneously to the increase of protein quantity, a decrease of the storage compounds was observed. Whereas carbohydrate amount tended to decrease in microalgal species studied (**Otero and Fábregas, 1997**).

The live food plays a vital role in the production of crustaceans, fish, and mollusks. Rotifers are filter feeders zooplanktonic organisms that feed on a wide variety of food sources (**Yin and Zhao, 2008**), especially algae, that are considered by many authors to provide better results in respect to growth and increasing contribution of FAs to the rotifers used in aquaculture (**Torzillo and Vonshak, 2013**). The most widely used green algal species in the culture of rotifers are the genera *Nannochloropsis*, *Nannochloris* and *Chlorella* which have been commonly used in massive cultures by nourishing a high nutritional quality to the rotifer (**Hee-Bae and Bum-Hur, 2011**).

The results of present study showed that the essential fatty acids composition (20:4n-6, 20:5n-3 and 22:6n-3) of enriched *B. plicatilis* showed a very good relationship with the contribution of each diet. Therefore, high levels of 20:4n-6, 20:5n-3 in rotifer enriched with *N. salina* cultured on N starved media was reflected in the corresponding EFA levels of rotifers fed on that diet. The FA composition of the rotifers changed in response to algal feeding, and algal culture media. The results demonstrated that *B. plicatilis* enriched with *N. salina* cultured on N starved media had high levels of 20:5n3 (18.57 µg/g) and 22:6n3

(31.95 µg.g/1), reflecting the high levels of these FAs. The levels of these long-chain PUFAs are markedly higher than reported previously for enriched rotifers (**Olsen et al., 1993**). *B. plicatilis* cultivated by this method are supposed to meet the nutritional requirement of the fish larvae for these essential n3 fatty acids. Arachidonic acid (20:4n6) represented 5.16 µg.g/1 of the total lipids in *B. plicatilis* enriched with *N. salina* cultured on N starved media. The requirement of n6 fatty acids for marine larvae is not established, but a small amount of C20:4(n-6) is probably required (**Sargent et al., 1989**). While, DHA/EPA ratio, used as a diet quality index, was higher when rotifers were fed *T. chuii* cultured on N starved media.

Protein content of rotifers is mainly related to the specific food ration made attainable for the rotifer density during cultivation. Our results demonstrate that protein is a major fraction of *B. plicatilis* (67.82±0.28%) enriched with *N. salina* cultured on N starved media. On the other hand, carbohydrate and lipid showed the minor contents. **Brown et al. (1997)** concluded that *Nannochloropsis* is a genus of poor nutritional value for molluscs and *Artemia* probably due to its hard cell wall. Carbohydrate content of *N. gaditana* decreased continuously. This is a common response to the increase of nutrient availability in continuous and semi continuous nutrient-limited cultures (**Sukenik et al., 1993**). It has been observed that the increase of protein and the decrease of carbohydrate and lipid contents of the microalgal feed results in better somatic or populational growth of filter feeders (**Ferreira et al., 2008**).

CONCLUSION

The results indicated that protein content was significantly higher in N starved *N. salina* culture when compared with control (F/2), and DHA/EPA ratio was highest in rotifers enriched with N starved *N. salina* culture. Therefore, an alternative option for commercial microalgal production is to N starved culture to higher production. Therefore, the productive response and quality of microalgae depended on the medium in which they grew; also, the productive response and proximate composition of rotifers depended upon the nutritional quality of algal species.

REFERENCES

- Abugrara, A. M.; El-Sayed, H. S.; Zaki, M. A. A. and Nour, A. M. (2019).** Utilization of *Nannochloropsis oceanica* alga for biodiesel production and the de-lipidated biomass for improving Red tilapia aquaculture. Egypt. Aquat. Biol. Fish., 23(4): 421-436.
- Ashour, M.; Elshobary, M. E.; El-Shenody, R.; Kamil, A. and Abomohra, A. (2019).** Evaluation of a native oleaginous marine microalga *Nannochloropsis oceanica* for dual use in biodiesel production and aquaculture feed. Biomass and Bioenerg., 120: 439-447.
- Barbarino, E. and Lourenço, S. O. (2005).** An evaluation of methods for extraction and quantification of protein from marine macro-and microalgae. J. Appl. Phycol., 17: 447-460.
- Bligh, E. G. and Dyer, W. J. (1959).** A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

