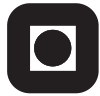

NTNU – Trondheim
Norwegian University of
Science and Technology

Time kinetics of fatty acids changes in total lipids and phospholipids of *A. franciscana* following enrichment and starvation, with a main focus on docosahexaenoic acid (DHA)

Yang Jin

Marine Coastal Development
Submission date: May 2013
Supervisor: Yngvar Olsen, IBI

Norwegian University of Science and Technology
Department of Biology

Acknowledgements

The work presented in this thesis was initiated when I started my master's study in Norwagian Universiy of Science and Technology (NTNU) in 2011, and all of the experimental work was performed at NTNU Centre of Fisheries and Aquaculture and SINTEF Fisheries and Aquaculture in Trondheim, from 2011 to 2013.

First, I want to thank my supervisor Professor Yngvar Olsen for his enthusiastic support throughout my work, and for his numerous and invaluable comments during and after my experiments. I would also like to thank my co-supervisor Keshuai Li for his excellent guidance and supervision under the entire duration of the project.

Furthermore, I would like to thank the employees at SINTEF and NTNU Sealab for the help and suggestions. I appreciate the guidance and knowledge of Jan Ove Evjemo, the technical help from Yngve Attramadal and the analytical help from Maria Bergvik and Kjersti Rennan Dahl.

Finally, I want to thank my parents and my girlfriend Li Gao for encouraging and supporting me, and also the Chinese friends in Trondheim and China for moral support.

Trondheim, 28. Feb 2013

Yang Jin

Abstract

HUFA in PL has been considered to be easily digested by marine fish larvae, which will result in better growth, survival and development (e.g., pigmentation, stress resistance and gut maturation). As one of the most commonly used live preys in aquaculture, *Artemia* sp. contain low amount of highly unsaturated fatty acids (HUFA), especially of docosahexaenoic acid (DHA, 22:6n-3) in phospholipids (PL) even after enrichment. The objective of the present thesis is to study time kinetics of change in important HUFA in total lipid (TL), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of *A. franciscana* nauplii and juveniles following short-term enrichment, subsequent starvation and long-term cultivation, with a main focus on DHA.

No DHA was found in PC and PE during the first hour of the short-term enrichment of *Artemia* nauplii. The content of HUFA did not accumulate in PC and PE during the first hour of enrichment, but significantly ($p<0.05$) increased in TL. After 1h, the content of HUFA in PC and PE increased gradually and the regression analysis showed that the content of DHA in PC and PE during short-term enrichment and following starvation, respectively, increased and decreased significant slower than that in TL. Moreover, relatively low content of DHA in TL was found during long-term cultivation of juvenile *Artemia* when compared with nauplii, whereas the content of DHA in PC and PE was similar. The contents of EPA and ARA in TL, PC and PE increased steadily during short-term enrichment, following starvation of nauplii, and long-term cultivation of juvenile *Artemia*. From the results we assumed that the accumulation of HUFA in PL during short-term enrichment of nauplii was probably due to fatty acids retailoring between PL and TAG, and an additional amount of DHA might be incorporated into PL through PL-synthesis pathway during long-term cultivation of juvenile *Artemia.*

Table of Content

1 Introduction 1

2 Materials and Methods 3

2.1 Production of nauplii and juvenile Artemia 3

2.2 Lipid and fatty acid analysis 5

2.2 Statistics 6

3 Results 7

3.1 Characterization of the diets and Artemia 7

3.2 Change of HUFA composition in the early phase of enrichment 7

3.3 Other important fatty acids in TL, PC and PE 12

4 Discussions 15

4.1 *Artemia vs*. diets 15

4.2 HUFA assimilation in Artemia 16

4.3 HUFA dissimilation in Artemia 18

4.4 Other important fatty acids in Artemia 18

4.5 Suggestions for Artemia production 20

References 22

Appendix 26

1 Introduction

The importance and essential function of highly unsaturated fatty acids (HUFA) such as DHA (docosahexaenoic acid, 22:6n-3), EPA (eicosapentaenoic acid, 20:5n-3) and ARA (arachidonic acid, 20:4n-6) in fish larvae nutrition has been demonstrated for long time (Tocher, *et al.*, 1989; Bell, *et al.*, 1999; Sargent, *et al.*, 1999; Lee, 2001; Sargent, *et al.*, 2002). These requirements are probably because marine fish larvae have limited ability in converting 18:3n-3 to DHA and EPA (Tocher, *et al.*, 1989; Sargent, *et al.*, 2002). Phospholipids (PL) and triacylglyceride (TAG) are main dietary HUFA suppliers for marine fish larvae. In larvae of many species, such as herring (*Clupea harengus*), haddock (*Melanogrammus aeglefinus*), cod (*Gadus morhua*) and halibut (*Hippoglossus hippoglossus*), PL is considered as the primary HUFA supplier which therefore plays an important role in growth, survival and larval development (e.g., pigmentation, stress resistance and gut maturation) (Kanazawa, 1997; Gisbert, *et al.*, 2005; Cahu, *et al.*, 2009).

The brine shrimp, *Artemia* sp. is widely used as a live feed organism for marine and freshwater fish larvae and juveniles in aquaculture all around the world. The most commonly used species is *Artemia franciscana,* which is the dominant *Artemia* population in Great Salt Lake and exploited for industrial purposes (Evjemo and Olsen, 1999). *Artemia* sp. is considered as an insufficient source of n-3 HUFA, especially of DHA and EPA for marine larvae (Leger, *et al.*, 1986; Navarro, *et al.*, 1992). The contents of these fatty acids can be increased by feeding, or enriching, the *Artemia* with HUFA-rich products, such as microalgae, microparticulate diets and lipid emulsions (Leger, *et al.*, 1986; Coutteau and Sorgeloos, 1997). However, the fatty acid composition of the live prey can only be manipulated to a limited extent and the result of the enrichment is largely depended on the enrichment diet and environmental conditions such as temperature, enrichment time and salinity (Leger, *et al.*, 1987; Dhert, *et al.*, 1993; Coutteau and Mourente, 1997). The nutritional problem of low HUFA contents seems to become more strongly expressed when applying

Artemia to species at higher latitudes, such as Atlantic cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus*). For example, halibut larvae fed marine copepods *T. longicornis* for 35 days contained over 4 times higher DHA in total lipid (TL) than those fed enriched *A. franciscana* with higher DHA contents than the copepods (Evjemo, *et al.*, 2003). It has been suggested that this may be because the n-3 HUFA in *Artemia*, especially DHA, is mostly accumulated in TAG after enrichment, whereas marine copepods forming the natural diet often contain high amount of HUFA in PL (Coutteau and Mourente, 1997; Bell, *et al.*, 2003; Ando, *et al.*, 2004).

The larger juvenile *Artemia* has been considered as a good alternative live feed for many fish species, such as *P. monodon* and *Lates calcarifer* (Dhert, *et al.*, 1993). In particular, different sizes of *Artemia* juveniles have frequently been used as live feed for Atlantic halibut *(Hippoglossus hippoglossus)*, giving a better pigmentation and metamorphosis of the fish than *Artemia* nauplii (Olsen, *et al.*, 1999a; Olsen, *et al.*, 1999b). Juvenile *Artemia* are rich in protein (Lim, 2001), but low in fat, especially HUFA such as DHA, EPA and ARA (Smith, *et al.*, 2002). This can be manipulated by short-term enrichment by emulsified diets such as DHA Selco after the cultivation of Juvenile *Artemia* (Smith, *et al.*, 2002).

It has been suggested that the metabolic pathways of lipids are the same in fish as in mammals (Sargent, *et al.*, 2002; Tocher, 2003; Tocher, *et al.*, 2008). Lipase converts TAG into free fatty acids (FFA) and monoacylglycerols (MAG) and phospholipase digests PL into FFA and lyso-PL (Tocher, *et al.*, 2008). The lyso-PL, MAG and FFA are carried in micelles to the intestinal epithelium and then absorbed by enterocytes. The TAG is re-synthesized in the enterocytes from MAG and FFA, following the same pathway in fish as in higher terrestrial mammals (Sargent, *et al.*, 2002). PL can be synthesized *de novo* by utilizing MAG, FFA and a polar head group, or re-synthesized through lyso-PL and FFA (Sargent, *et al.*, 1999; Tocher, *et al.*, 2008). It is assumed that at least some species of fish larvae may have limited ability in

synthesizing PL *de novo*, but may utilize the latter pathway (Bell, *et al.*, 2003; Tocher, *et al.*, 2008). Therefore, the HUFA may be transferred from diets into TAG and PL through synthetic pathways. Moreover, it has been suggested that the fatty acids incorporated in PL may also be retailored through combined acyl exchange since the required enzymes have been reported in fish (Tocher, *et al.*, 2008).

Few studies have focused on these metabolic pathways in live feed organisms such as rotifers and *Artemia.* It is not known how, for example, HUFA in diets is transferred into PL of rotifers and *Artemia*. We assume that the HUFA level in PL of *Artemia* can be manipulated by using enrichment diet, but how fast the level can increase in PL is unknown. The objective of the present thesis is to study time kinetics of change in important HUFA in total lipid (TL), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of *A. franciscana* nauplii and juveniles following short-term enrichment, subsequent starvation and long-term cultivation, with a main focus on DHA.

2 Materials and Methods

2.1 Production of nauplii and juvenile Artemia

Artemia franciscana cysts (Great Salt Lake, Utah, USA; INVE Aquaculture, Belgium) were decapsulated using hypochlorite, followed by rinsing and transferring into 60L conical plastic tanks with seawater (26℃) and strong aeration for hatching. After 24h, hatched *Artemia* were separated into 6 tanks for following fatty acid enrichment.

3 conical plastic tanks with 60L of seawater (34‰ salinity, 28℃) and strong aeration at the bottom of each tank were used for the enrichment of *Artemia* nauplii. The starting density was 120 ind mL^{-1}. *Artemia* were enriched with Multigain (BioMar Group, Denmark) given in a concentration of 200mg L^{-1} at the beginning of the

enrichment (Time 0) for 8h. The feed were consumed after 8h, and *Artemia* was thereafter starved for another 16h.

Juvenile *Artemia* were cultivated in 3 plastic tanks with 80L seawater at 28℃ for 4 days. The starting density was 30 ind. mL^{-1}. *Artemia* were fed with a combined diet that contained the algae paste Pavlova 1800 (Brown Biflagellate *Prymnesiophyceae*, Instant Algae®, Reed Mariculture Inc., USA; 0.19 ml L^{-1} $24h^{-1}$) and Multigain (1.3mg L^{-1} $12h^{-1}$). The fatty acid composition of the algae paste was analyzed by Reed Mariculture Inc. (Appendix Table 4 and 5). The fatty acid composition of the combined diet was estimated as the weighted mean of Pavlova 1800 and Multigain (Equation 1 and 2, below), where DW (Pav) and DW (Mul) are the feed ration for *Artemia* of Pavlova 1800 and Multigain, respectively, FAp and FAm are the absolute contents of fatty acid to be estimated in the mixed diet of Pavlova 1800 and Multigain, respectively. The terms mg g^{-1} DW is the absolute values and % is the relative composition. TFA is the absolute values of the total fatty acid of total lipid.

$$\text{FA (mg g}^{-1}\text{ DW)} = \frac{DW(Pav)*FAp(mg\ g{-}1DW)+DW(Mul)*FAm(mg\ g{-}1\ DW)}{DW(Pav)+DW(Mul)} \quad (1)$$

$$\text{FA \%} = \frac{DW(Pav)*TFA(Pav)*FAp(mg\ g{-}1\ DW)+DW(Mul)*TFA(Mul)*FAm(mg\ g{-}1\ DW)}{DW(Pav)*TFA(Pav)+TFA(Mul)*DW(Mul)} \quad (2)$$

The density was counted at the beginning and the end of the experiments for nauplii and every day after sampling for the juvenile *Artemia*. Samples were then taken from the center of the tanks by a hollow plastic pipe and mixed well before counting. Ten 0.1mL droplets were counted under a stereo-microscope to determine the number of live *Artemia* and for total numbers after fixation with Lugol's solution. Survival was calculated by Equation 3 below. 10 Juvenile *Artemia* individuals were sampled every day for the measurement of body lengths under 25X magnification in a stereo-microscope.

$$\text{Survival \%} = \frac{\text{number of live } A.franciscana}{\text{total number of } A.franciscana} \quad (3)$$

2.2 Lipid and fatty acid analysis

Artemia samples for lipid and fatty acid analysis were sampled at time 0h, 0.5h, 1h, 2h, 4h, 8h, 12h, 24h, day 2, day 3 and day 4. *Artemia* were sampled by a sieve with a mesh size of 64μm, rinsed by filtered seawater and tap water. Concentrated *Artemia* were scraped off into a 50ml plastic tube and were immediately frozen by liquid nitrogen. All samples were freeze dried and stored at -80℃ under nitrogen atmosphere before analysis.

