各種栄養強化剤を摂取した広塩性ツボワムシBrachionus plicatilis複合種2種の個体群増殖，脂肪酸組成およびタンパク質含量の比較

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Enrichment effect on two *Brachionus plicatilis* sp. complex morphotypes fed three nutritional enrichment diets

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Abstract: Information on effects of different enrichment on different morphotypes within the *Brachionus plicatilis* sp. complex is limited. This study investigated the effects of different enrichment diets on population growth, fatty acid content and protein content of L and SS types *B. plicatilis* sp. complex. Three commercial rotifer enrichment formulations were tested: docosahexaenoic acid (DHA) enriched *Chlorella vulgaris*, frozen *Nannochloropsis oculata*, salmon roe emulsion oil, against a control diet of normal *C. vulgaris*. DHA-enriched C. vulgaris and Frozen *N. oculata* had significantly higher population growth, fatty acid composition and soluble protein in both morphotypes. However, population growth under salmon roe emulsion treatment was affected negatively despite having high essential fatty acid content in both morphotypes. Variations in fatty acid assimilation in non-polar and polar lipids between morphotypes were observed highlighting enrichment effects and possible dissimilar biochemical roles of these compounds in tissue metabolism in each morphotype. Significantly higher levels of soluble protein were detected in DHA-enriched *C. vulgaris* and control treatment. SS-type had significantly higher soluble protein content except under the frozen *N. oculata* treatment. Culture method, enrichment type and dosage are important considerations as different rotifer morphotypes respond differently to different enrichment.

Key words: *Brachionus plicatilis*; Morphotypes; Enrichment; Fatty acid

Since the introduction of euryhaline rotifers in the field of aquaculture, there have been many developments in its mass culture (Fu et al. 1997; Hagiwara et al. 1998; Dhert et al. 2001; Hamre 2016). The importance of rotifers, especially those belonging to the *Brachionus plicatilis* species complex, is often highlighted as they are often used as the preferred first-feed during marine finfish larviculture (Watanabe et al. 1978; Tomoda et al. 2004; Park et al. 2006). This is due to favorable characteristics such as its small size (70−340 μm), high reproduction rate, planktonic nature, suitability for mass culture and ability for artificial manipulation of its nutritional qualities (Yoshimura et al. 1997; Dhert et al. 2001; Kotani et al. 2009). The genera *Brachionus* include several morphotypes; culturists describe them according to lorica size L (large, 130−340 μm), and SS (super small, 90−110 μm) types and based on several classification criteria e.g. morphology, allozyme pattern, karyotypes (Hagiwara et al. 2001, 2014; Kotani et al. 2005). Morphologically, Fu et al. (1991) described L-type as larger and having obtuse-angled anterior spines compared to the smaller and rounded lorica with pointy anterior spines on the S-type. Furthermore, Segers (1995) also classified *B. plicatilis* as L-type and smaller varieties as *B. rotundiformis*. Kotani et al. (1997) demonstrated that a strong species boundary between L-type *B. plicatilis* and SS-type *B. rotundiformis* is present. Mills et

