

Exigent of Micro Algae for the Enrichment of *Artemia salina*

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Abstract: The nauplii of most strains of *Artemia* are lacking in certain biochemical elements required by some species of fish. The cysts from commercial traders test positive for DHA, however they are extremely difficult to obtain and very expensive. Most strains also have a low energy to protein ratio. These deficiencies can be remedied by an enrichment process. *Artemia salina* was enriched with three different algal species (*Nannochloropsis salina*, *Chlorella salina* and *Spirulina subsalsa*) and commercially available media A1 DHA SELCO media. After enrichment process, proximate analysis was carried out by Lowry's (protein), Anthrone (carbohydrate) and Phenol-Chloroform Method (lipid). *Nannochloropsis salina* and *C. salina* enriched *Artemia* be full of high lipid which is the nutrient content of both algae. *Spirulina subsalsa* enriched *Artemia* showed the high protein which is the nutrient content of *S. subsalsa*. *Chlorella salina* and *N. salina* enriched *Artemia* illustrated that good result in both protein and lipid. Concentration of the *Artemia* biomass was directly proportional with hours of enrichment. From this study, researchers can use algae (*N. salina*, *C. salina* and *S. subsalsa*) as a enrichment media instead of commercial A1 DHA SELCO media which give same nutrition profile to *Artemia*.

Key words: *Artemia*, protein, lipid, carbohydrate, nutrient, India

INTRODUCTION

Live diets used in the aquaculture of fish, brine shrimp (*Artemia*) nauplii are the most widely used food (Dhont *et al.*, 1993). Over 85% of all marine animals' culture utilizes *Artemia* as a partial or sole diet (Hoff and Snell, 1997). They have been used as a vector for the delivery of die-rent materials such as nutrients (Watanabe *et al.*, 1983) and probiotics (Gatesoupe, 1991) and all these may have a positive effect on host organism by improving properties of the native microflora.

The enrichment processes for *Artemia nauplii* significantly elevate all of the fatty acids found in the nauplii most notable is the elevations of C20:5n-3 (EPA) as well as infusion of C22:6n-3 (DHA). Both of these have been implicated to be essential for larval growth and development in a number of fish species (Sorgeloos *et al.*, 2001).

The essential Highly Unsaturated Fatty Acids (HUFAs) 20:5n-3 or Eicosapentaenoate (EPA) and 22:6n-3 or Docosa Hexaenoate (DHA) are significantly higher in *Artemia nauplii* that have been enriched. The varying amounts of EPA and DHA reflect the differences in the amount of enrichment media used as well as quantitative and qualitative differences in the sources of these fatty acids (e.g., fish oil or algae) that make up the commercially

prepared enrichment media. The enrichment process is time dependent. As the fatty acids and protein are taken up by the *Artemia*, their fatty acid profiles and protein concentrations change according to the duration of the enrichment period. The length of enrichment process should also be considered when preparing *Artemia nauplii* as a food for the larvae of ornamental fish (Tamaru *et al.*, 2004).

Microalgae strains are recognised as excellent sources of proteins, carbohydrates, lipids and vitamins to be used as food and feed additives (Rocha *et al.*, 2003). *Nannochloropsis* sp. is well known as a source of EPA, an important polyunsaturated fatty acid (Hu and Gao, 2003). *Chlorella* sp., also identified as source of EPA (Yongmanitchai and Ward, 1991) and a well known fact that *Spirulina* sp. is responsible for protein (Babadzhanov *et al.*, 2004).

Aim of this study is to enlarge the protein and lipid content of *Artemia* which is obligatory for fishes by the enrichment practice. Micro algae such as *Nannochloropsis* sp., *Chlorella* sp. and *Spirulina* sp. were used as a natural enrichment medium. In that each algae responsible for their respective nutrients which discussed before. And commercially available A1 DHA SELCO medium also used for standard. Due to this type of natural enrichment, researchers expect the nutrition

level of *Artemia* also increasing devoid of any toxicity of chemicals. The nutrition quality viz. protein, lipid and carbohydrate levels of the harvested cysts were then studied to understand the process of enrichment and value added by way of enrichment.