Lipid extraction was prepared following a modified method of Bligh and Dyer (1959). Each 30mg freeze dried sample was homogenized in a blendor (IKA® T10 basic Ultra-Turrax®) for 1 min with 0.8mL distilled water (dH_2O), 2mL methanol and 1ml chloroform. After that, the mixture was added 1mL chloroform and blending for 20 seconds. 1mL dH_2O was added and blending continued for another 20 seconds. The homogenate was then centrifuged (Hettich universal 32R) at 4000rpm for 10min at 5℃. After centrifugation, 1ml of chloroform was transferred into a tube (40×8.2mm HOLGER TEKNOLOGI) and dried under N_2 at 40℃ in the sample concentrator (DB3D TECHNE DRI-BLOCK®). Concentrated samples were weighted on analytical balance (UM×2 METTLER TOLEDO) after 24h in the drying vessel for total lipid.

Lipid separation of PC and PE was undertaken using thin-layer chromatography (TLC) based on a method of Fraser, *et al.* (1985). Extracted total lipid was sprayed on silica plates (PLC silica gel 60 F254, 0.5mm, 20×20cm, MERCKKGaA), and separated by using mixture solvent chloroform/methanol/water (67/30/3, v/v/v) for 45 min. The PC and PE zones were detected under ultraviolet light, scrapped off and put into 11mL quick fit test tubes. The elution was done by adding 3 ml chloroform/methanol (2/1) and centrifuged 2 min (5℃,4000rpm Hettich universal 32R) for three times. The

upper layer without silica was collected and evaporated at 60℃ under N_2.

Extracted total lipid, PC and PE was then used for preparing Fatty acid methyl esters (FAMEs) for fatty acids analysis following the method of Metcalfe, *et al.* (1966). The FAMEs were determined quantitatively by a gas chromatograph (Auto system XL PERKIN ELMER). An internal standard (IS) 19:0 methyl ester was added to the samples before extraction for analyzing the absolute amount of fatty acids in total lipid. A modified method from Abdulkadir and Tsuchiya (2008) was used for analyzing fatty acids in PC and PE of *Artemia* juveniles. The scrapped PC and PE with silica were added 0.5 mL isooctane, followed by 0.2 mL 14% BF_3 in methanol, and heated at 100℃ for 2h. After cooling on ice, 0.1 mL isooctane and 0.2 mL distilled water were added into the tubes. After centrifugation for 3 min (5℃,4000 rpm Hettich universal 32R), the upper layer was transferred and analyzed on a gas chromatograph (Auto system XL PERKIN ELMER). The method of direct methylation of PC and PE with silica was validated by comparing with the conventional methods of Fraser, *et al.* (1985) and Metcalfe, *et al.* (1966). 8 replicates was taken from one freeze dried sample, extracted total lipid and sprayed on silica plate for TLC. Separated PC and PE were scrapped and put into 8 quick fit test tubes, 4 for conventional methods and 4 for direct methylation. No significant difference ($p>0.05$, data not shown) was observed for the percent of fatty acids in PC and PE by using the two methods.

2.3 Statistics

The experimental data were tested for statistical significance by using one-way analysis of variance (ANOVA) with Tukey's multiple comparison test, and differences were considered significant at the $P<0.05$ level.

All the statistical tests were performed by SPSS 19.0 for windows. All tables were made in word 2010, and figures were made by Sigma plot 12.0.

3 Results

3.1 Characterization of the diets and Artemia

Table 1 shows the contents of highly unsaturated fatty acids (HUFA), total fatty acids (TFA) and total lipid (TL) in *Artemia* diets. Multigain was used for the short-term enrichment of *Artemia* nauplii and the combined diet with Pavlova and Multigain was used for long-term cultivation of juvenile *Artemia.* Both diets contained high amount of DHA and relatively low amount of EPA and ARA. Multigain contained 1.8 times higher TL, 2.1 times higher TFA and more than 5 times higher HUFA contents than the combined diet.

Short-term enrichment of *Artemia* nauplii by Multigain resulted in a significant ($p<0.05$) increase of TL content by 10% after 8h of incubation (Table 2). The content of TL decreased thereafter during the starvation period and the final content of TL (24h) was significantly ($p<0.05$) lower than that in the newly hatched nauplii (0h). In juvenile *Artemia,* the content of TL decreased significantly ($p<0.05$) from day 2 to day 3, but increased again significantly ($p<0.05$) on day 4. The content of TL was throughout significantly ($p<0.05$) higher in nauplii than in juvenile *Artemia*. The time kinetics of change of TFA in *Artemia* was in accordance with that of TL. The percent content of TFA remained above 70% in nauplii, whereas it was between 50% and 60% in juvenile *Artemia*. The body length did not change significantly ($p>0.05$) during the first 24h, but it increased significantly ($p<0.05$) during long-term cultivation (day 2 to day 4). The survival rates were higher than 90% during both nauplii and juvenile cultivation.

3.2 Change of HUFA composition in the early phase of enrichment

The percent contents of HUFA in TL, PC and PE of *Artemia* during the first part of the short-term enrichment process are shown in Fig. 1. Similar curves were observed

Table 1. Contents of HUFAs, TFA and TL of the diets and the combine diet (mg g^{-1} DW (% of TFA))

	DHA	EPA	ARA	TFA	TL
Multigain	122 (33.1)	5.3 (1.4)	6.0 (1.6)	367	427
Pavlova (*Prymnesiophyceae*)	14.2 (11.2)	0.3 (0.2)	0.5 (0.4)	126	200
Combined Diet[a]	22.5 (15.5)	0.6 (0.4)	0.9 (0.6)	172	242

[a] Combined Diet of Multigain and algae paste Pavlova (Weighted Average)

Table 2. Body length (mm), Survival (%), TFA (mg g^{-1} DW) and TL content (mg g^{-1} DW) of *A. franciscana* nauplii and juveniles

	Body Length	Survival	TFA	TL
A. franciscana nauplii				
0h	0.93±0.07^{a}	100^{a}	128±0.5^{a}	166±0.3^{a}
8h		92.7±2.5^{b}	142±9.3^{b}	182±0.8^{b}
24h	0.91±0.09^{a}	91.9±3.8^{b}	96.1±8.9^{c}	135±1.3^{c}
A. franciscana juveniles				
2 day	1.08±0.12^{a}	93.6±0.7^{a}	69.1±3.8^{a}	121±6.6^{a}
3 day	1.21±0.10^{b}	90.6±2.4^{a}	49.3±4.1^{b}	96.5±7.9^{b}
4 day	1.28±0.11^{c}	90.3±3.9^{a}	55.5±1.5^{c}	109±4.6^{c}

Different subscript letters within a column indicate significant difference ($p<0.05$).

between quantitative (Fig. 1 A) and relative (Fig. 1 B) contents of DHA, EPA and ARA in TL, and between relative contents of the fatty acids in PC (Fig. 1 C) and PE (Fig. 1 D). No DHA and relatively low contents of EPA and ARA were found in TL of newly hatched nauplii (Fig. 1 A, B). The absolute and relative contents of DHA, EPA and ARA in TL increased significantly ($p<0.05$) between 0h and 1h (Fig. 1 A, B). Moreover, the absolute and relative contents of DHA in TL increased much faster than that of EPA and ARA (Fig. 1 A, B), which was in agreement with the high content of DHA in Multigain (Table 1). In PC and PE, however, the content of DHA remained at 0% during the first hour of the enrichment and increased gradually thereafter (Fig. 1 C,

D). A similar delay was observed for the percent contents of EPA and ARA in PC and PE, which also increased gradually after one hour (Fig. 1 C, D). The percent contents of DHA in PC and PE were significantly (p<0.05) lower than the contents of EPA and ARA (Fig. 1 C, D). The percent contents of EPA in TL, PC and PE were throughout significantly (p<0.05) higher than the ARA percentage (Fig. 1 A, B, C, D).

The time kinetics of changes in the contents of HUFA following enrichment beyond 1h (1-8h), through successive starvation (8-24h) of nauplii, and through long-term cultivation of juvenile *Artemia* are shown in Figure 2. Similar curves were observed between quantitative (Fig. 2 A, B and C) and relative (Fig. 2 D, E and F) contents of DHA, EPA and ARA in TL of *Artemia* nauplii and juveniles. During enrichment period, the absolute and relative contents of DHA in TL increased rapidly with time after 1h and reached the maximum (11.4±1.3 mg g^{-1} DW; 8.00±0.5%) at 8h (Fig. 2 A and D; Appendix Table 4 and 5). During the starvation period, the content of DHA in TL sharply decreased to 3.67±1.2 mg g^{-1} DW (3.83±1.1%) at 24h (Fig. 2 B and E; Appendix Table 4, 5). A slight increase of the relative content of DHA was also found in TL of juvenile *Artemia* between day 2 and day 4 (Fig 2 F), whereas the quantitative content of the fatty acid in TL was almost constant during the same period (Fig 2 C). The absolute and relative contents of EPA and ARA increased steadily in TL during enrichment, starvation and long-term cultivation (Fig. 2 A-F) except that the absolute content of EPA was almost constant (p>0.05) between day 2 and day 4 (Fig. 2 C, Appendix Table 4). The absolute and relative contents of DHA were throughout higher than the contents of EPA and ARA in nauplii (Fig. 2 A, B, D and E), but significantly (p<0.05) lower than EPA and ARA in juvenile *Artemia* (Fig. 2 C and F). Similar curves were also observed between the relative contents of DHA, EPA and ARA in PC (Fig. 2 G, H and I) and PE (Fig. 2 J, K and L). In PC and PE, the DHA percentages increased gradually beyond 1h and the maximum contents were also found at 8h (Fig. 2 G and J), where after it decreased gradually reaching 1.54±0.1% and 1.70±0.1% after starvation for PC and PE, respectively (Fig. 2 H and K; Appendix Table 6 and 7). The percent contents of DHA in PC and PE also increased

during long-term cultivation and the rates of increase were 130% and 173%, respectively (Fig. 2 I and L, Appendix Table 6 and 7). An increase of the EPA and ARA contents was also found in PC and PE of *Artemia* during enrichment, starvation and long-term cultivation (Fig 2 G-L). The percent contents of DHA in PC and PE were throughout significantly lower than the contents of EPA in nauplii and juvenile *Artemia* (Fig 2 G, H, I, J, K and L). The contents of EPA in TL, PC and PE of nauplii and juvenile *Artemia* were throughout higher than the contents of ARA (Fig. 2 A-L). The percent contents of EPA and ARA in *Artemia* juvenile (Fig.2 C, F, I and L) were higher than that in nauplii (Fig. 2 A, B, D, E, G, H, J and K).

