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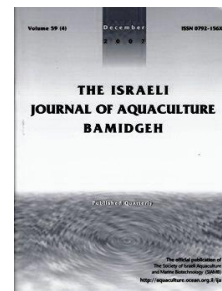
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Highly Unsaturated Fatty Acid (HUFA) Retention in the Freshwater Cladoceran, *Moina macrocopa*, Enriched With Lipid Emulsions

Jiun Yan Loh^{1,2}, Han Kiat Alan Ong³, Yii Siang Hii⁴, Thomas J. Smith⁵, Malcolm W. Lock⁵, Gideon Khoo^{2*}

¹ Faculty of Science & Biotechnology, Universiti Selangor, Jalan Timur Tambahan, 45600 Bestari Jaya, Selangor, Malaysia

² Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia

³ Faculty of Medicine & Health Sciences, Universiti Tunku Abdul Rahman, Bandar Sungai Long, Cheras, 43000 Kajang, Selangor, Malaysia

⁴ Faculty of Agro-technology & Food Science, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

⁵ Biomedical Research Centre, Sheffield Hallam University, Sheffield, United Kingdom

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Key words: arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, *Moina macrocopa*, lipid enrichment

Abstract

Fatty acid profiles of *Moina macrocopa* enriched with lipids from inexpensive squid or canola oil were compared with profiles of *Moina* enriched with a commercial enrichment diet, A1 DHA Selco[®] (ADS). Arachidonic acid (AA) significantly increased from <0.01 to 0.28 mg/g body weight in *Moina* enriched with ADS at 1 g/l for 12 h and to 0.38 mg/g in *Moina* enriched in 2 g/l canola oil for 12 h. The highest increase in eicosapentaenoic acid (EPA; from 0.15 to 0.38 mg/g) was obtained when *Moina* were enriched in 1 g/l ADS for 12 h. The highest increases in docosahexaenoic acid (DHA; originally <0.01) were obtained in *Moina* enriched in 1 g/l ADS for 12 h (increased to 0.09) or in 2 g/l ADS for 24 h (rose to 0.8 mg/g). The AA:EPA ratio was highest in *Moina* treated with 2 g/l canola oil for 12 h or 2 g/l squid oil for 24 h. The AA:DHA ratio was highest in *Moina* enriched with 2 g/l canola oil for 12 h while the DHA:EPA ratio was highest in *Moina* enriched with 2 g/l squid oil for 24 h.

* Corresponding author. E-mail: gideonkhoo@utar.edu.my

Introduction

Artemia nauplius is the most widely used live food species for feeding fish larvae, and has been used as bioencapsulated media to deliver specific nutrients or drugs to the targeted larvae (Sargent et al., 1997, 1999a). However, it is costly to import this live food and, as a saltwater food for cultured freshwater species, *Artemia* nauplii usually die within or soon after an hour, creating the need for intermittent feeding (Merchie, 1996). The development of freshwater zooplankton, such as *Moina* spp., can overcome these problems (Alam et al., 1993).

Moina macrocopa is one of the most abundant zooplankton in southeast Asian aquatic ecosystems such as paddies, ponds, swamps, rivers, and mangroves. This freshwater cladoceran is rich in protein and other nutrients, making it a superior live food for fish and prawn larvae compared to other live foods such as *Artemia* (Alam et al., 1993). However, fatty acid contents vary in *Moina* cultured in different media (Watanabe and Kiron, 1994) and, like *Artemia*, *Moina* do not meet the highly unsaturated fatty acids (HUFA) requirements of fish and crustacean larvae (He et al., 2001). Fatty acids play a major role as an energy source and may influence cellular membrane functions that are vital for cell growth, differentiation, and metabolism (Sargent et al., 1999b; Cahu et al., 2003; Tocher, 2003). Increasing the levels of dietary HUFA in fish larvae helps to increase stress resistance against starvation and osmotic shock (Koven et al., 2003), assists in skeletal formation (Cahu et al., 2003), aids the development of neural and visual systems (Sargent et al., 1997, 1999a), improves the survival rate (Watanabe, 1993), and enhances pigmentation (Bransden et al., 2005).

Many HUFA, especially arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), are necessary for the physiological development of fish and crustaceans (Watanabe, 1993). Fatty acid ratios such as n-6:n-3 are of considerable importance for determining the effects of HUFA fed to fish (Sargent et al., 1999b; Corraze, 2001) because they influence the production of hormone-like substances that are critical in a wide range of physiological processes in aquatic biota (Logue et al., 2000; Lund et al., 2007).

Fatty acid contents in *Artemia* nauplii, particularly essential HUFA, can be improved (Alam et al., 1993; Sargent et al., 1997, 1999a). The fatty acid composition of *Artemia* and other live foods such as rotifers can be enriched using microalgae, microalgae paste, and marine fish oils (Papandroulakis et al., 2002). The nutritional value of *Moina* can be enhanced by enrichment in various culture media as demonstrated for *Artemia* nauplii and rotifers (Watanabe et al., 1993; Das et al., 2007).