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al. (2016) further reported that the *Brachionus plicatilis* species complex could be further divided into 15 species and it was clarified that rotifer populations could include more varieties than previously thought. Consideration of rotifer size is important in seedling production, as an important factor during first feeding larvae is prey selection according to prey size and mouth gape (Shirota, 1970). The moment of first exogenous feeding is an important period as factors such as starvation and subsequent mortality usually accompanies this period. Food deprivation after yolk reabsorption has been reported to be linked to abnormality in behavior and morphological development, alimentary tract deterioration and trunk musculature along with reduction in decreased food utilization efficiency and feeding activity (Heming et al. 1982; Taylor and Freeberg, 1984; Rice et al. 1987; Gisbert et al. 2004). Dependent on the conditions of culture and types of enrichment, several nutrients may be present in insufficient levels in rotifers (Hamre et al. 2008). Levels of nutrients, e.g. protein, amino acids and essential fatty acids, are also determined by the metabolism of the feed organism and therefore good knowledge of nutritional levels in enrichments and also larval nutritional requirements throughout ontogeny would contribute to diet optimization and improved feeding protocols (Hamre 2016). Arachidonic acid (C20:4 *n*-6; ArA), eicosapentaenoic acid (C20:5 *n*-3; EPA) and docohexaenoic acid (C22:6 *n*-3; DHA) are three important highly unsaturated fatty acids (HUFA) that are vital for the development and survival of marine fish larvae during its early stages (Watanabe et al. 1983; Koven et al. 1992). Although it is now possible to produce rotifers at a large scale and of high nutritional value, their quality is often far from optimal which may be due to the low hygienic quality and overall culture condition which often results in low swimming activity, low reproduction rate and low ingestion rate (Dhert et al. 2001; Hagiwara et al. 2007). These factors have to be considered during the enrichment process as it may induce stress in the rotifer mesocosm, which may lead to other stressors such as increase in free ammonia levels, change of bacterial flora and contamination by protozoan species (Hagiwara et al. 1998). To ensure high larval survival and growth at the onset of exogenous feeding during subsequent larviculture, maintenance of feed quality, feed culture conditions and nutritional values of rotifers is crucial (Hagiwara et al. 2007). In addition to understanding differences in fatty acid composition, the impacts of enrichment on essential fatty acids for larviculture (e.g. ArA, EPA and DHA) and how they differ between different morphotypes is also an important consideration.

Information on the effect of different enrichment on different morphotypes within the commercially valuable *B. plicatilis* sp. complex is limited therefore this study aims to provide a preliminary insight into the suitability of enrichment and its impacts on two morphotypes commonly used during seedling production. This study focused on evaluating nutritional enrichment effect on growth ratio, egg bearing, fatty acid and protein content of the Indonesian strain, formerly known as SS type, and Obama strain rotifers, formerly known as L type, using three common Japanese commercial enrichment diets; a control diet of *Chlorella vulgaris*-V12 against (i) DHA-enriched *Chlorella vulgaris*-V12, (ii) Frozen *Nannochloropsis oculata* and (iii) Salmon roe emulsion oil.

Materials and Methods

Experimental design

L type rotifer Obama strain stock population was obtained from the National Research Institute of Aquaculture, Fisheries Research Agency, Minami-Ise, Mie, Japan. SS type rotifers Indonesian strain stock population was obtained from Nagasaki University, Nagasaki Prefecture, Japan. Both morphotypes were cultured in the continuous system following protocols outlined by Kotani et al. (2009). The continuous system was used as the primary rotifer cultivation and would serve as the supply stock for the enrichment cultures and provide baseline nutritional levels. Concentrated *C. vulgaris* (Chlorella Industry Co. Ltd, Tokyo,

Japan) was used as the control feed for the culture protocols. Water temperature was adjusted and maintained at 25 ± 1℃ using heaters for both SS-type and L-type rotifer cultures. Seawater used in culture was pumped from Kagoshima Bay surrounding the Kamoike On-shore Laboratory, Education and Research Centre for Marine Resources and Environment at Kagoshima University, Kagoshima, Japan. The filtered seawater was then diluted with tap water and the salinity was adjusted to 20 ± 1 psu and was left standing under strong aeration for 24 h before use. Confirmations of salinity were undertaken daily using a refractometer. Levels of dissolved oxygen (DO), DO saturation ratio, temperature and pH were measured twice daily, before enrichment and after the 24 h enrichment period. Measurements of DO, saturation ratio and temperature were done using a portable DO meter (Horiba D-75 pH/ORP/DO Meter, Horiba Ltd., Kyoto, Japan) and pH was measured using a pH Meter (Horiba D-51 pH meter, Horiba Ltd., Kyoto, Japan). The environmental conditions were kept as uniform as possible to prevent differences in response that would arise from variables other than that of those caused by the enrichment.