The regression analysis of DHA contents in TL, PC and PE of nauplii and juvenile *Artemia* is shown in Table 3. The slopes of the regression lines are a measure of the rate of increase or decrease (if the slope<0) of DHA in specific lipid classes (TL, PC and PE) during the specific period (enrichment, starvation and long-term

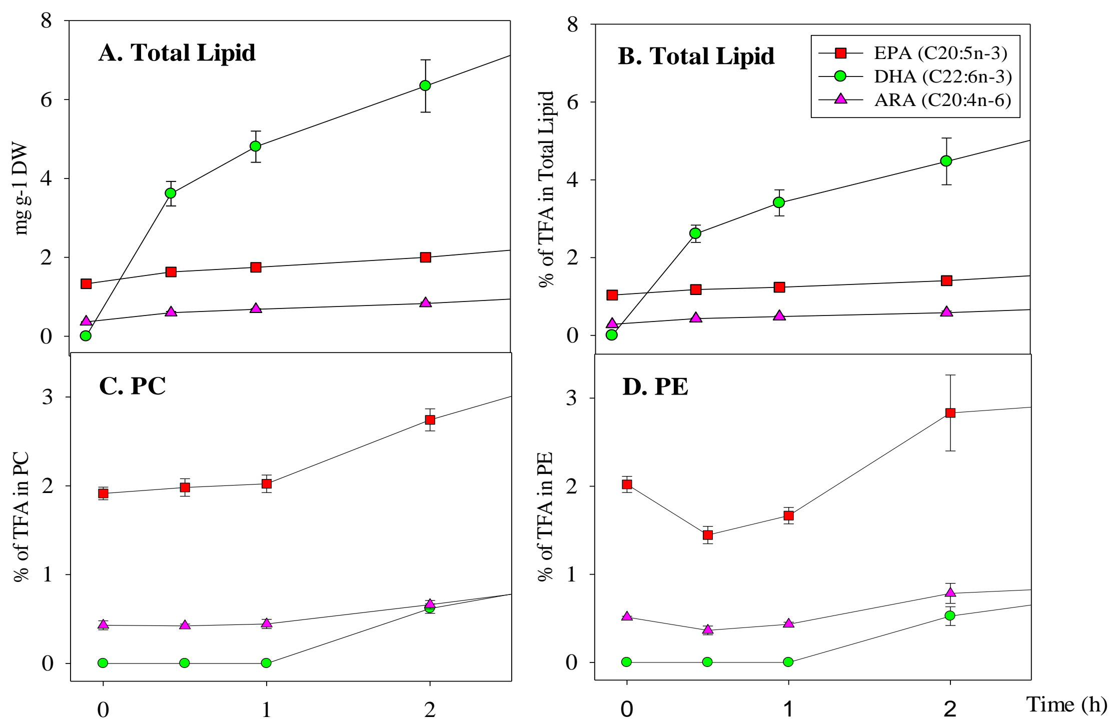

Figure 1 The contents of HUFAs in TL, PC and PE of *A. franciscana* following the early phase of enrichment by Multigain (0-2h). Error bars indicate SE from 2 replicates analyses at time 0, 3 replicate cultures with 2 replicate analyses each in later sampling.

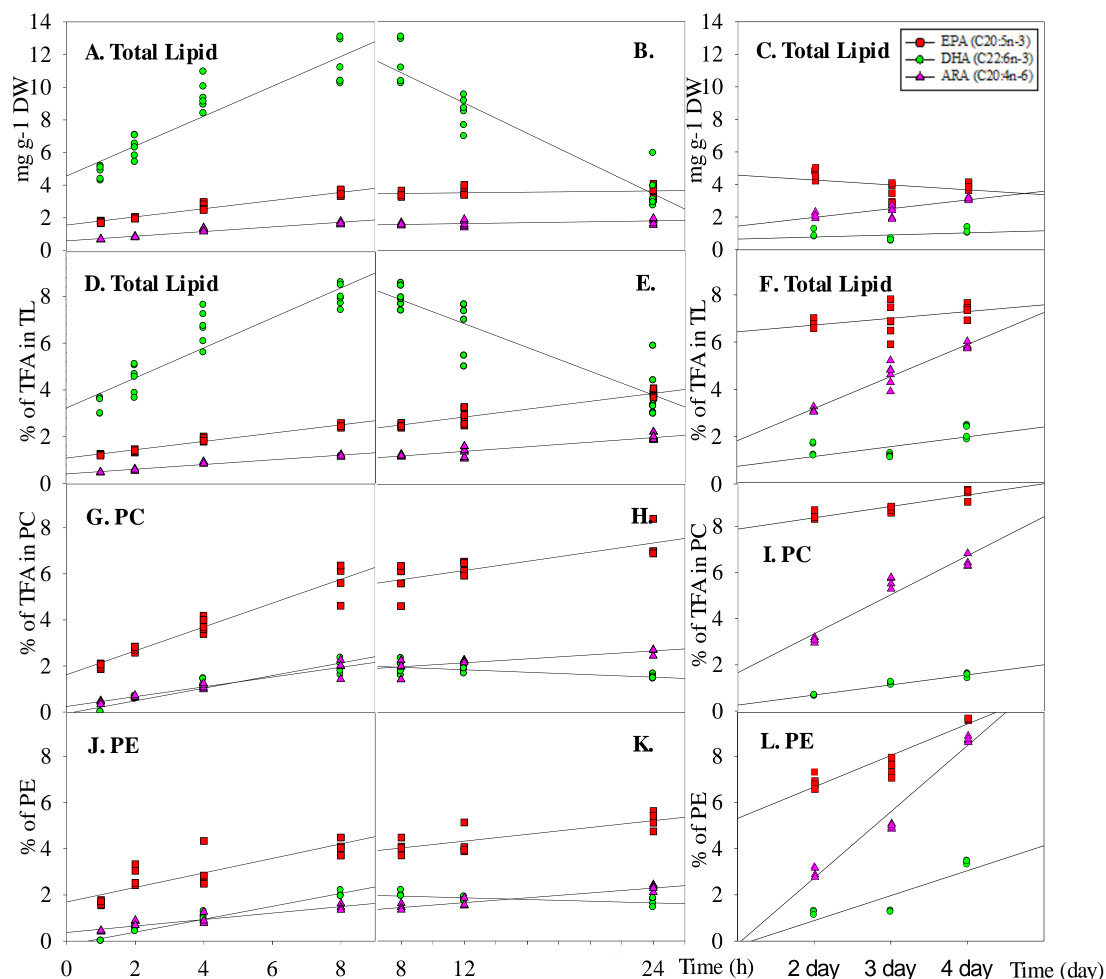

Figure 2 Change of HUFAs contents in TL, PC and PE of *A. franciscana* during enrichment (1-8h), starvation (8-24h) and long-term cultivation (2-4 day).

cultivation). All components showed a linear change in the DHA content with increasing time except that a non-linear change was found for the quantitative content of DHA in TL during long-term cultivation of juvenile *Artemia* (Table 3). The slope coefficients for absolute content of DHA in TL of *Artemia* nauplii during enrichment (1-8h) and starvation (8-24h) period were, respectively, significantly (p<0.05) higher than those for relative DHA contents during the same period. The rates of increase of the absolute and relative contents of DHA in TL during enrichment were higher than the rates of decrease during starvation. The slope coefficients for absolute and relative contents of DHA in TL of *Artemia* juvenile were significantly lower than those of *Artemia* nauplii during enrichment. For PC and PE, the slope coefficients during

enrichment and starvation were significantly ($p<0.05$) lower than those for TL within the same period. Moreover, the rates of increase for the contents of DHA in PC and PE during enrichment were more than 9 times higher than the rates of decrease during starvation period. The slope coefficient for the content of DHA in PE of juvenile *Artemia* was significantly ($p<0.05$) higher than that in TL and PC.

3.3 Other important fatty acids in TL, PC and PE

Figure 3 shows the absolute and relative contents of other important fatty acids in *Artemia* during short-term enrichment and starvation. The major fatty acids in TL, PC and PE of *Artemia* nauplii were C18:3n-3, C18:1n-9 and C16:0. The absolute and relative contents of C18:1n-9 and C18:3n-3 in TL decreased all through enrichment and starvation (0-24h) (Fig.3 A, B). The absolute amount (mg g^{-1} DW) of C16:0 in TL increased by 42.6% during enrichment and decreased thereafter during starvation (Fig.3 A). However, the percent content of C16:0 increased significantly ($p<0.05$) from the start of enrichment period (0h) to the end of the starvation period (24h) (Fig.3 B). The content of C18:3n-3 was significantly ($p<0.05$) higher than that of C18:1n-9 in PC of newly hatched nauplii, and it decreased by 28% all through enrichment and starvation (0-24h) (Fig. 3 C). On the other hand, the content of C18:1n-9 in PC remained almost unchanged during the same period ($p>0.05$) (Fig. 3 C). No significant difference ($p>0.05$) was found for the contents of C18:3n-3 and C18:1n-9 at 24h (Appendix Table 6). The percent content of C18:1n-9 was throughout significantly ($p<0.05$) higher than C18:3n-3 in PE (Fig.3 D). Both of the fatty acids decreased in PE during the enrichment and starvation. The percent content of C16:0 in PC increased steadily during enrichment and starvation period (Fig.3 C). However, it varied unsystematically with time in PE (Fig. 3 D).

Similar amounts ($p>0.05$) of C18:3n-3 and C18:1n-9 were found in TL of juvenile *Artemia* on day 2 (Fig. 4 A, B). However, the absolute and relative contents of C18:1n-9 were significantly ($p<0.05$) higher than the contents of C18:3n-3 on day 4

(Fig. 4 A, B). The absolute amount of C16:0 in TL significantly ($p<0.05$) decreased from day 2 to day 4 (Fig. 4 A) whereas the relative amount of C16:0 in TL remained stable ($p>0.05$) (Fig. 4 B). The contents of C18:1n-9 in PC and PE were throughout significantly higher than the contents of C18:3n-3 ($p<0.05$) (Fig. 4 C, D). The percent content of C16:0 significantly decreased in PC ($p<0.05$), but remained unchanged ($p>0.05$) in PE from day 2 to day 4 (Fig. 4 C, D). A significantly ($p<0.05$) higher percent content of C16:0 was observed in PC (more than 14%) than in PE (less than 5%) (Appendix Table 6 and 7).

Table 3 Regression analysis of DHA content in TL, PC and PE of *A. franciscana* nauplii and juvenile (DHA content = At + B; t is the time (h); A is the slope of the regression line (h^{-1}))

	A	r^2	p	n
A. franciscana nauplii (1-8h)				
TL[1], mg g^{-1} DW	0.92±0.09[a]	0.83	<0.001	6
TL[2], % of TFA	0.64±0.06[b]	0.85	<0.001	6
PC, % of TFA	0.27±0.02[c]	0.88	<0.001	4
PE, % of TFA	0.28±0.01[c]	0.96	<0.001	4
A. franciscana nauplii (8-24h)				
TL[1], mg g^{-1} DW	-0.46±0.04[a]	0.88	<0.001	6
TL[2], % of TFA	-0.26±0.03[b]	0.79	<0.001	6
PC, % of TFA	-0.03±0.01[c]	0.49	0.011	4
PE, % of TFA	-0.02±0.01[c]	0.47	0.014	4
A. franciscana juveniles (2-4 day)				
TL[1], mg g^{-1} DW	0.01±0.01[a]	0.10	0.260	4
TL[2], % of TFA	0.02±0.01[b]	0.42	0.010	4
PC, % of TFA	0.02±0.00[b]	0.96	<0.001	4
PE, % of TFA	0.05±0.01[c]	0.76	<0.001	4

[1]Absolut DHA Content (mg g^{-1} DW) [2]Relative DHA Content (% of TL)

Different subscript letters within a column indicate significant difference ($p<0.05$).