Live food enrichment by oil emulsion is common in fish and crustacean hatcheries. Commercial enrichment products and methodologies have been formulated for *Artemia* nauplii and rotifers (Agh and Sorgeloos, 2005) but not for *M. macrocopa*. To formulate an enrichment protocol for improving specific lipid contents in live foods, information regarding the fatty acid retention time and biosynthesis in the gut is required. In the present study, we determined the retention quality and HUFA ratio improvement in *M. macrocopa* enriched in 1 g/l and 2 g/l lipid emulsions of squid oil and canola oil for 12, 24, and 36 h, and compared the resulting fatty acid profiles with those of *M. macrocopa* fed a commercial enrichment diet, A1 DHA Selco[®].

Materials and Methods

Preparation of diets. Canola oil (Sime Darby Edible Product Ltd., Singapore), squid oil (Asia Star Laboratory Ltd., Thailand), and a commercial formulated emulsion, A1 DHA Selco[®] (ADS; INVE Ltd., Belgium) were selected for this experiment. ADS served as a reference diet. All treatment diets were prepared at concentrations of 1.0 and 2.0 g/l. The 1.0 g/l concentration was based on the standard protocol for the ADS commercial diet, whilst the 2.0 g/l concentration was selected to determine if doubling the concentration would significantly increase HUFA levels.

Emulsions of canola oil and squid oil were prepared based on methods modified from Estévez et al (1998), and Agh and Sorgeloos (2005). Soybean-extracted emulsifier, L- α -phosphatidylcholine (Sigma-Aldrich[™], USA), was added to the oils at a ratio of 1:4

(w:w). The mixtures were blended vigorously with dechlorinated water at 40-60°C using an electric blender (Waring Commercial®, USA) at maximum speed for 3 min. The ADS emulsion was prepared according to the manufacturer's instructions, where the ADS emulsion was weighed and mixed with dechlorinated water to the required concentrations at ambient temperature.

Enrichment experiment. An 80-l plastic barrel was filled with tap water that had been dechlorinated by strong aeration for several days prior to use. Wild-type *M. macrocopa* were obtained from oxidation ponds located in residential areas in Kuala Lumpur, Malaysia. The *M. macrocopa* were acclimatized in the barrel overnight before the start of the experiment. Mild aeration was provided during acclimatization to maintain the dissolved oxygen level at ≥ 4 ppm.

Only actively moving *Moina* individuals were selected for the experiment. During selection, aeration was turned off to reduce the water current in the tank. *Moina* were collected with a fine sieve near the tank edge. Adults were concentrated in a glass beaker for quantification. For counting, 40 ml of test organisms were transferred by syringe to a petri dish and counted under a dissecting microscope. After counting, one ml containing approximately 300 *Moina* was transferred to each 1.5-l aquarium for the enrichment treatment. Mild aeration was provided, and dissolved oxygen was 5.0 ± 0.1 ppm. The water temperature ranged 26-30°C and pH ranged 7.2-8.1. The experiments were conducted in the natural photoperiod (12 h light:12 h dark). Since HUFA levels of wild *M. macrocopa* greatly vary depending on food source (unpublished data), the *Moina* were unfed for 12 h before the experiment to obtain uniform and standardized HUFA levels in the unenriched samples.

Enriched *Moina* were collected after 12, 24, and 36 h incubation in the respective experimental diet. Samples were thoroughly rinsed with distilled water to remove excess oil emulsion prior to storage in 1.5-ml tubes at -20°C for further analysis.

Lipid extraction and fatty acid determination. Fatty acid methyl ester (FAME) of the *M. macrocopa* was performed according to a method modified from AOAC (1995). Qualitative and quantitative analyses by gas chromatography were conducted by a certified commercial analytical food chemistry laboratory (Chemical Laboratory Sdn. Bhd., Malaysia).

Statistical analysis. Fatty acid data are the means of four data sets; experimental diet data are the means of two data sets. The data was tested for normality and homogeneity-of-variance, and analyzed by 2-way ANOVA (with Tukey's test) to determine the effects of diet level (1.0 and 2.0 g/l), enrichment period (12, 24, 36 h), and the interaction of these factors. A 95% significance level ($p < 0.05$) was used. Statistical analyses were performed using Minitab ver. 13.

Results

The fatty acid contents of the unenriched *M. macrocopa* and oil emulsions differed (Table 1). Linoleic acid (18:2n-6) was 0.23 mg/g body weight in the unenriched *M. macrocopa* but reached the highest values - 0.50 mg/g and 0.40 mg/g - when fed the 1 g/l squid oil diet for 12 h (Table 2) and 0.40 mg/g when fed the 2 g/l canola oil diet for 36 h (Table 3). Linolenic acid (18:3n-3) was 0.14 mg/g body weight in unenriched *M. macrocopa* but reached the highest values - 0.70 mg/g and 0.86 mg/g - when fed 2 g/l squid oil and 2 g/l canola oil, respectively, for 12 h.

The essential fatty acids, arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), were low in unenriched *M. macrocopa*. AA (20:4n-6) reached its maximum in the 12-h 1 g/l ADS and 2 g/l canola oil treatments. EPA (20:5n-3) was significantly highest in the 12-h 1 g/l ADS treatment. DHA (22:6n-3) was highest when enriched with 1 g/l ADS for 12 h and 2 g/l ADS for 24 h.