Continuous culture

The system used was a slight modification of the system outlined by Kuwada (2000) and Kotani et al. (2009) (Fig. 1). The continuous culture system for SS-type rotifers consisted of three 55 *l* cylindro-conical tanks with (i) a tank supplying seawater, (ii) a tank for rotifer cultivation and (iii) a tank for harvesting the rotifers. In addition, the tanks were connected through the use of two quantitative pumps (Iwaki EWN-B11 Metering Pump, Iwaki Co. Ltd., Japan) were clear rubber hosing linked to a refrigerator that contained an 18 *l* barrel filled with a mixture of freshwater (15 *l*) and *C. vulgaris* (200 m*l*) at 20 psu. The pumps would supply the cultivation tank with seawater (33 psu) from the supply tank at a rate of (6 m*l*/min) whilst the other would supply the culture with the mixture of freshwater and *C. vulgaris* (at 4 m*l*/min; 15×10^9 cells/m*l*).

The continuous culture system for L-type rotifers consisted of three 200 *l* cylindro-conical tanks with each tank serving similar purposes as outlined for the SS-type system. The two quantitative pumps would supply the cultivation tank with seawater (33 psu) from the supply tank at a rate of (60 m*l*/min) whilst the

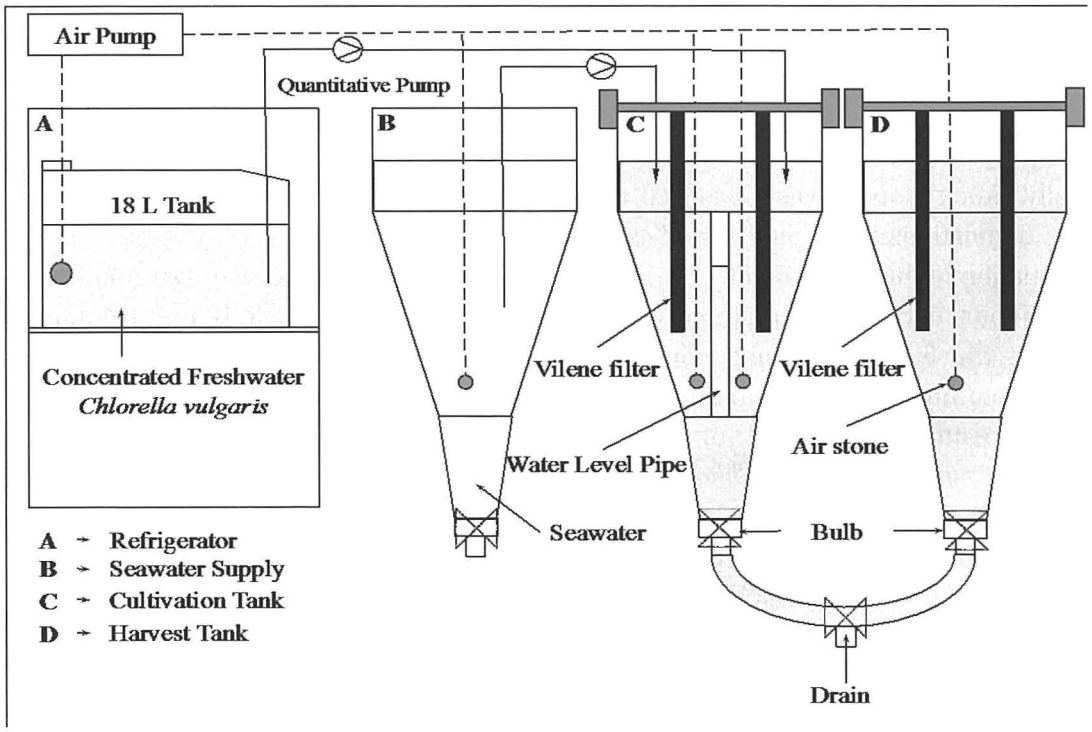

Fig. 1. Schematic overview of the modified continuous culture system of Kuwada (2000) and Kotani et al. (2009).

other would supply the culture with concentrated *C. vulgaris* in freshwater (at 40 m*l*/min; 15×10^9 cells/m*l*).

In both systems of continuous culture, aeration was provided by ceramic air-stones suspended at approximately 5 cm from the center of the tank bottom and 2 large sheets of Vilene mat filter (Japan Vilene Company Ltd., Saitama, Japan) were suspended vertically in the cultivation and harvest tanks to remove large particulate wastes. Dependent of density, approximately 20–30 *l* was harvested directly from the harvest tank during sampling as part of the control treatment. A second collection of 20–30 *l* was harvested from the continuous culture, rinsed and placed into a 15 *l* tank filled with 60 percent seawater (20 psu) then split evenly into 3 consignments of 5 *l* batch cultures.