MATERIALS AND METHODS

Culture collection: Microalgae (*Nannochloropsis salina*, *Chlorella salina* and *Spirulina subsalsa*) were obtained from the CAS in Marine Biology, Annamalai University. Stock cultures of these organisms were maintained using 25% Conway medium (Walne, 1970) at 28±2°C, 24:0 h dark/light cycle with white fluorescent light at an intensity of 40 µmol photon/m²/sec under continuous aeration. Batch cultures were scaled >20-30 L and cultured until reaching exponential growth before transfer to the microalgae culture tank of the continuous culture system.

Composition of A1 DHA SELCO: A1 DHA SELCO media is a product of Artemia International LLC, USA and composition is: Moisture (30%); crude ash (1%); crude lipids (67%); phosphorus (0.2%), vitamin A (1,500,000 IU kg⁻¹); vitamin D3 (150,000 IU kg kg⁻¹); vitamin E (3600 mg kg⁻¹); vitamin C (800 mg kg⁻¹); Antioxidants (ethoxyquin).

Enrichment of Artemia: About 50-100 g of A1 DHA SELCO was taken for 1 L of water and 1 L of different microalgae emulsified by mixing vigorously for 3 min.

Procedure for production of Artemia: About 1 g of cyst of *Artemia salina* was taken in sea water. The dry cyst was placed in water to be hydrated. The embryos inside the cyst started their metabolic activity. The free swimming nauplius, called instar 1, hatched out after about 20-24 h. Salinity of the sea water should be 35 ppt. Water temperature was maintained at 28-30°C and pH over 8. Strong aeration was provided to ensure vigorous water agitation to keep cysts in suspension. Air is provided through the open end of a half inch PVC pipe placed close to the tank bottom. Dissolved oxygen level measured above 4 ppm. A strong illumination of 200 lux was provided by two neon tubes (2×58 w) placed just above the tank rim. Cyst density for incubation was maintained 2.5g L⁻¹.

Collection of harvested Artemia: Unhatched cysts were collected by sunk and opening the drain valve for a few seconds. During the settling time, the oxygen level was monitored which should not drop <2 ppm. If necessary prior to harvest injected pure oxygen in to the culture tank for 10-15 min to raised the dissolved oxygen content >10 ppm. Nauplii were rinsed with fresh water and they

changed to temporary container filled with a known volume of filtered and sterilized seawater. Approximately 4 million nauplii were taken in 1 L and temperature was maintained between 5-10°C for forbid the loss of nutritional value of *Artemia*.

Enrichment and storage: Four different concentration of A1 DHA SELCO (0.2, 0.4, 0.6, 0.8 mL/2 L) was taken in 4 buckets and taken 1 bucket without media as control. Then freshly hatched *Artemia salina* was added each bucket. Finally, samples were collected at different intervals (6, 12, 18 and 24 h).

Freshly hatched *Artemia salina* was enriched with concentration of 1 L in each green waters (*N. salina*, *C. salina* and *S. subsalsa*). Finally, samples were collected at different intervals (6, 12, 18 and 24 h). Enriched metanauplii rapidly lose their nutritional value at room temperature as it happens to rotifers unless they are stored in cold seawater (4-10°C) (Bengtson *et al.*, 1991).

Proximate analysis of Artemia: Stored *Artemia* were taken and dried using oven then protein lipids and carbohydrates was estimated. The estimation of protein was done by Lowry *et al.* (1951)'s Method. The estimation of carbohydrates was done by Anthrone Method (Yemm and Willis, 1954). The estimation of lipid was done by Phenol and Chloroform Method (Morton *et al.*, 1991).

Statistical analysis: The collected data were subjected to statistical tests of Pearson multiple correlation analysis and ANOVA for the variance analysis to know the significant variation in the concentration levels. Probabilities p<0.05 were considered statistically significant. All statistical analyses were done by SPSS (v. 16) software for windows.

RESULTS

The concentration of crude protein, lipid and carbohydrates from *Artemia* enriched with different algae (*N. salina*, *C. salina* and *S. subsalsa*) were shown in Table 1. Protein, lipid and carbohydrates concentration of each algae were compared. Table 1 showed the protein concentration of *Artemia* enriched with *S. subsalsa* (spirulina) showed the maximum (0.33, 0.57, 0.86 and 1.05 mg g⁻¹), *N. salina* and *C. salina* were next to the *S. subsalsa*, respectively.