The AA:EPA ratio of *M. macrocopa* enriched with 1 g/l canola or squid oil reached a maximum after 12 h enrichment but subsequently dropped (Fig. 1). When increased to 2 g/l, enrichment in canola oil was highest after treatment for 12 h and in squid oil after 24 h (Fig. 2). When enriched in 1 g/l oil emulsions, the AA:DHA and DHA:EPA ratios showed similar trends.

Table 1. Fatty acid compositions of unenriched *Moina macrocopa* (12 h) and oil emulsions.

Fatty acid	Unenriched <i>Moina</i>	Oil emulsions		
		A1 DHA Selco ¹	Squid oil ²	Canola oil ³
12:0	-	-	-	0.01±<0.01
14:0	0.02±<0.01	-	0.01±<0.01	0.01±<0.01
14:1	0.01±<0.01	-	0.01±<0.01	0.02±<0.01
15:0	0.10±<0.01	0.23±<0.01	0.05±<0.01	0.03±<0.01
16:0	0.04±<0.01	0.04±<0.01	0.02±<0.01	-
17:0	0.40±0.05	0.95±<0.01	-	0.11±<0.01
18:0	0.31±0.03	0.05±<0.01	0.07±<0.01	0.07±<0.01
18:1n-9	0.07±0.01	0.03±<0.01	0.03±<0.01	0.03±<0.01
18:2n-6 (linoleic)	0.23±0.02	-	1.00±<0.01	0.79±<0.01
20:0	0.03±<0.01	-	0.03±<0.01	0.01±<0.01
18:3cis-6	0.47±0.01	-	0.90±<0.01	0.84±<0.01
18:3n-3 (linolenic)	0.14±0.01	-	0.01±<0.01	0.01±<0.01
22:0	0.01±<0.01	0.03±<0.01	0.01±<0.01	0.01±<0.01
20:3n-6	-	-	0.04±<0.01	0.06±<0.01
20:3n-3	0.04±<0.01	-	0.08±<0.01	0.09±<0.01
20:4n-6 (arachidonic)	-	-	0.01±<0.01	-
20:5n-3 (eicosapentaenoic)	0.15±0.01	-	0.05±<0.01	0.07±<0.01
22:6n-3 (docosahexaenoic)	-	0.04±<0.01	0.03±<0.01	0.01±<0.01
Σ SAFA	277.92±4.96	508.60±0.40	68.76±0.66	113.96±0.14
Σ MUFA	308.61±9.07	193.67±0.07	576.79±0.22	504.96±0.15
Σ PUFA	413.47±4.15	297.73±0.47	354.46±0.88	381.08±0.28
Σ n-3 PUFA	90.14±1.52	3.19±<0.01	27.55±0.05	35.41±0.25
Σ n-6 PUFA	1.51±0.05	1.13±0.01	8.10±0.01	12.50±0.11
AA:EPA	0.41±0.05	8.33±0.24	2.50±0.01	0.61±0.01
AA:DHA	3.13±0.18	12.50±0.35	4.81±0.03	3.21±0.02
DHA:EPA	0.66±0.08	6.67±<0.01	5.20±0.01	1.91±0.01

Values are means±SEM of four data sets for *M. macrocopa* (control) and two data sets for oil emulsions.

Hyphen (-) indicates non-detectable or <0.01 fatty acid.

¹ INVE, Belgium

² Asia Star Lab. Ltd., Thailand

³ Sime Darby Edible Product Ltd., Singapore

Σ SAFA = total saturated fatty acids

Σ MUFA = total monounsaturated fatty acids

Σ PUFA = total polyunsaturated fatty acids

Table 2. Fatty acid compositions (mg/g body weight) of *Moina macrocopa* enriched with 1 g/l A1 DHA Selco, squid oil, or canola oil for 12, 24, or 36 hours.