Nutritional enrichment

The enrichment trials were performed in 5 *l* cultures. Rotifers harvested from the continuous culture were split into triplicates with initial stocking density of approximately 1300 ± 100 individuals in each culture. The cultures were placed in a heated water bath (27 ± 1°C) in order to maintain the culture water temperature in all cultures. Aeration was provided by ceramic air-stones suspended at approximately 5 cm from the center of the tank bottom. A small strip of Vilene mat filter (10 cm × 25 cm) was suspended vertically in the 5 *l* tanks to remove large particulate wastes, which was cleaned daily. Stock density was measured and the rotifer populations were harvested after 24 h of inoculation in the enrichment.

Three nutritional enrichment treatments were undertaken in the 5 *l* cultures and compared to the stock culture in continuous culture. The control treatment consisted of concentrated *Chlorella vulgaris* (Fresh *Chlorella*-V12, Chlorella Industry Co. Ltd., Tokyo, Japan) that was being fed automatically in the continuous culture system. The trialed enrichment treatments were: (i) concentrated DHA-enriched *Chlorella vulgaris* (Super Fresh *Chlorella*-V12, Chlorella Industry Co. Ltd., Tokyo, Japan) was fed at 46,000 cells/rotifer according to stock density at inoculation into the culture tanks. The volume of concentrated *Chlorella* to be fed was adjusted using the formula:

$$\text{Volume of } C.\ vulgaris\ (\text{m}l) = \frac{(\text{stock density} \times 0.46 \times \text{culture volume})}{150}$$

(ii) frozen *N. oculata* (K2 Frozen *Nannochloropsis*, Chlorella Industry Co. Ltd., Tokyo, Japan) was fed to rotifers at 0.6 g/*l* of culture volume (3.63×10^{10} cells/g); (iii) following the manufacturers instruction, salmon roe emulsion oil (Sujiko Nyuka, MarineTech Co. Ltd., Aichi, Japan) was fed at 0.06 g/*l* of culture volume. All 5 *l* batch treatments were enriched twice (0800, 1700) and were harvested 24 h after inoculation of rotifers. After enrichment, the populations were rinsed with freshwater on a 63 μm mesh net. The mesh net was patted dry using paper towels and the samples filled and weighed in separate tubes and stored at −80°C.

Population density was measured daily in each culture and the growth ratio from the previous day in the batch culture was calculated using:

$$\text{Growth ratio (\%)} = \left(\frac{D_p - D_{p-1}}{D_{p-1}}\right) \times 100$$

(Kotani et al. 2009)

where D_p is the population density after enrichment and D_{p-1} is the population density at inoculation. Using an adaptation of the above equation, egg ratio was calculated using:

$$\text{Egg bearing ratio (\%)} = \left(\frac{R_2 - R_1}{R_1}\right) \times 100$$

Where R_1 is the average number of rotifers not bearing eggs; R_2 is the average number of rotifers bearing eggs (i.e. eggs and rotifer with 1,2 or 3 eggs are all counted). The formula is an adaptation of the similar formula used in growth ratio (Kotani et al. 2010) and similar to that used by Wilde et al. (2010) for egg rate.

Measurement of rotifer lorica length

The Keyence VHX-S90F free-angle observation system with the Keyence VH-Z20R digital microscope (Keyence Corporation, Osaka, Japan) was used to measure the lorica length of

100 individuals from each rotifer type. Rotifer samples were immobilized using Lugol's solution (I_3K) before measurement. Measurements of (i) lorica length (from lorica crown spines (base of corona) extending to the lower extremity of the lorica and (ii) lorica width (across laterally the widest points of the lorica) were undertaken as outlined by Fu et al. (1991) (Fig. 2).