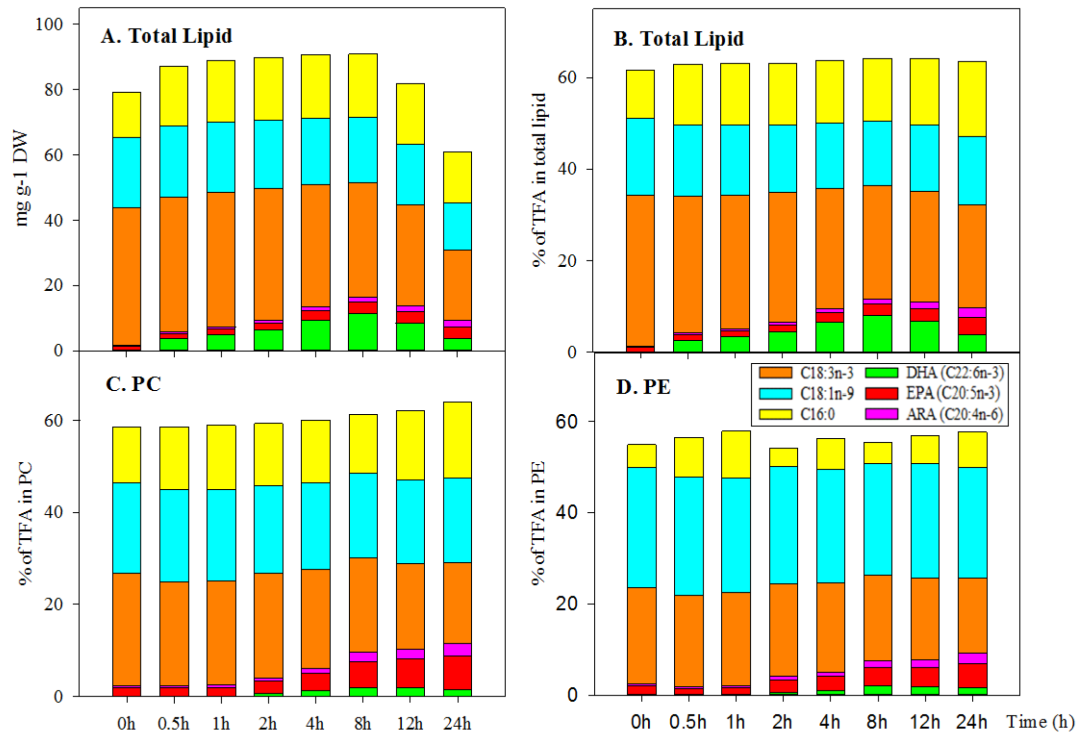

Fig. 3 Contents of important HUFAs in TL, PC and PE of *A. franciscana* nauplii following short-term enrichment (0-8h) with Multigain and following starvation (8-24h).

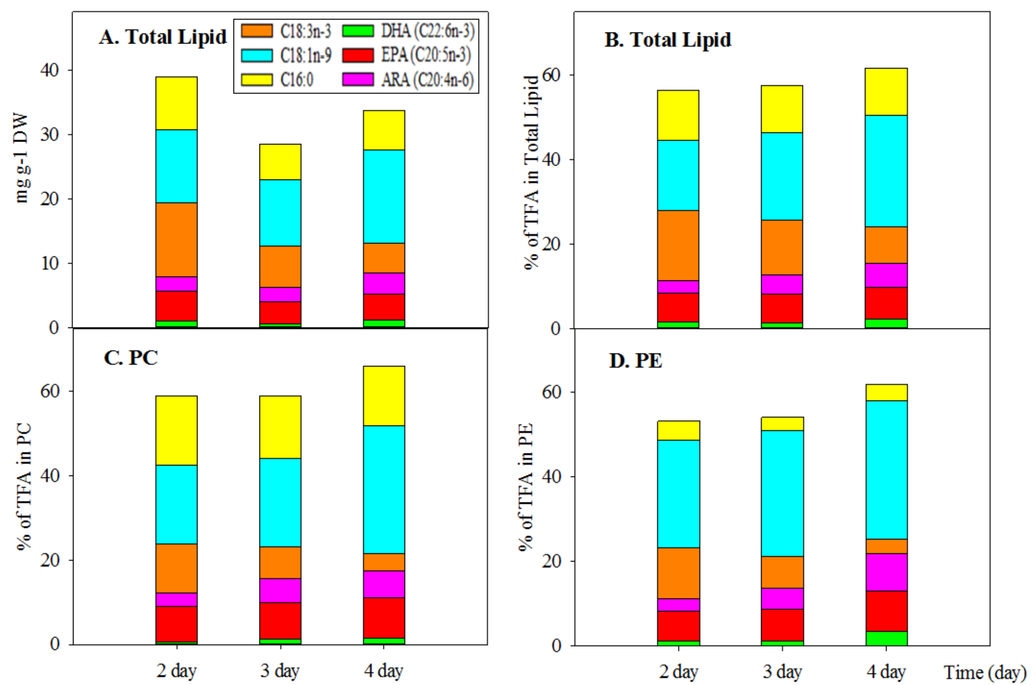

Fig. 4 Contents of important HUFAs in TL, PC and PE of *A. franciscana* juveniles as a function of cultivation time by the combined diet.

4 Discussions

Few published data has reported on the development of HUFA contents of PL in *Artemia* sp. during the periods of enrichment and starvation (Coutteau and Mourente, 1997). The present study was focused on the time kinetics of HUFA contents in PC and PE of *Artemia* after short-term enrichment, subsequent starvation and through long-term cultivation of juvenile *Artemia*.

4.1 Artemia vs. diets

The content of TL in newly hatched *Artemia* sp. nauplii is largely depended on the genetic characteristics of the strain and the quality of the cysts. In present study, the content of TL in newly hatched *Artemia* nauplii (16%, Table 2) was in agreement with the studies from Evjemo, *et al.* (1997) and Harel, *et al.* (1999), whereas Boglino, *et al.* (2012) and McEvoy, *et al.* (1996) reported on nauplii which contained only 12% TL. The nauplii of the same species that were used in Coutteau and Mourente (1997) contained higher amount of TL (20%). Other species like *A. sinica*, studied by Evjemo, *et al.* (1997), contained about 13% of TL in newly hatched nauplii.

As compared with the present study, Boglino, *et al.* (2012) also used Multigain (BioMar Group, Denmark) as enrichment diet for enriching *Artemia* nauplii. The differences of the two studies might be due to the different enrichment time (8h versus 24h) and an additional supply of Multigain at 12h in the study of Boglino, *et al.* (2012). Similar dosage of Multigain (1.5 *versus* 1.67µg ind^{-1}) and same water temperature (26℃) were used in the two studies. The study from Boglino, *et al.* (2012) has resulted in lower content of TL (160 mg g^{-1} DW) with higher percentage of DHA in TL (16.9 %) after 24h enrichment *versus* our value of 182 mg g^{-1} DW and 8.0 % after 8h. Similar percentages of EPA and ARA in TL were observed in both studies.

Juvenile *Artemia* may attain an optimal growth rate which is determined by food

concentration, animal density and environmental conditions (i.e., temperature, salinity and water quality) (Evjemo and Olsen, 1999). Sick (1976) has found that *Artemia* grown on five different species of algae at 25℃ exhibited significantly different growth rate and survival. Evjemo and Olsen (1999) obtained 10-12% higher growth rate than Sick (1976) when *Artemia* was fed *I. galbana* at 28℃. Comparing with the six species of algae that were used in the former studies, the growth rate of *Artemia* juveniles after 4 days cultivation fed with Pavlova paste at 26℃ in present study was higher than those fed with *chlorella conductrix* and *nitzschia closterium* from Sick (1976), but lower than those fed other four species of algae from Sick (1976) and Evjemo and Olsen (1999). The differences might be due to the various cysts quality of *Artemia* and different enrichment diets.

4.2 HUFA assimilation in Artemia

PC and PE are the two main components of PL of *Artemia* (Navarro*, et al.*, 1991). Similar experiment was done by Coutteau and Mourente (1997) for fatty acid analysis of PC and PE in 24h *Artemia* enriched by triacylglycerol (TAG) and ethyl esters (EE). The study has resulted more than 1.5 times higher content of TL with 1.4 times higher content of DHA in TL after 24h enrichment as compared with the present study. However, trace amounts of DHA in PC (2.6% and 1.9% for TAG and EE enrichment, respectively) and PE (2.0% for both enrichments) were found after enrichment in their experiment, which were similar to the values at 8h in the present study (Figure 2, Appendix Table 6 and 7). Unpublished data from McEvoy and Navarro (1994) cited in Bell*, et al.* (2003) also showed that *Artemia* contained 2.4% and 0.6% of DHA in PL after 24h enriched by tuna orbital oil/herring roe phospholipid (88/12 w/w) and Super Selco^{TM}, respectively. All three studies have illustrated that only limiting amount of DHA could be enriched into PC (less than 3%) and PE (around 2%) of *Artemia* regardless of the level of TL and DHA in TL after enrichment. The percentages of EPA in PC and PE from the other two studies were more than 2 times higher than those in the present study after enrichment. The percent content of ARA

from McEvoy and Navarro (1994, unpublished) was around 2% after enrichment, which was in accordance with the result from the present study. The present study also showed that the DHA/EPA ratio of TL of *Artemia* nauplii was >2 after short-term enrichment (Appendix Table 4 and 5), which was similar to the ratio in TL of Calanoid copepods (Sargent, *et al.*, 2002). On the other hand, the DHA/EPA ratio in PC and PE in the present study was around 0.35 after enrichment (Appendix Table 6, 7), which was similar to the studies from others on the same species of *Artemia*. (Coutteau and Mourente, 1997; McEvoy and Navarro, 1994, unpublished data)

As PL is the main constitution of cell membrane bilayers, the synthesis of PL in *Artemia* is assumed to be related to their growth. Therefore, the 8h enrichment of *Artemia* nauplii in the present study might not result in significant increase of PL. The study from Coutteau and Mourente (1997) have shown that the quantitative content of PC and PE in *Artemia* nauplii remained constant ($p>0.05$) during 24h enrichment with TAG and EE. Similar result was obtained by McEvoy, *et al.* (1996) that the quantitative content of PC and PE in *Artemia* nauplii remained relatively stable after 16h enrichment with 12% herring roe in tuna orbital oil, Super Selco (Artemia Systems, INVE, Ghent) and baker's yeast. Rainuzzo, *et al.* (1994) also found that the quantitative content of PL in rotifers seems to be independent of enrichment diet and method.