Fatty acid	A1 DHA Selco			Squid oil			Canola oil		
	12 h	24 h	36 h	12 h	24 h	36 h	12 h	24 h	36 h
12:0	<0.01	0.03±<0.01	<0.01	0.02±<0.01	0.01±<0.01	<0.01	- ^g	0.01±<0.01 ^h	- ^g
14:0	0.02±<0.01	0.01±<0.01	0.01±<0.01	0.01±<0.01	<0.01	<0.01	0.44±0.01 ^h	0.02±<0.01 ^g	- ^g
14:1	0.01±<0.01 ^b	<0.01 ^a	<0.01 ^{ab}	<0.01	<0.01	<0.01	0.04±<0.01 ^h	- ^g	0.01±<0.01 ^g
15:0	1.36±0.12 ^a	0.11±<0.01 ^b	0.44±0.05 ^{ab}	0.01±<0.01	<0.01	<0.01	0.29±0.01 ^h	- ^g	0.03±<0.01 ^g
16:0	0.36±0.03 ^b	0.02±<0.01 ^a	0.05±<0.01 ^a	0.01±<0.01	<0.01	<0.01	3.22±0.16 ^h	- ^g	0.03±<0.01 ^g
17:0	8.50±0.70 ^b	1.36±0.14 ^a	1.78±0.16 ^a	0.05±0.01	0.01±<0.01	<0.01	0.10±0.01	-	0.56±0.07
18:0	1.37±0.12 ^b	0.05±<0.01 ^a	0.23±0.03 ^a	0.07±0.01	0.03±<0.01	<0.01	-	0.04±<0.01	0.08±<0.01
18:1n-9	0.16±0.01	0.41±0.05	0.52±0.06	0.49±0.03 ^e	0.45±0.01 ^e	0.02±<0.01 ^d	0.48±0.03 ^h	0.13±0.01 ^g	0.01±<0.01 ^g
18:2n-6	0.21±0.01	0.28±0.03	0.08±0.01	0.50±0.06	0.01±<0.01	<0.01	0.05±<0.01	0.01±<0.01	0.15±<0.01
20:0	0.20±0.02	0.06±0.01	0.01±<0.01	0.01±<0.01	0.14±0.01	<0.01	0.04±<0.01	-	0.17±0.02
18:3cis-6	0.87±0.01 ^b	0.10±<0.01 ^a	0.06±<0.01 ^a	0.05±<0.01	0.04±<0.01	<0.01	-	-	1.07±0.12
18:3n-3	0.01±<0.01	0.01±<0.01	<0.01	0.01±<0.01	0.01±<0.01	<0.01	0.02±<0.01	-	0.06±<0.01
22:0	0.08±0.01 ^b	<0.01 ^a	0.02±<0.01 ^a	0.01±<0.01	0.02±<0.01	<0.01	- ^g	0.10±0.01 ^h	0.01±<0.01 ^g
20:3n-6	0.37±0.04	0.10±0.01	0.07±0.01	0.07±0.01	0.03±<0.01	<0.01	-	-	0.01±<0.01
20:3n-3	<0.01	<0.01	<0.01	0.02±<0.01	0.02±<0.01	<0.01	-	-	-
20:4n-6	0.28±0.01 ^b	0.03±<0.01 ^a	0.04±<0.01 ^a	0.01±<0.01	0.01±<0.01	<0.01	0.01±<0.01	0.02±<0.01	0.01±<0.01
20:5n-3	0.38±0.02 ^b	0.03±<0.01 ^a	0.05±<0.01 ^a	0.02±<0.01	0.01±<0.01	<0.01	-	0.01±<0.01	0.01±<0.01
22:6n-3	0.09±0.01 ^b	<0.01 ^a	<0.01 ^a	<0.01	<0.01	<0.01	-	0.01±<0.01	-
ΣSAFA	447.73±2.99	410.63±30.59	411.29±22.91	250.49±10.09 ^d	764.95±19.78 ^e	968.20±3.68 ^e	673.68±22.49 ^h	343.60±4.57 ^g	121.16±2.76 ^g
ΣMUFA	268.05±8.80 ^a	423.65±38.69 ^{ab}	510.32±26.06 ^b	594.25±19.01 ^e	222.18±19.77 ^d	24.83±2.87 ^d	290.76±21.56 ^g	577.51±2.35 ^h	214.18±15.96 ^g
ΣPUFA	284.22±11.67 ^b	165.65±8.15 ^{ab}	78.38±3.16 ^a	155.26±9.13	12.86±0.22	6.96±0.81	35.56±0.95 ^g	78.89±6.58 ^g	664.65±13.97 ^h
Σn-3PUFA	27.65±1.86 ^b	6.25±0.20 ^a	6.26±0.24 ^a	7.94±0.59	3.49±0.09	1.35±0.16	4.97±0.36	2.43±0.10	11.14±0.72
Σn-6PUFA	21.22±0.39	24.78±2.48	15.50±0.95	18.59±1.42	3.87±0.07	2.77±0.33	2.49±0.16	2.86±0.18	3.34±0.14
AA:EPA	7.35±0.10	11.31±0.44	8.94±0.35	14.00±0.71	10.52±0.20	2.50±<0.01	46.50±4.94	40.45±5.03	11.35±0.41
AA:DHA	4.27±0.18	<0.01	<0.01	25.00±1.41	<0.01	<0.01	25.25±2.09	30.03±2.30	-
DHA:EPA	4.23±0.49	<0.01	<0.01	2.08±0.25	<0.01	<0.01	13.63±0.93	16.00±1.12	-

Values are means±SEM (n = 4). Hyphen (-) indicates non-detectable or <0.01.

Superscripts indicate significant variation (p<0.05) within the enrichment periods for the individual diet.

Table 3. Fatty acid composition (mg/g body weight) of *Moina macrocopa* enriched with 2 g/l A1 DHA Selco, squid oil, or canola oil for 12, 24, or 36 hours.