These results reflected the nutrient content present in the spirulina. *Spirulina subsalsa* contains about 60% (51-71%) protein (Babadzhanov *et al.*, 2004). It is a complete protein containing essential amino acids, though with reduced amounts of methionine, cysteine and lysine when compared to the proteins of meat, eggs and milk. It

Table 1: Biochemical composition of *A. salina* enriched by green water

Time (h)	<i>Nannochloropsis salina</i> (mg g ⁻¹)			<i>Chlorella salina</i> (mg g ⁻¹)			<i>Spirulina subsalsa</i> (mg g ⁻¹)		
	Protein	Lipid	Carbohydrates	Protein	Lipid	Carbohydrates	Protein	Lipid	Carbohydrates
6	0.17±0.230	0.34±0.002	0.035±0.01	0.13±0.040	0.27±0.71	0.15±0.51	0.33±0.07	0.17±0.22	0.04±0.11
12	0.21±0.520	0.47±0.050	0.130±0.21	0.29±0.430	0.47±0.45	0.19±0.02	0.57±0.51	0.28±0.32	0.19±0.16
18	0.46±0.003	0.75±0.480	0.320±0.43	0.36±0.070	0.76±0.02	0.22±0.33	0.86±0.82	0.49±0.01	0.27±0.27
24	0.65±0.270	0.97±0.720	0.370±0.17	0.45±0.005	1.31±0.61	0.34±0.28	1.05±0.04	0.57±0.10	0.31±0.19

Table 2: Biochemical composition of *A. salina* enriched by A1 DHA

Time (h)	Concentration of enrichment media (mL L ⁻¹)					
	C	0.2	0.4	0.6	0.8	1.0
Concentration of protein (mg g⁻¹)						
6	0.080±0.01	0.14±0.230	0.21±0.17	0.18±0.23	0.20±0.200	0.21±0.070
12	0.085±0.20	0.29±0.020	0.31±0.03	0.35±0.53.0	0.37±0.060	0.41±0.210
18	0.090±0.04	0.40±0.630	0.47±0.25	0.49±0.22	0.52±0.009	0.61±0.650
24	0.092±0.34	0.70±0.520	0.84±0.34	0.81±0.11	0.91±0.100	0.94±0.020
Concentration of lipid (mg g⁻¹)						
6	0.070±0.21	0.15±0.330	0.16±0.41	0.19±0.48	0.20±0.320	0.22±0.110
12	0.070±0.45	0.27±0.040	0.39±0.07	0.37±0.45	0.39±0.280	0.41±0.003
18	0.078±0.33	0.39±0.210	0.45±0.27	0.47±0.43	0.51±0.220	0.51±0.002
24	0.070±0.21	0.40±0.110	0.53±0.17	0.57±0.55	0.61±0.210	0.62±0.150
Concentration of carbohydrate (mg g⁻¹)						
6	0.10±0.330	0.19±0.040	0.20±1.02	0.16±0.45	0.18±0.340	0.28±0.250
12	0.15±0.070	0.28±0.450	0.34±0.76	0.26±0.36	0.26±0.320	0.34±0.880
18	0.20±0.550	0.40±0.550	0.38±0.56	0.35±0.11	0.35±0.470	0.44±0.430
24	0.19±0.170	0.42±0.003	0.53±0.23	0.52±0.21	0.52±1.050	0.60±0.010

is however superior to typical plant protein such as that from legumes (Babadzhanov *et al.*, 2004). Overall while spirulina is often marketed as an excellent source of protein and is approximately 30 times more expensive per gram of protein.

From Table 1, lipid content of Artemia enriched with algae showed that *N. salina* and *C. salina* were taken the superior place. *Spirulina subsalsa* had small quantity of lipid than compare to others. But in 24 h enriched Artemia of *C. salina* (1.31±0.61 mg g⁻¹) showed the highest lipid concentration among the all. Chlorella has yielded EPA at 39.9% of total lipids (Belasco, 1997). Chlorella is considered as a protein source also (Belasco, 1997). *N. salina* considered as a promising algae for industrial applications because of its ability to accumulate high levels of polyunsaturated fatty acids. It is mainly used as an energy rich food source for fish larvae and rotifers (Sukenic *et al.*, 1989).