The following questions arise: How does HUFA (e.g., DHA, EPA and ARA) in diets assimilate into PC and PE? Mourente, *et al.* (1991) has found that acylases and transacylases do not have specificities for esterifying particular fatty acids into PL, which means that an exchange of fatty acids between and within PL and TAG could happen if there is a concentration gradient (Sargent, *et al.*, 1999). A review from Tocher (2003) has showed that many enzymes for the PL remodeling have been demonstrated in fish. In the present study, high content of DHA in Multigain (Table 1) may result in high absorbed DHA in TAG of *Artemia* nauplii, which was suggested to be the main lipid class that accumulate DHA (Coutteau and Mourente, 1997; Bell, *et*

al., 2003; Ando*, et al.*, 2004). The remodeling of PL in *Artemia* nauplii might happen and the exchanged fatty acids were probably between DHA in TAG and 18:3n-3 in PL (Fig. 3). The slow increase of DHA in PC and PE could be explained by the specificity of fatty acids during remodeling that DHA was competing with other fatty acids such as EPA and ARA for reacylating into PC and PE. Although the contents of EPA and ARA in Multigain were relatively low (Table 1), an increase of EPA and ARA was still observed in PC and PE of *Artemia* during enrichment. This could be explained by: (a) unlike DHA, EPA and ARA were naturally present in PC and PE of un-enriched *Artemia* nauplii; and (b) the retroconversion of DHA to EPA during enrichment (Navarro*, et al.*, 1999). A time delay of approximately 1h was found (Figure 3) before the exchange of HUFA started in PC and PE (Figure 3). This is compatible with the fact that it will take some time to digest, accumulate and reacylate HUFA into PC and PE.

Unlike the 24h enriched and starved nauplii, the juvenile *Artemia* contained less than 2% of DHA in TL (Fig. 4), which was probably due to the low DHA content in the combined diet (Table 1). This means that the content of DHA that are reacylated into PC and PE becomes lower because of the low concentration of DHA in TAG. However, similar content of DHA was found in PC and PE of juvenile *Artemia* and nauplii (Fig.4). This was probably because an additional content of DHA was incorporated into PL through synthetic pathway since juvenile *Artemia* require an abundance of PL for new tissue growth during long-term cultivation. In addition, a lower increase rate of DHA was found in *Artemia* juvenile when compared with nauplii. That was probably because of the lower DHA concentration in the combined diet as compared to the enrichment diet (Table 1).

4.3 HUFA dissimilation in Artemia

During the starvation period, the contents of lipids and fatty acids in *Artemia* are likely to become reduced and the degree of reduction is mostly depended on

temperature and time (Evjemo*, et al.*, 2001). Evjemo*, et al.* (2001) have found that the loss rate of TL content in *Artemia* was 1.42% h^{-1} at 26℃ and 0.46% h^{-1} at 12℃. Moreover, DHA in TL was lost at a rate more than twice than that of TL (Coutteau and Mourente, 1997; Evjemo*, et al.*, 2001). Our present study showed loss rate of TL and DHA of 1.64% h^{-1} and 4.7% h^{-1}, respectively, at 26℃. The rapid decrease of DHA in TL might be because: (a) *Artemia* are naturally deficient in DHA, which might make it easily to be excluded (Figure 1); (b) there was a continuous and rapid retroconversion of DHA to EPA during starvation (Navarro*, et al.*, 1999); and (c) 91% of the total content of DHA was concentrated in TAG and the majority of the content was esterifed in *sn*-3 position instead of sn-2 position (Ando*, et al.*, 2004), which exposed DHA to be easily catabolized.

On the other hand, the content of EPA was still increasing during the starvation period in the present study (Figure 3). That was probably because: (a) the retroconversion from DHA (Navarro*, et al.*, 1999); and (b) EPA was mainly esterified in *sn*-2 position of TAG (Ando*, et al.*, 2004) and was relatively protected in this position.

Relatively few studies have been done on the contents of HUFA in TL of *Artemia* during starvation (Evjemo*, et al.*, 1997; Estevez*, et al.*, 1998; Evjemo*, et al.*, 2001), even less studies were focusing on the contents of HUFA in PC and PE (Coutteau and Mourente, 1997). In the present study, the content of DHA in PC and PE decreased, but the contents of EPA and ARA increased during the starvation period (Figure 2), which was in accordance with the study carried out by Coutteau and Mourente (1997). However, slightly higher DHA (1.5% *versus* 1.4% of PC; 1.7% *versus* 1.0% of PE), but more than 2 times lower EPA in PC and PE were found in the present study rather than the study from Coutteau and Mourente (1997). This was probably due to the lower temperature (12℃) and longer starvation time (72h) in his study rather than in the present study (26℃ and 18h).

In present study, the content of DHA in TL reduced significantly ($p<0.05$) faster than

that in PC and PE of *Artemia* nauplii (Table 3). The reason for the loss of DHA in PC and PE might also be the remodeling, meaning that DHA in PC and PE could have been exchanged by other fatty acids as the concentration of DHA in TAG was decreased during the starvation period. The slow loss of DHA content in PC and PE might be because the position of HUFA like DHA were preferentially esterified in *sn*-2 position (Tocher*, et al.*, 2008), which made DHA less available for being catabolized as compared with its position in TAG. The percentages of EPA and ARA in PC and PE increased during the same period, in agreement with their concentration in TL which was still high.

4.4 Other important fatty acids in Artemia

The increase and decrease of C16:0, C18:1n-9 and C18:3n-3 in TL of *Artemia* nauplii (Fig. 3) was related to the fatty acid composition of the enrichment diet (Appendix, Table 4, 5). The rates of change of the three fatty acids in PC and PE of *Artemia* nauplii were in accordance with that in TL (Fig. 3 C, D), which was probably due to the remodeling of fatty acids in PC and PE. The content of C16:0 in PC of juvenile *Artemia* was significantly higher than that in PE (Figure 3, 4). The reason for this has been suggested by Tocher (2003) that different specificities of the enzymes involved in deacylation/reacylation may cause different fatty acids distribution among PC and PE.

4.5 Suggestions for Artemia production

The present study has revealed that different enrichment time may affect the TL content and fatty acids composition in TL and PL of *Artemia* nauplii. Based on the present study, we suggest that the enrichment time of *Artemia* nauplii should be longer than 8h to ensure that a sufficient amount of HUFA, especially DHA, are incorporated into PL, which can be more beneficial for marine fish larvae (Sargent*, et al.*, 2002; Wold*, et al.*, 2007; Tocher*, et al.*, 2008; Cahu*, et al.*, 2009; Wold*, et al.*,

2009). Moreover, the fact that HUFA did not accumulate in PL during the first hour of enrichment, but mainly become incorporated in TAG, suggests that it is possible to produce *Artemia* for scientific experiments with variable HUFA enrichments in PL and TAG.

Artemia juveniles may be a good alternative live feed for marine fish species since it contained low levels of TL (Table 1) and high levels of HUFA in PL (Fig. 4). The relative low levels of DHA in TL of *Artemia* juveniles can additionally be manipulated by short-time enrichment by emulsified diet such as DHA Selco after cultivation (Smith*, et al.*, 2002). An obstacle for applying juvenile *Artemia* in marine fish larval rearing might be the relatively longer body length of juvenile *Artemia*, the higher bacteria load in culture (Olsen*, et al.*, 1999a), and the extra cost for cultivation.

References

Abdulkadir, S., Tsuchiya, M., 2008. One-step method for quantitative and qualitative analysis of fatty acids in marine animal samples. J Exp Mar Biol Ecol. 354, 1-8.

Ando, Y., Samoto, H., Murayama, Y., 2004. Positional distribution of DHA and EPA in triacyl-sn-glycerols (TAG) of *Artemia franciscana* nauplii enriched with fish oils ethyl esters and TAG. Aquaculture. 233, 321-335.

Bell, J.G., Tocher, D.R., Farndale, B.M., McVicar, A.H., Sargent, J.R., 1999. Effects of essential fatty acid-deficient diets on growth, mortality, tissue histopathology and fatty acid compositions in juvenile turbot (*Scophthalmus maximus*). Fish Physiol Biochem. 20, 263-277.

Bell, J.G., McEvoy, L.A., Estevez, A., Shields, R.J., Sargent, J.R., 2003. Optimising lipid nutrition in first-feeding flatfish larvae. Aquaculture. 227, 211-220.

Boglino, A., Darias, M.J., Ortiz-Delgado, J.B., Ozcan, F., Estevez, A., Andree, K.B., Hontoria, F., Sarasquete, C., Gisbert, E., 2012. Commercial products for *Artemia* enrichment affect growth performance, digestive system maturation, ossification and incidence of skeletal deformities in Senegalese sole (*Solea senegalensis*) larvae. Aquaculture. 324, 290-302.

Cahu, C.L., Gisbert, E., Villeneuve, L.A.N., Morais, S., Hamza, N., Wold, P.A., Infante, J.L.Z., 2009. Influence of dietary phospholipids on early ontogenesis of fish. Aquac Res. 40, 989-999.

Coutteau, P., Sorgeloos, P., 1997. Manipulation of dietary lipids, fatty acids and vitamins in zooplankton cultures. Freshwater Biol. 38, 501-512.

Coutteau, P., Mourente, G., 1997. Lipid classes and their content of n-3 highly unsaturated fatty acids (HUFA) in *Artemia franciscana* after hatching, HUFA-enrichment and subsequent starvation. Mar Biol. 130, 81-91.

Dhert, P., Sorgeloos, P., Devresse, B., 1993. Contributions towards a specific DHA enrichment in the live food *Brachionus plicatilis* and *Artemia* Sp. Fish Farming Technology, 109-115.

Estevez, A., McEvoy, L.A., Bell, J.G., Sargent, J.R., 1998. Effects of temperature and

starvation time on the pattern and rate of loss of essential fatty acids in *Artemia* nauplii previously enriched using arachidonic acid and eicosapentaenoic acid-rich emulsions. Aquaculture. 165, 295-311.

Evjemo, J.O., Olsen, Y., 1999. Effect of food concentration on the growth and production rate of *Artemia franciscana* feeding on algae (*T-iso*). J Exp Mar Biol Ecol. 242, 273-296.

Evjemo, J.O., Danielsen, T.L., Olsen, Y., 2001. Losses of lipid, protein and n-3 fatty acids in enriched *Artemia franciscana* starved at different temperatures. Aquaculture. 193, 65-80.

Evjemo, J.O., Reitan, K.I., Olsen, Y., 2003. Copepods as live food organisms in the larval rearing of halibut larvae (*Hippoglossus hippoglossus* L.) with special emphasis on the nutritional value. Aquaculture. 227, 191-210.

Evjemo, J.O., Coutteau, P., Olsen, Y., Sorgeloos, P., 1997. The stability of docosahexaenoic acid in two *Artemia* species following enrichment and subsequent starvation. Aquaculture. 155, 135-148.

Fraser, A.J., Tocher, D.R., Sargent, J.R., 1985. Thin-layer chromatography flame ionization detection and the quantitation of marine neutral lipids and phospholipids. J Exp Mar Biol Ecol. 88, 91-99.

Gisbert, E., Villeneuve, L., Zambonino-Infante, J.L., Quazuguel, P., Cahu, C.L., 2005. Dietary phospholipids are more efficient than neutral lipids for long-chain polyunsaturated fatty acid supply in European sea bass *Dicentrarchus labrax* larval development. Lipids. 40, 609-618.

Harel, M., Ozkizilcik, S., Lund, E., Behrens, P., Place, A.R., 1999. Enhanced absorption of docosahexaenoic acid (DHA, 22: 6n-3) in *Artemia* nauplii using a dietary combination of DHA-rich phospholipids and DHA-sodium salts. Comp Biochem Phys B. 124, 169-176.

Kanazawa, A., 1997. Effects of docosahexaenoic acid and phospholipids on stress tolerance of fish. Aquaculture. 155, 129-134.