Fatty acid	A1 DHA Selco			Squid oil			Canola oil		
	12 h	24 h	36 h	12 h	24 h	36 h	12 h	24 h	36 h
12:0	-	0.01±<0.01	-	-	0.01±<0.01	0.01±<0.01	0.01±<0.01 ^g	0.01±<0.01 ^h	0.01±<0.01 ^h
14:0	0.01±<0.01	0.01±<0.01	0.03±<0.01	- ^d	0.03±<0.01 ^e	0.04±<0.01 ^e	-	0.02±<0.01	0.04±<0.01
14:1	0.01±<0.01 ^a	- ^a	0.01±<0.01 ^b	- ^d	0.01±<0.01 ^e	0.01±<0.01 ^e	-	0.01±<0.01	0.01±<0.01
15:0	0.22±<0.01	0.23±<0.01	0.32±<0.01	- ^d	0.21±<0.01 ^e	0.16±<0.01 ^e	- ^g	0.13±0.01 ^h	0.12±<0.01 ^g
16:0	0.09±<0.01	0.09±<0.01	0.11±<0.01	0.03±<0.01 ^d	0.11±<0.01 ^e	0.09±<0.01 ^e	0.06±<0.01	0.09±<0.01	0.07±<0.01
17:0	1.70±0.01	1.81±0.02	2.37±<0.01	- ^d	4.33±0.02 ^e	3.83±0.04 ^e	- ^g	2.21±0.13 ^h	2.68±0.06 ^h
18:0	0.16±<0.01	0.17±<0.01	0.14±<0.01	1.29±0.08 ^e	0.14±<0.01 ^d	0.11±<0.01 ^d	2.31±0.12 ^h	0.11±0.01 ^g	0.09±<0.01 ^g
18:1n-9	0.05±<0.01	0.01±<0.01	0.02±<0.01	0.05±<0.01	0.04±<0.01	0.04±<0.01	0.11±<0.01	0.01±<0.01	0.09±0.01
18:2n-6	0.08±<0.01	0.09±<0.01	0.08±<0.01	0.22±0.02	0.12±<0.01	0.19±<0.01	0.05±<0.01	0.18±<0.01	0.40±0.01
20:0	0.01±<0.01	0.02±<0.01	0.02±<0.01	1.55±0.13 ^e	0.02±<0.01 ^d	0.02±<0.01 ^d	2.24±0.06 ^h	0.02±<0.01 ^g	0.03±<0.01 ^g
18:3cis-6	0.61±<0.01 ^b	0.65±<0.01 ^b	0.10±0.01 ^a	0.78±0.08 ^e	0.05±<0.01 ^d	0.08±<0.01 ^d	1.19±0.17	1.04±0.12	0.14±0.01
18:3n-3	0.02±<0.01	0.02±<0.01	-	0.70±0.12	0.01±<0.01	0.01±<0.01	0.86±0.10 ^h	0.04±<0.01 ^g	0.01±<0.01 ^g
22:0	0.01±<0.01	0.01±<0.01	0.02±<0.01	0.06±0.01	0.02±<0.01	0.02±<0.01	0.06±<0.01	0.02±<0.01	0.02±<0.01
20:3n-6	0.02±<0.01	0.01±<0.01	0.09±0.01	0.03±<0.01 ^d	0.20±<0.01 ^e	0.35±0.01 ^f	0.03±<0.01 ^g	0.01±<0.01 ^g	0.79±0.01 ^h
20:3n-3	0.01±<0.01	-	-	-	0.01±<0.01	-	-	0.01±<0.01	-
20:4n-6	0.04±<0.01	0.02±<0.01	0.07±<0.01	0.01±<0.01 ^d	0.14±<0.01 ^e	0.14±<0.01 ^e	0.38±0.03 ^h	0.03±<0.01 ^g	0.03±<0.01 ^g
20:5n-3	0.10±<0.01	0.14±<0.01	0.05±<0.01	-	0.01±<0.01	0.03±<0.01	-	0.10±<0.01	0.08±<0.01
22:6n-3	0.04±<0.01	0.08±<0.01	-	0.04±<0.01	0.01±<0.01	0.01±<0.01	-	0.01±<0.01	-
ΣSAFA	384.22±1.01	376.27±1.17	485.66±0.92	300.12±3.99	532.77±0.27	504.66±1.08	511.76±14.78	250.63±13.89	222.18±3.34
ΣMUFA	202.32±0.25	179.70±0.37	207.66±0.48	330.16±24.52	207.65±2.44	209.30±1.76	96.83±2.17	122.15±5.99	122.83±1.45
ΣPUFA	413.46±0.87	444.03±1.37	306.68±0.51	369.72±27.54	259.58±2.58	286.03±2.51	391.40±12.66 ^g	627.22±19.87 ^g	654.99±4.67 ^h
Σn-3PUFA	27.36±0.20 ^b	36.32±0.29 ^b	8.99±0.04 ^a	17.63±0.98	4.71±0.10	6.03±0.29	69.34±7.92	13.97±0.08	6.67±0.10
Σn-6PUFA	9.88±0.53	4.60±0.07	25.32±1.21	3.73±0.31 ^d	35.70±0.37 ^e	54.88±1.10 ^f	39.33±3.11 ^h	3.13±0.23 ^g	55.18±0.95 ^h
AA:EPA	4.47±0.34 ^a	1.57±0.05 ^a	14.06±0.40 ^b	70.00±19.80	167.18±8.54	80.01±7.64	2476.25±275.42	3.04±0.27	3.21±0.04
AA:DHA	10.84±0.86 ^a	2.63±0.06 ^a	271.07±23.06 ^b	14.82±2.20 ^d	172.50±9.84 ^e	191.14±2.37 ^e	856.25±48.14 ^h	53.89±2.47 ^g	75.75±6.33 ^g
DHA:EPA	4.25±0.02 ^{bc}	5.86±0.07 ^b	0.93±0.07 ^{bc}	210.00±50.91	12.73±1.16	4.46±0.44	25.00±4.24	0.87±0.09	0.58±0.03

Values are means±SEM (n = 4). Hyphen (-) indicates non-detectable or <0.01.