In case of carbohydrate concentration in Artemia, the results showed that all these algae contain very low quantity of carbohydrates than compare to A1 DHA SELCO media (Table 2). The enrichment process is time dependent. As the fatty acids and protein are taken up by the Artemia, their fatty acid profiles and protein concentrations change according to the duration of the enrichment period the length of enrichment process should also be considered when preparing *Artemia nauplii* as a food for the larvae of ornamental fish. The concentration of crude protein, lipid and carbohydrates from Artemia enriched with A1 DHA SELCO media were

shown in Table 2. From this result, the concentration and enrichment duration played a vital role. All the 4 concentrations (0.2, 0.4, 0.8 and 1.0 mL L⁻¹) were compared with control. About 24 h and 1.0 mL L⁻¹ enriched Artemia showed the best one.

The concentration of each nutrients was shown in Table 1 and 2 and it was made statistically significant by using the significant Pearson correlation (p<0.05) and significantly varied in each samples (one-way ANOVA p<0.05).

DISCUSSION

Resemblance of enrichment by *N. salina*, *C. salina* and *S. subsalsa* were done by 17 sec bray curtis similarity index from the software of Primer 6 (v. 6) (Fig. 1-3). Figure 1 showed the concentration of protein resemblance between *N. salina*, *S. subsalsa* and *C. salina*. In these *N. salina* and *C. salina* were resembled at 81.38%. *N. salina* and *C. salina* were highly resembled at 93.71% in lipid concentration (Fig. 2). In case of carbohydrate concentration, *N. salina* and *S. subsalsa* resembled at 90.24% (Fig. 3).

Brine shrimp are typically filter feeders that consume organic detritus, microscopic algae and bacteria. Blooms of microscopic algae are favorite habitats of Artemia and large populations develop in such areas where they feed on the algae and heterotrophic bacteria that are produced by these blooms. Brine shrimp populations have done

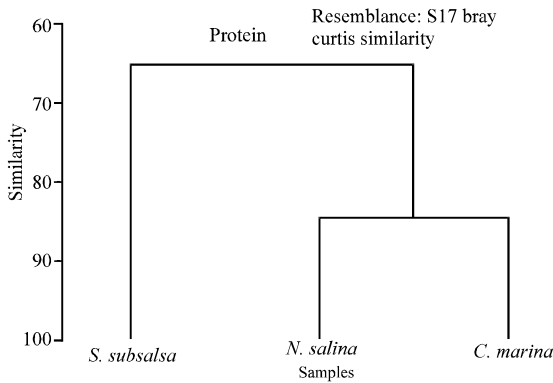


Fig. 1: Similarity index of *N. salina*, *C. salina* and *S. subsalsa* based on the protein concentration

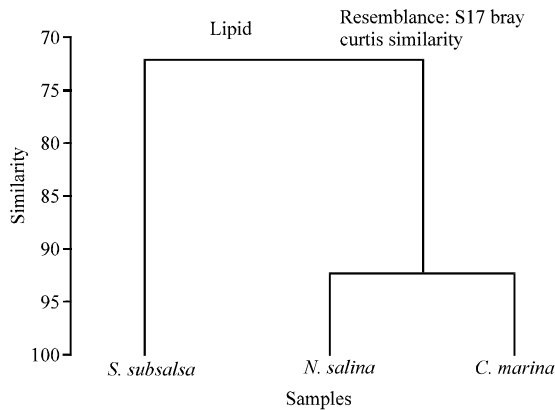


Fig. 2: Similarity index of *N. salina*, *C. salina* and *S. subsalsa* based on the lipid concentration

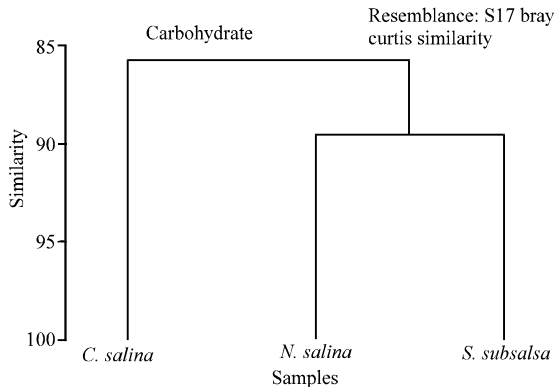


Fig. 3: Similarity index of *N. salina*, *C. salina* and *S. subsalsa* based on the carbohydrate concentration

well in cultures when fed algae, rice bran (Lavens and Sorgeloos, 2000), soybean meal or whey powder. The nauplii do not need food for 4 days after hatching.