Lee, S.M., 2001. Review of the lipid and essential fatty acid requirements of rockfish (*Sebastes schlegeli*). Aquac Res. 32, 8-17.

Leger, P., Naessens-Foucquaert, E., Sorgeloos, P., 1987. International study on *Artemia*

XXXV. Techniques to manipulate the fatty acid profile in *Artemia* nauplii and the effect on its nutritional effectiveness for the marine crustacean *Mysidopsis bahia* (M). in: Sorgeloos, P., Bengtson, D.A., Decleir, W., Jaspers, E. (Ed.), *Artemia* Research and its Applications. Universa Press, Wetteren, Belgium, pp. 411-424.

Leger, P., Bengtson, D.A., Simpson, K.L., Sorgeloos, P., 1986. The Use and Nutritional-Value of *Artemia* as a Food Source. Oceanogr Mar Biol. 24, 521-623.

Lim, L.C., Soh, A., Dhert, P., Sorgeloos, P.,, 2001. Production and application of on-grown *Artemia* in freshwater ornamental fish farm. Aquaculture Economics & Management. 5, 18.

McEvoy, L.A., Navarro, J.C., Hontoria, F., Amat, F., Sargent, J.R., 1996. Two novel *Artemia* enrichment diets containing polar lipid. Aquaculture. 144, 339-352.

Metcalfe, L.D., Schmitz, A.A., Pelka, J.R., 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal Chem. 38, 514-&.

Mourente, G., Tocher, D.R., Sargent, J.R., 1991. Specific accumulation of docosahexaenoic acid (22-6n-3) in brain lipids during development of juvenile turbot *Scophthalmus maximus* L. Lipids. 26, 871-877.

Navarro, J.C., Amat, F., Sargent, J.R., 1991. A study of the variations in lipid-levels, lipid class composition and fatty-acid composition in the 1st stages of *Artemia* sp. Mar Biol. 111, 461-465.

Navarro, J.C., Amat, F., Sargent, J.R., 1992. Fatty-acid composition of coastal and inland *Artemia* sp. populations from Spain. Aquaculture. 102, 219-230.

Navarro, J.C., Henderson, R.J., McEvoy, L.A., Bell, M.V., Amat, F., 1999. Lipid conversions during enrichment of *Artemia*. Aquaculture. 174, 155-166.

Olsen, A.I., Attramadal, Y., Jensen, A., Olsen, Y., 1999a. Influence of size and nutritional value of *Artemia franciscana* on growth and quality of halibut larvae (*Hippoglossus hippoglossus*) during the live feed period. Aquaculture. 179, 475-487.

Olsen, Y., Evjemo, J.O., Olsen, A., 1999b. Status of the cultivation technology for production of Atlantic halibut (*Hippoglossus hippoglossus*) juveniles in Norway Europe. Aquaculture. 176, 3-13.

Rainuzzo, J., Reitan, K., Olsen, Y., 1994. Effect of short- and long-term lipid enrichment on

total lipids, lipid class and fatty acid composition in rotifers. Aquacult Int. 2, 19-32.

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., Tocher, D., 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture. 179, 217-229.

Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The Lipids. in: Halver, J.G., Hardy, R.W. (Eds.), Fish Nutrition. Elsevier Inc., USA.

Sick, L.V., 1976. Nutritional effect of 5 species of marine-algae on growth, development, and survival of brine shrimp *Artemia-Salina*. Mar Biol. 35, 69-78.

Smith, G.G., Ritar, A.J., Phleger, C.F., Nelson, M.M., Mooney, B., Nichols, P.D., Hart, P.R., 2002. Changes in gut content and composition of juvenile *Artemia* after oil enrichment and during starvation. Aquaculture. 208, 137-158.

Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev Fish Sci. 11, 107-184.

Tocher, D.R., Carr, J., Sargent, J.R., 1989. Poly-unsaturated fatty-acid metabolism in fish cells - differential metabolism of (n-3) and (n-6) series acids by cultured-cells originating from a fresh-water teleost fish and from a marine teleost fish. Comp Biochem Phys B. 94, 367-374.

Tocher, D.R., Bendiksen, E.A., Campbell, P.J., Bell, J.G., 2008. The role of phospholipids in nutrition and metabolism of teleost fish. Aquaculture. 280, 21-34.

Wold, P.A., Hoehne-Reitan, K., Cahu, C.L., Infante, J.Z., Rainuzzo, J., Kjorsvik, E., 2007. Phospholipids *vs*. neutral lipids: Effects on digestive enzymes in Atlantic cod (*Gadus morhua*) larvae. Aquaculture. 272, 502-513.

Wold, P.A., Hoehne-Reitan, K., Cahu, C.L., Infante, J.Z., Rainuzzo, J., Kjorsvik, E., 2009. Comparison of dietary phospholipids and neutral lipids: effects on gut, liver and pancreas histology in Atlantic cod (*Gadus morha* L.) larvae. Aquacult Nutr. 15, 73-84.

Appendix

Table 4 All Fatty Acids Contents (mg/g DW) in Total lipid of diets (Multigain and Combination), *Artemia*nauplii (0-24h) and juvenile (2-4 day)

	0h(n=2)	0.5h(n=6)	1h(n=6)	2h(n=6)	4h(n=6)	8h(n=6)	12h(n=6)	24h(n=6)	D2(n=4)	D3(n=6)	D4(n=5)	Mul(n=2)	Pav	Comb(n=2)
C14:0	0.73±0.0	1.50±0.1	1.65±0.0	1.71±0.1	1.84±0.1	1.76±0.2	1.54±0.1	1.23±0.2	1.18±0.2	0.57±0.0	0.71±0.1	24.0±0.9	15.8	16.5±0.1
C16:0	13.6 ± 0.1^{a}	18.3 ± 0.8^{b}	19.0 ± 0.6^{b}	19.1 ± 0.7^{b}	19.4 ± 1.1^{b}	19.4 ± 1.5^{b}	18.5 ± 1.0^{b}	15.6 ± 0.9^{a}	8.14 ± 0.5^{c}	5.53 ± 0.2^{d}	6.11 ± 0.5^{d}	111 ± 1.8^{e}	11.7	19.3 ± 0.1^{b}
C18:0	6.41±0.0	6.75±0.5	6.85±0.2	6.84±0.2	6.95±0.3	6.63±0.3	6.28±0.3	6.53±0.6	6.00±0.2	4.81±0.3	3.89±0.1	3.59±0.2	0.38	0.63±0.0
C20:0	0.12±0.0	0.15±0.0	0.15±0.0	0.15±0.0	0.15±0.0	0.15±0.0	0.15±0.0	0.19±0.0	0.13±0.0	0.08±0.0	0.08±0.0	0.43±0.0	0.00	0.03±0.0
C22:0	0.18±0.0	0.19±0.0	0.22±0.0	0.21±0.0	0.25±0.0	0.25±0.0	0.23±0.0	0.27±0.0	0.21±0.0	0.17±0.0	0.18±0.0	0.44±0.0	0.00	0.03±0.0
C16:1n7	2.43±0.0	2.44±0.2	2.39±0.2	2.36±0.2	2.32±0.2	2.39±0.2	2.32±0.5	1.71±0.2	1.12±0.1	0.85±0.1	1.13±0.1	1.12±0.1	7.99	7.46±0.0
C18:1n7	6.94±0.0	7.19±0.5	7.16±0.3	6.94±0.6	6.96±0.4	6.97±0.3	6.82±0.8	6.22±0.5	5.31±0.2	5.03±0.5	4.60±0.1	1.92±0.1	2.03	2.02±0.0
C18:1n9	21.5 ± 0.1^{a}	21.6 ± 1.6^{a}	21.5 ± 0.9^{a}	21.0 ± 1.5^{a}	20.4 ± 1.1^{a}	19.8 ± 1.3^{a}	18.4 ± 2.8^{ab}	$14.3\pm1.7b^{c}$	11.5 ± 0.5^{cd}	10.2 ± 1.3^{d}	14.5 ± 0.2^{b}	9.98 ± 0.6^{d}	12.8	12.6 ± 0.0^{cd}
C20:1n9	0.59±0.0	0.59±0.1	0.59±0.0	0.59±0.0	0.59±0.0	0.59±0.0	0.55±0.0	0.50±0.1	0.31±0.0	0.24±0.0	0.24±0.0	0.00±0.0	2.16	1.99±0.0
C18:2n6	7.63±0.0	7.72±0.6	7.79±0.3	7.79±0.4	7.58±0.3	7.30±0.5	6.55±0.8	4.70±0.6	5.3±0.5	3.28±0.2	3.61±0.2	8.58±0.4	7.23	7.33±0.0
C18:3n3	42.2 ± 0.1^{a}	41.3 ± 3.5^{ab}	41.2 ± 1.4^{ac}	40.6 ± 1.7^{ac}	37.5 ± 1.5^{bcd}	35.1 ± 2.2^{bd}	31.0 ± 3.5^{d}	21.7 ± 2.7^{e}	11.5 ± 0.6^{f}	6.37 ± 0.5^{g}	4.68 ± 0.3^{h}	7.76 ± 0.6^{g}	6.97	7.03 ± 0.0^{g}
C18:4n3	8.26±0.0	8.02±0.7	7.98±0.3	7.79±0.4	7.00±0.2	6.30±0.5	5.19±0.8	2.94±0.5	2.90±0.3	1.19±0.1	0.82±0.1	2.50±0.2	22.6	21.0±0.0
C20:2n6	0.26±0.0	0.27±0.0	0.27±0.0	0.27±0.0	0.28±0.0	0.26±0.0	0.25±0.0	0.24±0.0	0.21±0.0	0.18±0.0	0.16±0.0	0.00±0.0	0.13	0.12±0.0
C20:3n3	1.69±0.0	1.68±0.1	1.71±0.1	1.70±0.0	1.65±0.1	1.55±0.1	1.37±0.1	1.11±0.2	0.52±0.0	0.36±0.0	0.22±0.0	0.81±0.1	1.14	1.12±0.0
C20:3n6	0.14±0.0	0.19±0.0	0.20±0.0	0.21±0.0	0.24±0.0	0.24±0.0	0.21±0.0	0.17±0.0	0.14±0.0	0.10±0.0	0.15±0.0	1.18±0.0	0.13	0.21±0.0
C20:4n3	1.32±0.0	1.40±0.1	1.43±0.0	1.44±0.0	1.39±0.0	1.32±0.1	1.11±0.1	0.67±0.1	0.40±0.1	0.16±0.0	0.15±0.0	2.83±0.1	0.13	0.33±0.0
C20:4n6	0.37 ± 0.0^{a}	0.60 ± 0.0^{b}	0.68 ± 0.0^{c}	0.83 ± 0.0^{d}	1.28 ± 0.1^{e}	1.67 ± 0.1^{f}	1.71 ± 0.2^{f}	1.88 ± 0.2^{f}	2.16 ± 0.2^{f}	2.29 ± 0.4^{f}	3.19 ± 0.1^{g}	5.99 ± 0.3^{h}	0.51	0.92 ± 0.0^{d}
C20:5n3	1.33 ± 0.0^{a}	1.63 ± 0.1^{b}	1.75 ± 0.1^{b}	2.00 ± 0.0^{c}	2.73 ± 0.2^{d}	3.49 ± 0.2^{e}	3.67 ± 0.3^{e}	3.68 ± 0.3^{e}	4.70 ± 0.3^{f}	3.42 ± 0.6^{de}	4.01 ± 0.2^{ef}	5.25 ± 0.3^{f}	0.25	0.64 ± 0.0^{g}
C22:5n6	0.00±0.0	1.40±0.1	1.84±0.1	2.38±0.2	3.61±0.3	4.47±0.4	3.55±0.5	1.74±0.5	0.23±0.0	0.20±0.0	0.40±0.1	47.5±1.9	2.16	5.64±0.1
C22:6n3	0.00 ± 0.0^{a}	3.61 ± 0.3^{b}	4.81 ± 0.4^{c}	6.34 ± 0.7^{d}	9.45 ± 0.9^{e}	11.4 ± 1.3^{e}	8.44 ± 0.9^{de}	3.67 ± 1.2^{f}	1.02 ± 0.3^{gh}	0.59 ± 0.1^{g}	1.22 ± 0.2^{h}	122 ± 5.2^{i}	14.2	22.5 ± 0.4^{j}
Unknow	12.5±0.0	12.0±1.0	12.0±0.7	12.0±0.7	11.0±0.6	10.5±0.7	9.60±1.5	6.75±0.9	6.17±0.8	3.65±0.2	4.56±0.3	11.8±0.2	14.6	14.4±0.0
SFA	21.1 ± 0.2^{a}	26.9 ± 1.3^{bc}	27.9 ± 0.7^{b}	28.0 ± 1.0^{b}	28.6 ± 1.5^{b}	28.3 ± 1.9^{b}	26.8 ± 1.2^{bc}	23.9 ± 1.4^{ac}	15.7 ± 0.9^{d}	11.2 ± 0.6^{e}	11.0 ± 0.7^{e}	139 ± 2.9^{f}	28.8	37.3 ± 0.2^{g}
MUFA	31.5 ± 0.2^{a}	31.8 ± 2.2^{a}	31.7 ± 1.3^{a}	30.9 ± 2.3^{a}	30.3 ± 1.6^{a}	29.8 ± 1.8^{a}	28.1 ± 4.0^{ab}	22.8 ± 2.3^{bc}	18.2 ± 0.7^{ce}	16.4 ± 1.9^{e}	20.4 ± 0.1^{ce}	13.0 ± 0.8^{e}	25.0	24.1 ± 0.1^{b}
PUFA	63.2 ± 0.1^{a}	67.8 ± 5.3^{ab}	69.7 ± 1.9^{b}	71.3 ± 1.8^{b}	72.7 ± 2.1^{b}	73.0 ± 5.3^{ab}	63.1 ± 4.1^{ab}	42.5 ± 5.1^{c}	29.0 ± 2.0^{d}	18.2 ± 1.7^{e}	18.6 ± 0.6^{e}	204 ± 9.1^{f}	58.4	69.6 ± 0.7^{b}
TFA	128 ± 0.5^{a}	139 ± 9.6^{ab}	141 ± 4.2^{b}	142 ± 5.3^{b}	143 ± 5.4^{b}	142 ± 9.3^{ab}	128 ± 10^{ab}	95.9 ± 8.9^{c}	69.1 ± 3.8^{d}	49.3 ± 4.1^{e}	$54.6+1.5^{e}$	368 ± 13^{f}	127	145 ± 1.0^{b}