Superscripts indicate significant variation (p<0.05) within the enrichment periods for the individual diet.

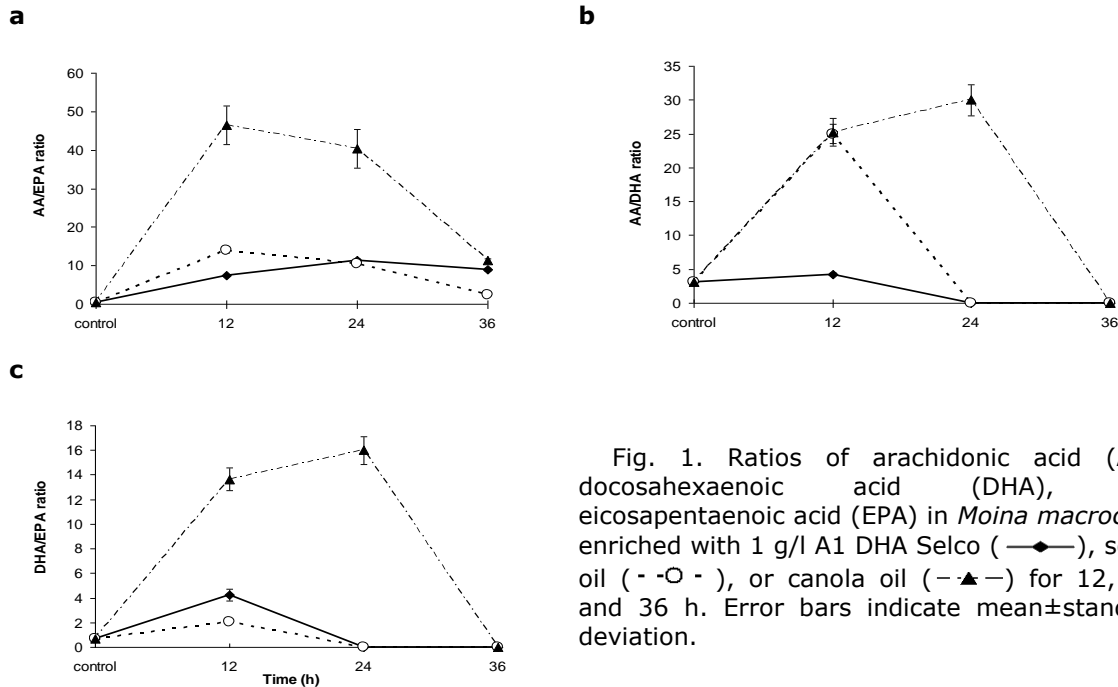


Fig. 1. Ratios of arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) in *Moina macrocopa* enriched with 1 g/l A1 DHA Selco (—●—), squid oil (-○-), or canola oil (-▲-) for 12, 24, and 36 h. Error bars indicate mean±standard deviation.

Concentration was the dominant factor affecting the fatty acid composition of the enriched *M. macrocopa* (Table 4). AA, EPA, and DHA significantly increased when the treatment concentration was doubled, with the exception of AA for ADS enrichment. The n-6:n-3 ratio was significantly affected by enrichment time in ADS. AA:DHA trends were positive for both squid and canola oil enrichment. Enrichment beyond 24 h in squid or canola oil did not alter the EPA and DHA levels or the AA:EPA and DHA:EPA ratio.