Crude protein content of *Artemia* was similar across all life stages (nauplii X = 58.4%, adults X = 60.0%, DM basis) and was higher than the level present in the diet

(42.7%). The values reported here are like those of nauplii reported by Wantanabe *et al.* (1983), X = 61.6% DM and Webster and Lovell (1990), 55.6% DM. Crude fat was almost 2 times higher in the nauplii stage than in adults. It appears that the crude fat content of the diet (20.22% DM) did not contribute to the crude fat content of *Artemia* in any stage or the level present was not adequate for the needs of *Artemia*. Fat values reported earlier for nauplii (X = 19.4% DM, Wantanabe *et al.*, 1983; 20.1% DM, Webster and Lovell, 1990) are comparable to the nauplii values from this study (X = 13.2% DM).

Soluble carbohydrates were higher in nauplii than in adults and lower in all life stages compared to the diet. Either the diet is not a significant source of carbohydrate or the level present in the diet is not adequate for the needs of *Artemia* in any life stage. Fiber constituents (NDF, ADF and lignin) were diluted by feeding in both life stages, perhaps due to the lack of any fiber in the yeast diet. It should be noted that in the 24 h post-hatch and the unfed adult *Artemia*, data indicated a downward trend for all nutrients tested. It is therefore essential that *Artemia* be utilized as quickly as possible in order to avoid nutrient degradation.

Previously, Ozkizilcik and Chu (1994) were evaluated three enrichment techniques for their efficiency in improving the dietary value of *Artemia nauplii* to striped bass larvae. Newly hatched *Artemia nauplii* from the Great Salt Lake (GSL) were enriched for 24 h using the following diets:

- Gelatin-acacia microcapsules containing menhaden oil rich in Ω -3 Polyunsaturated Fatty Acids (PUFA), primarily 20:5 ω 3 eicosapentaenoic acid
- An emulsion of Baker's yeast and menhaden oil
- Marine *Chlorella* sp.

Unfed San Francisco Bay (SFB) and GSL nauplii were used as controls. Enriched GSL (all three diets) and unfed SFB nauplii had significantly higher ($p < 0.05$) levels of 20:503 than the unfed nauplii from GSL. About 7 days post-hatched (day 0) striped bass larvae were reared for 21 days on enriched or unfed nauplii. On day 21, wet weight and total length of striped bass larvae fed enriched GSL nauplii and unfed SFB nauplii were significantly greater ($p < 0.05$) than those fed unenriched GSL nauplii. The enrichment of the GSL nauplii appeared to increase the eicosapentaenoic acid content and enhance the growth of the striped bass larvae.

CONCLUSION

In this study, the proximate composition analyses of crude protein, lipid and carbohydrate showed a linear

increase in concentration in the Artemia biomass which was directly proportional with hours of enrichment and increase in concentration of enrichment media. From the four media, *N. salina*, *C. salina* and *S. subsalsa* are the natural media. The results showed the nutrients such as protein, carbohydrate, lipid present in *N. salina*, *C. salina* and *S. subsalsa* were transferred to Artemia through the enrichment process. Surprisingly, *N. salina* and *C. salina* are the lipid content algae so the enriched Artemia also contain high lipids. *Spirulina subsalsa* enriched Artemia contains high protein. But *C. salina* showed the good result in protein also. A1 DHA SELCO also showed very good but the expense is very high compare to algae. From this study, researchers can use algae (*N. salina*, *C. salina* and *S. subsalsa*) as an enrichment media instead of commercial A1 DHA SELCO media which give same nutrition level.

ACKNOWLEDGEMENTS

The researchers are thankful to the authorities of Annamalai University for the facilities and the National Fisheries Development Board, Government of India for financial assistance.

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