Different subscript letters within a row indicate significant difference ($p<0.05$).

Table 5 All Fatty Acid Content (% of Total Fatty Acid) in Total lipid of diets (Multigain and Combination), *Artemia*nauplii (0-24h) and juvenile (2-4 day)

	0h(n=2)	0.5h(n=6)	1h(n=6)	2h(n=6)	4h(n=6)	8h(n=6)	12h(n=6)	24h(n=6)	D2(n=4)	D3(n=6)	D4(n=5)	Mul(n=2)	Pav	Comb(n=2)
C14:0	0.57±0.0	1.09±0.0	1.17±0.0	1.2±0.0	1.29±0.0	1.24±0.0	1.21±0.1	1.29±0.2	1.69±0.3	1.16±0.1	1.31±0.1	6.53±0.0	12.5	11.3±0.0
C16:0	10.7±0.1^{a}	13.3±0.6^{b}	13.5±0.4^{b}	13.4±0.4^{b}	13.6±0.4^{b}	13.8±0.5^{b}	14.6±0.8^{bc}	16.4±1.3^{c}	11.8±0.1^{a}	11.2±0.5^{a}	11.2±0.6^{a}	30.1±0.6^{d}	9.20	13.2±0.1^{b}
C18:0	5.00±0.0	4.87±0.0	4.85±0.0	4.81±0.1	4.87±0.1	4.68±0.1	4.93±0.2	6.82±0.2	8.69±0.3	9.77±0.4	7.12±0.1	0.98±0.0	0.30	0.43±0.0
C20:0	0.09±0.0	0.11±0.0	0.10±0.0	0.11±0.0	0.11±0.0	0.10±0.0	0.12±0.0	0.19±0.0	0.19±0.0	0.17±0.0	0.15±0.0	0.12±0.0	0.00	0.02±0.0
C22:0	0.14±0.0	0.14±0.0	0.15±0.0	0.14±0.0	0.18±0.0	0.18±0.0	0.18±0.0	0.28±0.0	0.31±0.0	0.34±0.1	0.33±0.1	0.12±0.0	0.00	0.02±0.0
C16:1n7	1.90±0.0	1.77±0.0	1.69±0.1	1.66±0.1	1.63±0.1	1.69±0.1	1.81±0.3	1.79±0.2	1.62±0.0	1.73±0.1	2.08±0.1	0.30±0.0	6.30	5.13±0.0
C18:1n7	5.41±0.0	5.19±0.1	5.07±0.1	4.88±0.3	4.88±0.1	4.93±0.1	5.33±0.2	6.5±0.4	7.70±0.3	10.2±0.3	8.44±0.4	0.52±0.0	1.60	1.39±0.0
C18:1n9	16.8±0.0^{a}	15.6±0.2^{b}	15.3±0.3^{b}	14.8±0.6^{bc}	14.3±0.3^{c}	14.0±0.1^{c}	14.4±1.0^{bc}	14.9±0.6^{bc}	16.6±1.2^{abc}	20.7±1.1^{d}	26.5±0.5^{e}	2.71±0.1^{f}	10.1	8.66±0.0^{g}
C20:1n9	0.46±0.0	0.43±0.0	0.42±0.0	0.42±0.0	0.41±0.0	0.42±0.0	0.43±0.0	0.53±0.0	0.44±0.0	0.48±0.0	0.44±0.0	0.00±0.0	1.70	1.37±0.0
C18:2n6	5.95±0.0	5.57±0.1	5.51±0.1	5.47±0.1	5.32±0.0	5.16±0.1	5.12±0.2	4.9±0.2	7.66±0.4	6.67±0.2	6.62±0.2	2.33±0.0	5.70	5.04±0.0
C18:3n3	32.9±0.1^{a}	29.8±0.5^{b}	29.2±0.2^{b}	28.5±0.3^{c}	26.3±0.3^{d}	24.8±0.2^{e}	24.3±0.8^{ef}	22.6±0.9^{f}	16.6±0.4^{g}	12.9±0.7^{h}	8.59±0.7^{i}	2.11±0.1^{j}	5.50	4.84±0.0^{k}
C18:4n3	6.44±0.0	5.78±0.1	5.65±0.1	5.48±0.1	4.91±0.1	4.44±0.1	4.05±0.3	3.05±0.2	4.19±0.2	2.42±0.2	1.51±0.1	0.68±0.0	17.8	14.5±0.0
C20:2n6	0.21±0.0	0.20±0.0	0.19±0.0	0.19±0.0	0.19±0.0	0.18±0.0	0.19±0.0	0.25±0.0	0.30±0.0	0.37±0.0	0.29±0.0	0.00±0.0	0.10	0.08±0.0
C20:3n3	1.32±0.0	1.21±0.0	1.21±0.0	1.20±0.0	1.16±0.0	1.09±0.0	1.08±0.1	1.15±0.1	0.76±0.1	0.73±0.0	0.41±0.0	0.22±0.0	0.90	0.77±0.0
C20:3n6	0.11±0.0	0.14±0.0	0.14±0.0	0.15±0.0	0.17±0.0	0.17±0.0	0.17±0.0	0.17±0.0	0.21±0.0	0.21±0.0	0.27±0.0	0.32±0.0	0.10	0.14±0.0
C20:4n3	1.03±0.0	1.01±0.0	1.01±0.0	1.01±0.0	0.98±0.0	0.93±0.0	0.87±0.0	0.69±0.0	0.57±0.1	0.33±0.0	0.27±0.0	0.77±0.0	0.10	0.23±0.0
C20:4n6	0.28±0.0^{a}	0.43±0.0^{b}	0.48±0.0^{c}	0.58±0.0^{d}	0.90±0.1^{e}	1.18±0.0^{f}	1.35±0.2^{fk}	1.96±0.1^{g}	3.12±0.1^{h}	4.62±0.5^{i}	5.85±0.1^{j}	1.63±0.0^{k}	0.40	0.64±0.0^{d}
C20:5n3	1.04±0.0^{a}	1.18±0.0^{b}	1.24±0.0^{b}	1.41±0.1^{c}	1.91±0.1^{d}	2.47±0.1^{e}	2.89±0.3^{e}	3.85±0.1^{f}	6.80±0.2^{g}	6.91±0.7^{g}	7.35±0.3^{g}	1.43±0.0^{c}	0.20	0.44±0.0^{h}
C22:5n6	0.00±0.0	1.01±0.1	1.3±0.1	1.68±0.2	2.54±0.3	3.15±0.1	2.81±0.5	1.82±0.4	0.34±0.0	0.41±0.1	0.74±0.2	12.9±0.1	1.70	3.88±0.0
C22:6n3	0.00±0.0^{a}	2.61±0.2^{b}	3.41±0.3^{c}	4.47±0.6^{d}	6.64±0.7^{e}	8.00±0.5^{e}	6.69±1.2^{e}	3.83±1.1^{bc}	1.46±0.3^{fg}	1.19±0.1^{f}	2.24±0.3^{bg}	33.1±0.3^{h}	11.2	15.5±0.1^{i}
Unknow	9.72±0.0	8.63±0.2	8.47±0.3	8.47±0.2	7.68±0.2	7.42±0.2	7.50±0.5	7.02±0.4	8.91±0.8	7.41±0.2	8.50±0.7	3.20±0.2	11,5	9.88±0.0
SFA	16.5±0.1^{a}	19.5±0.6^{b}	19.8±0.5^{b}	19.7±0.4^{b}	20.1±0.4^{b}	20.0±0.5^{b}	21.1±1.0^{bc}	25.0±1.4^{de}	22.7±0.1^{cd}	22.7±0.8^{cd}	20.6±1.1^{b}	37.8±0.5^{f}	22.7	25.6±0.1^{e}
MUFA	24.5±0.0^{a}	23.0±0.2^{b}	22.4±0.4^{b}	21.7±0.9^{bc}	21.2±0.5^{c}	21.0±0.3^{c}	21.9±1.4^{bc}	23.7±1.1^{ab}	26.0±1.6^{abc}	32.1±2.6^{d}	36.6±1.7^{e}	3.54±0.1^{f}	19.7	16.6±0.0^{g}
PUFA	49.3±0.1^{ac}	48.9±0.5^{ag}	49.4±0.2^{ac}	50.1±0.7^{ab}	51.0±0.9^{bc}	51.6±0.8^{b}	49.5±1.4^{abg}	44.3±1.7^{d}	42.2±0.7^{d}	37.5±2.2^{e}	34.7±1.3^{e}	55.4±0.6^{f}	46.1	47.9±0.1^{g}

Different subscript letters within a row indicate significant difference ($p<0.05$).