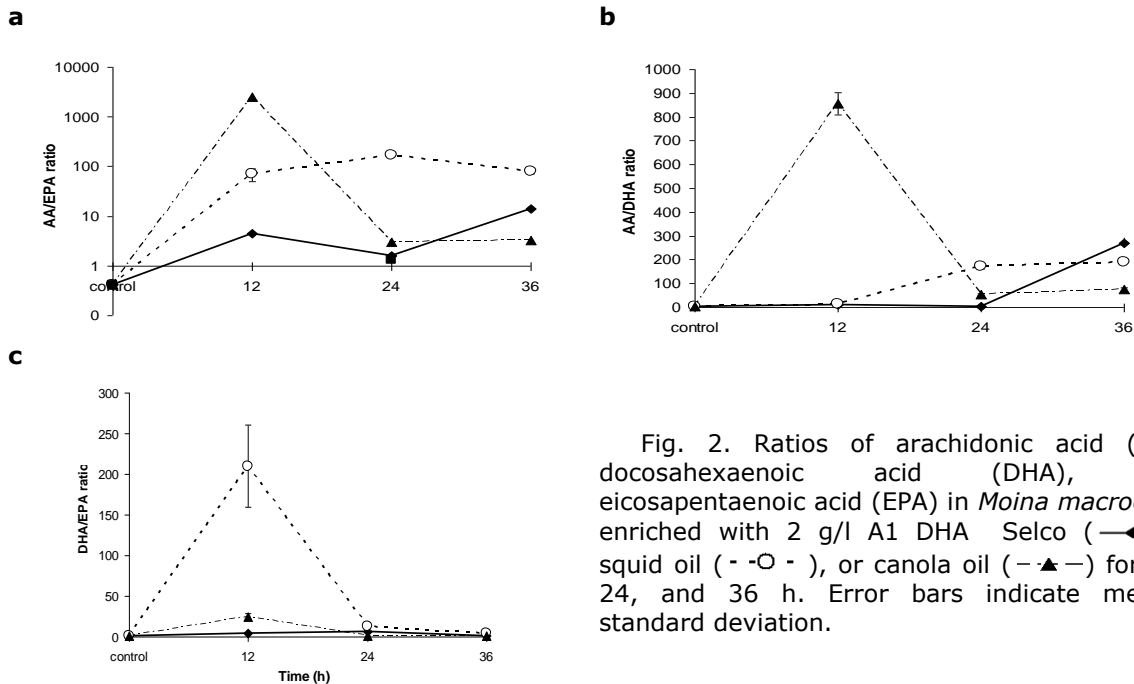


Fig. 2. Ratios of arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) in *Moina macrocopa* enriched with 2 g/l A1 DHA Selco (—●—), squid oil (-○-), or canola oil (-▲-) for 12, 24, and 36 h. Error bars indicate mean ± standard deviation.

Table 4. *F* and *P*-values derived from 2-way ANOVA analysis, with further comparisons using Tukey's test, to describe the effects of diet concentration, time, and the interaction of these two variables.

Fatty acid	Concentration		Time		Concentration × time	
	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value
<i>A1 DHA Selco</i>						
20:4n-6	14.76	***0.000	9.94	**0.001	10.71	***0.000
20:5n-3	1.29	0.292	9.17	**0.001	5.62	**0.002
22:6n-3	4.34	*0.023	4.71	*0.018	4.43	**0.007
AA:EPA	19.33	***0.000	4.01	*0.030	8.59	***0.000
AA:DHA	5.69	**0.009	4.84	*0.016	5.44	**0.002
DHA:EPA	8.42	**0.001	7.51	**0.003	3.99	*0.011
<i>Squid oil</i>						
20:4n-6	97.25	***0.000	18.88	***0.000	23.26	***0.000
20:5n-3	11.21	***0.000	0.37	0.697	0.66	0.622
22:6n-3	7.17	**0.003	2.95	0.069	2.66	0.054
AA:EPA	11.12	***0.000	1.17	0.324	1.00	0.424
AA:DHA	43.38	***0.000	7.12	**0.003	11.09	***0.000
DHA:EPA	1.39	0.266	1.14	0.335	1.11	0.374
<i>Canola oil</i>						
20:4n-6	7.04	**0.003	5.13	*0.013	5.26	**0.003
20:5n-3	9.11	**0.001	0.13	0.878	2.07	0.113
22:6n-3	4.14	*0.027	4.91	*0.015	0.72	0.586
AA:EPA	2.21	0.129	2.32	0.118	2.27	0.088
AA:DHA	15.23	***0.000	10.74	***0.000	10.48	***0.000
DHA:EPA	1.62	0.216	2.73	0.083	1.60	0.203

Significance level: **p*<0.05, ***p*<0.01, ****p*<0.001.

for cellular housekeeping functions, lipids are required for hormone synthesis and serve as carriers for fat soluble vitamins (Parker, 2002). In the present study, results clearly indicate that lipid levels in *M. macrocopa* can be improved by changes in the enrichment process, similar to *Artemia nauplii* (Das et al., 2007).

Enrichment with ADS produced the highest overall EPA and DHA contents, showing significant interactions between treatment concentration and enrichment time.

Discussion

Lipids play crucial roles in the physiological and biological functions of living organisms (Sargent et al., 1997, 1999a). Marine and freshwater fish require n-3 and n-6 fatty acids in their daily diet, especially during the gastrula stage of fish embryo development when lipids are the main energy source (Vetter et al., 1983). An inadequate lipid content in fish diets can adversely affect larvae performance during the growout stage since larvae often have low energy reserves and require substantial energy for their high somatic growth rate and body development (Fraser et al., 1988). Lipids contain 2.5 times the energy of carbohydrates and proteins per consumed weight (Parker, 2002). In addition, as the energy source

Diet and conditions (initial stage of neonate/nauplius; enrichment duration and dose) influence subsequent enrichment results (Léger et al., 1987). In our study, treatment concentration had a stronger effect on the fatty acid content of *M. macrocopa* than enrichment time. The desirable essential fatty acids - AA, EPA, and DHA - are low in unenriched *Moina*. When enriched for 12 h at 2 g/l, canola oil increased the AA level in *Moina*. AA is an essential dietary fatty acid that has been measured in relatively high amounts in neural and visual tissues of fish (Tocher and Harvie, 1988). This fatty acid is the main precursor of hormone-like compounds such as eicosanoids that are important in the development of neural tissues and the immune system, specifically for signal transduction (Sargent et al., 1999b) and inflammatory responses (Tocher, 2003).