- Brown, M. R.; Jeffrey, S. W.; Volkman, J. K. and Dunstan, G. A.** (1997). Nutritional properties of microalgae for mariculture. *Aquaculture*, 151: 315-331.
- Carvalho, A. P.; Monteiro C. M. and Malcata, F. X.** (2009). Simultaneous effect of irradiance and temperature on biochemical composition of the microalga *Pavlova lutheri*. *J. Appl. Phycol.*, 21(5): 543-552.
- Costard, G. S.; Machado, R. R.; Barbarino, E.; Martino, R. C. and Lourenço, S. O.** (2012). Chemical composition of five marine microalgae that occur on the Brazilian coast. *Int. J. Fish. Aquacult.*, 4(9): 191-201.
- Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A. and Smith, F.** (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, 28: 350-356.
- Durmaz, Y.; Donato, M.; Monteiro, M.; Gouveia, L.; Nunes, M. L.; Gama Pereira, T.; Gökpınar, Ş. and Bandarra, N. M.** (2009). Effect of temperature on α -tocopherol, fatty acid profile, and pigments of *Diatrypa vlkianum* (Haptophyceae). *Aquacult. Int.*, 17(4): 391-399.
- El-Kassas, H.Y.** (2013). Growth and fatty acid profile of the marine microalga *Picochlorum* sp. grown under nutrient stress conditions. *Egypt. J. Aquat. Res.*, 39(4): 233-239.
- Estévez, A.L.; McEvoy, A.; Bell, J. G. and Sargent, J. R.** (1999). Growth, survival, lipid composition and pigmentation of turbot (*Scophthalmus maximus*) larvae fed live-prey enriched in Arachidonic and Eicosapentaenoic acids. *Aquaculture*, 180: 321-343.
- Fan, J.; Cui, Y.; Wan, M.; Wang, W. and Li, Y.** (2014). Lipid accumulation and biosynthesis genes response of the oleaginous *Chlorella pyrenoidosa* under three nutrition stressors. *Biotechnol. Biofuels*, 7(1):17.
- Ferreira, F.; Apel, A. and Henderson, J. M.** (2008). Taking a new look at looking at nothing. *Trends Cogn. Sci.*, 12(11): 405-410.
- Fidalgo, J. P.; Cid, A.; Torres, E.; Sukenik, A. and Herrero, C.** (1998). Effects of nitrogen source and growth phase on proximate biochemical composition, lipid classes and fatty acid profile of the marine microalga *Isochrysis galbana*. *Aquaculture*, 166: 105-116.
- Folch, J.; Lees, M. and Stanley, G. H. S.** (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 226: 497-509.
- Fernandez-Reiriz, M. J.; Pdrez-Camacho, A.; Ferreira, M. J.; Blanco, J.; Planers, M.; Campos, M. J. and Labarta, U.** (1989). Biomass production and variation in the biochemical profile (total protein, carbohydrates, RNA, lipids and fatty acids) of seven species of marine microalgae. *Aquaculture*, 83: 17-37.
- Ghoname, R. M.; El-Sayed, H. S.; Ghozlan, H. A. and Sabry, S. A.** (2020). Application of probiotic bacteria for the improvement of sea bream (*Sparus aurata*) larval production. *Egypt. J. Aquat. Biol. Fish.*, 24(1): 371-398
- Guillard R. R.** (1975). Culture of phytoplankton for feeding marine invertebrates. In: "Culture of Marine Invertebrate Animals." Chanley, M. H. & Smith, W.L. (Eds.). Plenum Press, New York, pp. 29-60.
- Hee-Bae, J. and Bum-Hur, S.** (2011). Selection of suitable species of *Chlorella*, *Nannochloris*, and *Nannochloropsis* in high-and low-temperature seasons for mass culture of the rotifer *Brachionus plicatilis*. *Fish. Aquat. Sci.*, 14(4): 323-332.