Table 6 All Fatty Acid Content (% of Total Fatty Acid) in PC of *Artemia*nauplii (0-24h) and juvenile (2-4 day)

	0h (n=2)	0.5h (n=4)	1h (n=6)	2h (n=4)	4h (n=6)	8h (n=4)	12h (n=4)	24h (n=4)	2day	3day	4day
C14:0	0.40±0.0	0.41±0.0	0.45±0.1	0.54±0.1	0.62±0.1	0.61±0.1	0.72±0.1	0.66±0.1	1.40±0.1	1.51±0.1	1.28±0.1
C16:0	12.0±0.1^{a}	13.7±0.8^{ab}	13.9±1.0^{ab}	13.5±0.6^{ab}	13.6±1.1^{ab}	12.7±1.4^{ab}	15.1±1.0^{ac}	16.4±2.1^{ac}	16.5±0.3^{c}	14.9±0.5^{b}	14.2±0.8^{a}
C16:1n7	1.61±0.0	1.65±0.1	1.65±0.1	1.63±0.0	1.62±0.1	1.66±0.1	1.69±0.1	1.86±0.1	1.65±0.1	1.66±0.0	2.00±0.0
C18:0	9.20±0.3	9.69±0.4	9.64±0.5	9.99±0.1	9.93±0.3	9.53±0.3	9.96±0.4	9.87±1.1	11.3±0.1	11.4±0.2	8.16±0.2
C18:1n7	7.29±0.1	7.08±0.2	7.08±0.2	7.12±0.2	7.05±0.1	7.35±0.2	7.77±0.3	8.57±0.1	7.58±0.2	9.46±0.1	7.85±0.2
C18:1n9	19.8±0.2^{ab}	20.0±0.2^{b}	20.0±0.2^{b}	19.0±0.1^{a}	18.7±0.4^{a}	18.5±0.3^{a}	18.3±0.4^{a}	18.5±0.9^{ab}	18.8±0.9^{ab}	20.9±1.1^{ab}	30.3±0.9^{c}
C18:2n6	5.19±0.0	5.16±0.2	5.19±0.2	5.23±0.1	5.16±0.1	5.27±0.1	4.92±0.2	4.77±0.3	7.02±0.5	5.44±0.4	5.60±0.2
C18:3n3	24.4±0.2^{a}	22.5±1.1^{ab}	22.6±1.3^{ab}	22.7±0.3^{b}	21.5±0.7^{bc}	20.5±0.4^{bc}	18.4±1.1^{cd}	17.5±1.0^{d}	11.5±0.3^{e}	7.59±0.1^{f}	4.14±0.3^{g}
C18:4n3	8.94±0.2	8.26±0.3	8.14±0.5	8.10±0.2	7.13±0.3	5.89±0.3	4.49±0.1	2.96±0.4	0.20±0.0	0.20±0.0	0.13±0.0
C20:1n9	0.64±0.0	0.54±0.0	0.54±0.0	0.55±0.0	0.57±0.0	0.64±0.1	0.62±0.0	0.52±0.0	0.39±0.0	0.41±0.0	0.39±0.0
C20:2n6	0.30±0.0	0.28±0.0	0.28±0.0	0.31±0.0	0.31±0.0	0.32±0.0	0.35±0.1	0.30±0.0	0.30±0.0	0.37±0.0	0.30±0.0
C20:3n3	1.55±0.0	1.39±0.0	1.33±0.0	1.42±0.1	1.36±0.1	1.37±0.1	1.31±0.0	1.09±0.1	0.59±0.0	0.58±0.0	0.29±0.0
C20:4n3	0.96±0.1	0.61±0.1	0.61±0.1	0.63±0.1	0.70±0.1	0.66±0.1	0.53±0.1	0.33±0.0	0.30±0.0	0.20±0.0	0.14±0.0
C20:4n6	0.43±0.1^{a}	0.42±0.0^{a}	0.44±0.1^{a}	0.66±0.1^{b}	1.13±0.1^{c}	1.92±0.4^{d}	2.23±0.1^{d}	2.65±0.1^{de}	3.08±0.1^{e}	5.59±0.2^{f}	6.46±0.3^{g}
C20:5n3	1.91±0.1^{a}	1.98±0.1^{a}	2.02±0.1^{a}	2.74±0.1^{b}	3.81±0.3^{c}	5.68±0.8^{cd}	6.28±0.3^{d}	7.33±0.7^{de}	8.44±0.2^{e}	8.73±0.1^{f}	9.41±0.2^{f}
C22:6n3	0.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}	0.62±0.1^{b}	1.27±0.2^{c}	1.96±0.3^{cd}	1.86±0.1^{d}	1.54±0.1^{cd}	0.67±0.0^{b}	1.19±0.1^{c}	1.54±0.1^{cd}
unknown	5.40±0.1	6.37±0.7	6.16±0.5	5.28±0.6	5.42±0.3	5.04±0.1	5.08±0.6	4.86±0.3	6.68±0.5	7.83±0.6	6.81±0.4
SFA	21.6±0.4^{a}	23.8±1.0^{ab}	24.0±1.4^{ab}	24.1±0.6^{ab}	24.1±1.4^{ab}	22.9±1.8^{ab}	25.8±0.8^{bc}	27.0±3.2^{ac}	29.4±0.4^{c}	28.0±0.7^{c}	23.8±1.0^{a}
MUFA	29.3±0.1^{ac}	29.3±0.2^{ac}	29.2±0.1^{ac}	28.3±0.2^{b}	28.0±0.3^{b}	28.2±0.3^{b}	28.4±0.3^{b}	29.4±0.7^{abc}	28.4±0.6^{b}	32.4±1.2^{c}	40.5±1.0^{d}
PUFA	43.7±0.2^{a}	40.6±1.6^{a}	40.6±1.9^{a}	42.3±0.7^{a}	42.5±1.6^{a}	43.9±1.8^{a}	40.8±1.2^{a}	38.8±2.6^{ab}	35.5±1.0^{b}	31.7±0.3^{c}	29.0±0.6^{d}

Different subscript letters within a row indicate significant difference ($p<0.05$).

Table 7 All Fatty Acid Content (% of Total Fatty Acid) in PE of *Artemia*nauplii (0-24h) and juvenile (2-4 day)

	0h	0.5h	1h	2h	4h	8h	12h	24h	2day	3day	4day
C16:0	5.01±1.0^{a}	8.67±0.4^{b}	10.3±0.4^{c}	4.05±0.7^{a}	6.83±3.1^{abc}	4.56±0.9^{a}	6.23±1.6^{abc}	7.71±4.5^{abc}	4.40±0.3^{a}	3.23±0.0^{a}	3.90±0.1^{a}
C16:1n7	0.54±0.1	0.55±0.0	0.53±0.0	0.32±0.1	0.42±0.1	0.52±0.2	0.60±0.2	0.79±0.3	0.49±0.0	0.48±0.0	0.50±0.0
C18:0	8.06±0.1	8.49±0.4	8.09±0.1	8.50±0.6	8.48±0.6	8.67±0.1	9.13±0.6	8.01±2.0	11.5±0.2	11.3±0.4	9.69±0.2
C18:1n7	9.85±0.0	9.28±0.3	9.37±0.1	10.0±0.1	9.75±0.3	10.2±0.2	10.5±0.2	12.4±0.9	12.9±0.2	15.3±0.2	13.5±0.3
C18:1n9	26.3±0.7^{ab}	26.0±0.7^{b}	25.2±0.3^{b}	25.7±0.1^{b}	24.9±0.4^{b}	24.5±0.6^{b}	25.0±1.2^{b}	24.4±2.2^{bc}	25.6±1.1^{b}	29.6±1.1^{ac}	32.8±0.6^{d}
C18:2n6	6.17±0.0	5.97±0.2	6.06±0.0	6.07±0.3	5.98±0.3	6.06±0.1	6.12±0.1	5.93±0.3	9.25±0.4	8.61±0.8	6.54±0.3
C18:3n3	21.1±0.0^{a}	20.0±0.6^{b}	20.3±0.2^{b}	20.3±0.5^{ab}	19.5±0.7^{abc}	18.8±0.3^{cd}	18.0±0.5^{cd}	16.4±1.0^{d}	12.0±0.2^{e}	7.50±0.3^{f}	3.44±0.2^{g}
C18:4n3	10.0±0.6	8.70±0.4	8.92±0.1	10.3±0.6	8.88±1.2	7.25±0.2	6.18±0.9	3.71±0.5	3.80±0.3	1.86±0.1	1.11±0.1
C20:1n9	0.94±0.0	0.76±0.0	0.77±0.0	1.00±0.1	0.91±0.1	1.00±0.1	0.91±0.1	0.90±0.2	0.74±0.0	0.21±0.0	0.27±0.0
C20:2n6	0.51±0.0	0.39±0.0	0.77±0.0	0.61±0.1	0.72±0.1	0.55±0.1	0.70±0.2	0.70±0.1	0.58±0.0	0.63±0.0	0.59±0.0
C20:3n3	3.16±0.2	2.27±0.3	2.53±0.1	3.53±0.4	3.09±0.7	3.05±0.2	2.67±0.2	2.49±0.5	1.53±0.1	1.20±0.0	0.82±0.1
C20:4n3	0.60±0.0	0.39±0.1	0.48±0.0	0.67±0.1	0.61±0.1	0.59±0.1	0.53±0.1	0.43±0.1	0.26±0.0	0.25±0.0	0.36±0.0
C20:4n6	0.51±0.0^{a}	0.36±0.1^{b}	0.43±0.0^{b}	0.78±0.1^{ac}	0.95±0.2^{c}	1.47±0.1^{d}	1.63±0.2^{d}	2.30±0.1^{e}	3.03±0.2^{f}	5.00±0.1^{g}	8.76±0.1^{h}
C20:5n3	2.02±0.1^{a}	1.45±0.1^{a}	1.67±0.1^{a}	2.83±0.4^{ab}	3.09±0.8^{ab}	4.08±0.3^{b}	4.26±0.6^{bc}	5.24±0.4^{c}	6.93±0.3^{d}	7.53±0.4^{d}	9.65±0.0^{e}
C22:6n3	0.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}	0.53±0.1^{b}	1.03±0.2^{bc}	2.01±0.1^{d}	1.81±0.1^{d}	1.70±0.1^{de}	1.24±0.1^{ce}	1.28±0.0^{ce}	3.39±0.1^{f}
unknown	5.23±0.3	6.75±1.5	4.57±0.4	4.64±0.2	4.19±0.3	5.31±0.3	4.55±1.1	5.88±0.7	5.54±0.5	5.35±0.8	3.96±0.6
SFA	13.1±1.2^{abc}	17.2±0.5^{a}	18.4±0.4^{b}	12.6±1.3^{c}	15.3±3.5^{abc}	13.2±1.0^{c}	15.4±2.1^{abc}	15.7±6.4^{abc}	16.1±0.5^{ac}	15.3±0.4^{c}	14.3±0.2^{c}
MUFA	37.6±0.6^{abc}	36.6±0.8^{bc}	35.9±0.2^{b}	37.0±0.2^{c}	36.0±0.8^{bc}	36.2±0.7^{bc}	37.0±1.0^{abc}	38.5±3.0^{abc}	39.7±0.9^{a}	45.5±1.1^{d}	47.1±0.7^{e}
PUFA	44.1±1.0^{ab}	39.5±1.3^{b}	41.2±0.2^{b}	45.8±1.3^{a}	44.5±2.7^{ab}	45.3±0.9^{a}	43.1±1.4^{ab}	39.9±2.8^{abc}	38.6±1.7^{bc}	33.9±1.0^{c}	34.7±0.3^{c}

Different subscript letters within a row indicate significant difference ($p<0.05$).