Fatty acid composition

The extraction of lipids was undertaken following the methods outlined by Folch et al. (1957). Approximately 2 g (wet weight) of frozen samples are required for gas chromatography analyses and these were lyophilized using a freeze dryer (EYELA FDU-1200, Tokyo Rikaikai Co., Ltd, Japan). The samples were then suspended in chloroform and methanol solutions with varying concentration ratios (2:1, 1:1 v/v). These were then mixed thoroughly using a homogenizer (VH-10 1-8471-31 Violamo Homogenizer, AS ONE Laboratory Equipments and Supplies, Osaka, Japan) before being filtered twice through circular filter paper (Whatman 1001-110 Grade 1 Qualitative Filter Paper, GE Healthcare Companies, Buckinghamshire, United Kingdom). Crude lipids were separated using into non-polar lipid (NL) and polar lipid (PL) fractions by column chromatography on Sep-Pak cartridges (WAT020520 Sep-Pak Plus, Silica Cartridges, Waters Corporation, Massachussets, U.S.A) (Juaneda and Rocquelin, 1985) with chloroform:methanol (98:2 v/v) to obtain NL and methanol for PL. The extracted lipids were then transferred to test tube vials using a 0.5 m*l* mixture of C19:0 (internal standard) diluted in chloroform (2 mg/m*l*) after which 1 m*l* of 5% hydrogen chloride methanol solution was added. These were then incubated at 85 ± 1°C for 3 h. After cooling, 1 m*l* of hexane and 5.5 m*l* of double distilled water were added to the test tube vials and vortexed using a mixer (FLX-F60 Frontlab Mixer, AS ONE Laboratory Equipments and Supplies, Osaka, Japan) then centrifuged for 10 mins at 3300 rpm for 10 mins using a centrifuge (LC 06 Centrifuge, Tomy Seiko Co., Ltd, Tokyo, Japan). Fatty acid containing hexane layer was extracted, transferred to vials and stored at −80°C until gas chromatography analysis. When subjected to GC, the samples were injected automatically by an autosampler into the gas chromatographer (GC-2010 Plus; Shimazu, Kyoto, Japan) equipped with a hydrogen flame ionizing type detector (260°C). Helium was used as a carrier gas. Omegawax capillary gas ghromatography column (Sperco, 30 m × 0.32 mm × 0.25 μm) was used as the capillary column. Data were analyzed based on the nonadecanoic acid (C19:0) internal standard.

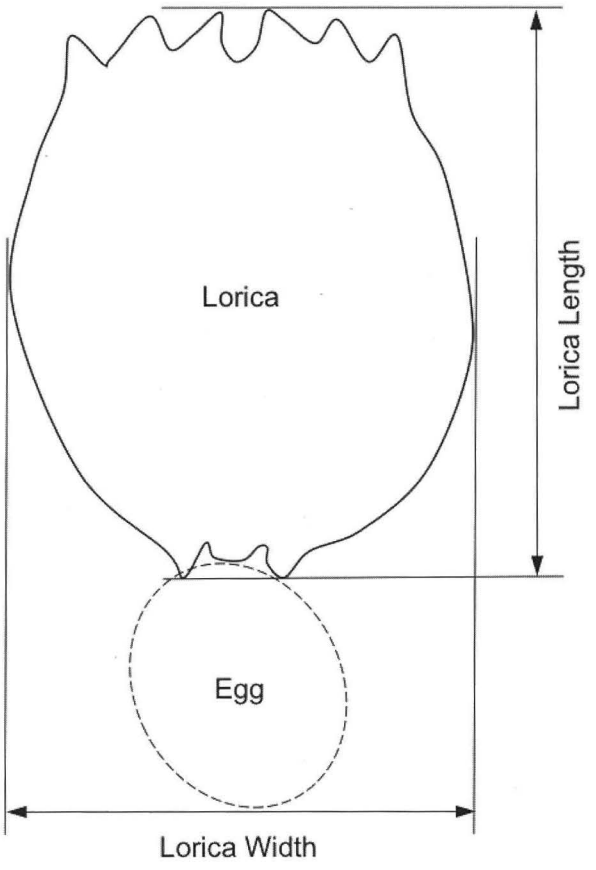

Fig. 2. Lorica length and width measurements (Waqalevu et al., 2018) following methods by Fu et al. (1991).