- Huerlimann, R.; de Nys, R. and Heimann, K.** (2010). Growth, Lipid Content, Productivity, and Fatty Acid Composition of Tropical Microalgae for Scale-Up Production. *Biotechnol. Bioeng.*, 107: 245-257.
- Lin, Q.; Lin, J.; Zhang, D. and Wang, Y.** (2009). Weaning of juvenile seahorses *Hippocampus erectus* Perry, 1810 from live to frozen food., 291: 224-229.
- Lubzens, E.** (1987). Raising rotifers for use in aquaculture. *Hydrobiologia*, 147: 245 -255
- Lowry, O.; Rosebrough, N.; Farr, A. and Randall, R.** (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193(1): 265-275.
- Olsen, Y.; Reitan, K. I. and Vadstein, O.** (1993). Dependence of temperature on loss rates of rotifers, lipids, and ω 3 fatty acids in starved *Brachionus plicatilis* cultures. *Hydrobiologia*, 255: 13-20.
- Otero, A. and Fábregas, J.** (1997). Changes in the nutrient composition of *Tetraselmis suecica* cultured semicontinuously with different nutrient concentrations and renewal rates. *Aquaculture*, 159: 111-123.
- Pérez-Legaspi, I. A.; Guzmán-Fermán, B. M.; Moha-León, J. D.; Ortega-Clemente, L. A. and Valadez-Rocha, V.** (2018). Effects of the biochemical composition of three microalgae on the life history of the rotifer *Brachionus plicatilis* (Alvarado strain): an assessment. *Ann. Limnol. Int. J. Lim.*, 54: 20-28.
- Øie, G.; Makridis, P.; Reitan, K. I. and Olsen, Y.** (1997). Protein and carbon utilisation of rotifers *Brachionus plicatilis*. in first feeding of turbot larvae - *Scophthalmus maximus* L.. *Aquaculture*, 153: 103-122.
- Sargent, J. R.; Henderson, R. J. and Tocher, D. R.** (1989). The Lipids. In: "Fish Nutrition, 2nd ed". Halver, J. (Ed.). Academic Press, New York, London, pp. 154-209.
- Sato, N. and Murata, N.** (1988). Membrane lipids. *Meth. Enzymol.*, 167: 251-259.
- Sukenik, A.; Zmorab, O. and Carmeli, Y.** (1993). Biochemical quality of marine unicellular algae with special emphasis on lipid composition. II. *Nannochloropsis* sp. *Aquaculture*, 117(3-4): 313-326.
- Thang, D. V.; Faruq, A.; Thomass-Hall, S. R.; Simon, Q.; Ekaterina, N. and Schenk, P. M.** (2015). High Protein- and High Lipid-Producing Microalgae from Northern Australia as Potential Feedstock for Animal Feed and Biodiesel. *Front. Bioeng. Biotechnol.*, 3: 106-110.
- Torzillo, G. and Vonshak, A.** (2013). Environmental stress physiology with reference to mass cultures. In: "Handbook of microalgal culture: applied phycology and biotechnology". Richmond, A. & Hu, Q. (Eds.). Blackwell Publishing Ltd., New Jersey, pp. 90-113.
- Watanabe, T.; Oowa, C.; Kitajima, C. and Fujita, S.** (1983). Nutritional values of live organisms used in Japan for mass propagation of fish: A review. *Aquaculture*, 34: 115-143.
- Yin, X. W. and Zhao, W.** (2008). Studies on life history characteristics of *Brachionus plicatilis* O.F. Müller (Rotifera) in relation to temperature, salinity and food algae. *Aquat. Ecol.*, 42: 165-176.
- Zar, J. H.** (1999). *Biostatistical Analysis*, 4th ed. Prentice Hall, Upper Saddle River.
- Zhu, S.; Wang, Y.; Shang, C.; Wang, Z.; Xu, J. and Yuan, Z.** (2015). Characterization of lipid and fatty acids composition of in response to nitrogen starvation. *J. Biosci. Bioeng.*, 120(2): 205-209.