The DHA level in unenriched *M. macrocopa* (<0.01 mg/g) indicates that this zooplankton is naturally deficient in this fatty acid. The EPA level was higher (0.15 mg/g) than the DHA level in unenriched *M. macrocopa*, as in *M. micrura* (Das et al., 2007). It has been hypothesized that *Moina* sp. cannot synthesize DHA *de novo* and metabolize PUFA as rapidly as *Artemia* (Estévez et al., 1998). The elevation of DHA generally increases the EPA level of organisms, as shown by the marked retroconversion of DHA to EPA that takes place during enrichment (Navarro et al., 1999). Such retroconversion occurred in *Moina* enriched with 1 and 2 g/l ADS, although this zooplankton took a longer time to synthesize and metabolize DHA from ADS. The DHA content in *Artemia* nauplii increased approximately two fold from 12 to 24 h of enrichment (Han et al., 2000). Although the DHA level did not greatly increase as the enrichment time increased, the DHA levels after enrichment with all three experimental diets were significantly higher than in unenriched *Moina* (0.01-0.08 mg/g). Since DHA is important for vision and membrane fluidity (Koven, 2003), an insufficient level of this essential fatty acid in diets for fish larvae is likely to impair neural and visual development (Sargent et al., 1999b). EPA contents dropped markedly after enrichment, except in the 1 g/l ADS treatment. During supplementation, it was anticipated that EPA would be metabolized to eicosanoids, particularly 3-series prostanoids and 5-series leukotrienes (Sargent et al., 1999b). However, EPA levels were not increased by squid or canola oil.

HUFA are important for survival, growth, and pigmentation in almost all fish and crustacean larvae (Watanabe, 1993). Nevertheless, the effects of HUFA cannot be determined solely by the absolute amount of a particular HUFA fed to larvae. The absolute requirement for any given HUFA in fish is proportionate to the absolute amounts of other HUFA in the diet due to competitive inhibition by fatty acids that can affect the quality of the provided HUFAs (Sargent et al., 1999b; Corraze, 2001). AA and EPA compete for binding to cyclooxygenase and lipoxygenase enzymes (Hamre et al., 2005). Such competition can result in biological imbalances that affect cell membrane receptors (Sargent et al., 1999b). EPA and DHA bind to cyclooxygenase and lipoxygenase in fish tissues and subsequently determine the n-2:n-3 eicosanoid ratio in the muscle of fish larvae. These two eicosanoids cause physiological changes (Logue et al., 2000) such as malpigmentation resulting from excessive production of eicosanoids that induce biochemical stress (Sargent et al., 1999a). It has been suggested that increasing the n-3:n-6 ratio could reduce or inhibit the synthesis of AA-derived prostaglandins, consequently influencing the rate and type of eicosanoids produced (Lund et al., 2007).

This study indicates that the lipid ratio in *M. macrocopa* is strongly influenced by the lipid ratio of the live foods it is fed. The commercial enrichment diet, ADS, was superior for enhancing DHA but did not efficiently improve the n-3:n-6 or n-6:n-3 ratios. AA:EPA and AA:DHA were better improved when *M. macrocopa* was enriched with 2 g/l canola oil for 12 h and 24 h, respectively. Malpigmentation in some species, e.g., turbot, can be mitigated if the n-6:n-3 ratio in the diet is increased during larval feeding (Reitan et al., 1994). In our study, the DHA:EPA ratio rose when *M. macrocopa* was enriched with 2 g/l squid oil at 12 h. A study on the megalopa stage of the Chinese mitten crab, *Eriocheir sinensis*, showed that it could tolerate a greater range of salinity fluctuation when the diet was supplemented with DHA:EPA (Sui et al., 2007). Increasing the HUFA ratio could also reduce solidification of the total bio-membrane lipid pool that acts as an anti-freeze

mechanism for many aquatic poikilotherms to protect them from cold water temperatures (Brett and Müller-Navarra, 1997).

The bio-encapsulated lipid ratios in *M. macrocopa* tissues during enrichment positively correlate with dietary levels. Fatty acids such as linoleic (LA) and linolenic (LnA) acids are relatively important for larval performance. The LA level was most improved (0.50 mg/g) when *M. macrocopa* were enriched with 1 g/l squid oil for 12 h; the LnA level was further improved (0.86 mg/g) by using 2 g/l canola oil for 12 h. LA is a metabolic precursor of γ -linolenic acid (GLA) to AA. It functions to prevent abnormal coloration in freshwater fish, while LnA can be elongated to DHA via EPA catabolism (Tocher, 2003). As demonstrated in this study, LnA-enriched *M. macrocopa* could be an alternative source of DHA and EPA for freshwater fish larvae.

In conclusion, this study suggests that the fatty acid content in *M. macrocopa* can be improved with lipid emulsions. Animal and plant-based oil emulsions, such as squid and canola oils, possess a competitive advantage for enhancing the distribution of fatty acids and increasing the n-3:n-6 lipid ratio of this zooplankton. Oil emulsions are less expensive than the commercial enrichment diet, A1 DHA Selco[®], thus making them a viable alternative for enriching *M. macrocopa*.

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