Protein content

To determine the amount of soluble protein present we homogenized 0.01 g (dry weight (dw)) of harvested rotifer in 200 μ*l* ice-cold phosphate buffer (pH 7.4) and the supernatant was extracted after centrifugation (at 4°C, 1700 g for 10 min). We extracted 20 μ*l* from the stock homogenate and any leftover supernatant was stored at −80°C for later use. The extracted

homogenate was then diluted 500 times due to the high amount of exhibited protein levels in the preliminary studies. The samples were then subject to the Bradford method of binding Coomasie Brilliant Blue G-250 (CBB) dye to proteins. Bovine serum albumin (BSA) was used as the standard and samples were analyzed using a Hitachi ratio beam spectrophotometer U-5100. Standard curves were then compared to sample data to ascertain protein content in each rotifer sample.

Crude protein levels were determined following the Kjeldahl method. Using 0.1 g dw of rotifer sample we digested the sample in 10 m*l* each of concentrated H_2SO_4 and 30% H_2O_2 for 90 min at 550°C. This was followed by distillation in 50 m*l* 30–40% NaOH using the KJELDAHL distilling apparatus (1002 Kjeltec System Tecator, Höganäs, Sweden). 150 m*l* of the solution was then distilled in H_3BO_3 solution, with methylene blue and methyl red indicators in ethanol that was titrated with 0.1 NH_2SO_4 until a neutral pH is reached. The percentage nitrogen (%N) and the percentage crude protein (%CP) were calculated using:

$$\% \text{ N} = \frac{(\text{S-B}) \times \text{N} \times 1.4007}{\text{W}}$$

$$\% \text{ Protein} = \% \text{ N} \times \text{Protein factor}$$

Where, S is the amount (H_2SO_4 (m*l*)) back titration of sample, B is the amount (H_2SO_4 (m*l*)) back titration of blank, N is the normality of H_2SO_4, W is the weight of the sample (g) and 1.4007 is the milliequivalent weight of nitrogen. A protein factor of 6.25 was used was in this study.

Statistical analysis

Statistical analysis was undertaken in SigmaPlot© version 11.0, from Systat Software, Inc., San Jose California USA. Non-parametric data were subject to Kruskal-Wallis ANOVA on ranks with any significant differences detected further subjected to a Student-Newman-Keuls multi comparison post-hoc test. For comparison between the two morphotypes Mann-Whitney *U*-test was conducted. Parametric data were subject to a one-way analysis of variance (ANOVA) and any significant differences were further subjected to a Tukey Honest Significant Difference post-hoc test. For comparison between the two morphotypes unpaired *t*-tests were conduct.

Results

Rotifer lorica length measurements

L-type rotifers were observed to be within the size range of lorica length: 173.48 ± 19.24 μm (L-type) and 123.21 ± 17.89 μm for SS-type (mean ± SD). Lorica width was found to be 81.11 ± 6.74 μm (L-type) and 61.85 ± 9.42 μm (SS-type). It can be seen that there is some overlap in size due to the close proximity in the upper size limit of SS-type and lower size limit of L-type.

Population growth

DHA-enriched *Chlorella* treatment was observed to have significantly higher growth and egg ratio for both species ($p < 0.05$). L-type were observed to have significantly higher growth ratio while SS-type had higher egg ratio under this treatment ($p < 0.05$) highlighting the performance of this treatment in terms of population growth but also the differences in reproductive response by each species (Table 1). In contrast, the salmon roe emulsion treatment was observed to have significantly low growth ratio in L and SS-type and also significantly low egg ratio in L-type ($p < 0.05$). SS-type had significantly lower egg ratio of the two morphotypes ($p < 0.05$) further highlighting the possible differences in reproductive responses to each treatment (Table 1). Frozen *N. oculata* treatment also experienced increases in growth and egg ratio and there were no significant differences observed between the two morphotypes. Population growth in the control culture remained relatively constant with minimal extreme fluctuations throughout the enrichment period: 10.91 ± 8.23% (L-type) and −7.23 ± 5.56% (SS-type).

Fatty acid composition

In the enrichment diets, significant differences were found in proportions of thirteen

fatty acid species in the NL region (16:0, 16:2, 16:3, 18:0, 18;1 *n*-9, 18:3 *n*-3, 18:4 *n*-3, 20:4 *n*-6, 20:4 *n*-3, 20:5 *n*-3, 22:0, 22:5 *n*-6, 22:6 *n*-3) and eleven fatty acid species in the PL (16:1, 16:2, 16:3, 18: 1 *n*-9, 18:2 *n*-6, 18:3 *n*-3, 20:4 *n*-6, 20:5 *n*-3, 22:0, 22:5 *n*-6; 22:6 *n*-3 (Table 2). DHA (22:6 *n*-3; $p < 0.01$) was not detected in the control and frozen *N. oculata* whereas significantly high proportions observed in both the DHA-enriched *C. vulgaris* (NL: 13.97 ± 0.40%; PL: 3.94 ± 0.55%) and salmon roe emulsion (NL: 15.22 ± 0.32%; PL: 26.14 ± 0.36%). Eicosapentaenoic acid (EPA, 20:5 *n*-3; $p < 0.01$) was significantly high in the frozen *N. oculata* (NL: 42.27 ± 2.44%; PL: 34.12 ± 5.03%). Arachidonic acid (ArA, 20: 4 *n*-6; $p < 0.05$) was found to be significantly high in both the NL and PL of the microalgal diets compared to the salmon roe emulsion diet. Salmon roe emulsion was found to have significantly higher ($p < 0.01$) fatty acid content (mg/g) of ArA, DHA and EPA compared to the microalgal based diets, with

Table 1. Growth ratio and egg ratio of SS-type rotifer at inoculation and after 24 h enrichment

Rotifer Species Population Growth	Rotifer morphotypes			
	L-Type		SS-Type	
	Growth ratio* (%)	Egg ratio* (%)	Growth ratio* (%)	Egg ratio (%)
Chlorella vulgaris (Control)	10.91 ± 8.23 ^{ab}	5.64 ± 1.14 ^{b}	(-)7.23 ± 5.51 ^{b}	3.76 ± 1.18
DHA-enriched *C. vulgaris*	55.24 ± 24.35 $^{b+}$	7.31 ± 5.86 ^{b}	25.34 ± 10.87 ^{b}	7.83 ± 2.01 $^{+}$
Frozen *N. oculata*	20.44 ± 8.37 ^{b}	5.99 ± 0.27 ^{b}	14.87 ± 3.96 ^{b}	6.07 ± 1.91
Salmon egg emulsion oil	(-)41.07 ± 5.65 ^{a}	(-)14.53 ± 4.95 ^{a}	(-)63.88 ± 6.68 $^{a+}$	(-)9.33 ± 3.43

Values indicate mean ± SD in that treatment. Asterisks indicate significant differences among treatments in that fatty acid (One-Way ANOVA, *: $p < 0.05$). Crosses indicate significant differences between same treatments among rotifer type in that fatty acid (Unpaired *T*-test, +: $p < 0.05$). Alphabetical superscripts indicate result of a Tukey pairwise multiple comparison test among treatment ($p < 0.05$, $b > a$).

Table 2. Fatty acid composition (area %) and contents (mg/g dry weight) of enrichment diets

Enrichment		Non-Polar Lipid					Polar Lipid			
		Chlorella vulgaris (Control)	DHA-enriched *C. vulgaris*	Frozen *N. oculata*	Salmon roe emulsion oil		*Chlorella vulgaris* (Control)	DHA-enriched *C. vulgaris*	Frozen *N. oculata*	Salmon roe emulsion oil
Fatty acid (%)										
14:0		3.46 ± 0.25	5.03 ± 0.72	4.33 ± 1.26	3.67 ± 0.11		2.82 ± 1.39	5.19 ± 2.12	5.42 ± 4.23	7.50 ± 0.05
16:0	*	17.22 ± 1.09 ^{c}	18.17 ± 2.60 ^{c}	12.96 ± 3.72 ^{b}	9.46 ± 0.05 ^{b}		26.15 ± 1.56	16.66 ± 3.22	9.14 ± 3.67	16.50 ± 0.18
16:1		2.48 ± 0.21	3.53 ± 0.30	5.69 ± 0.21	6.79 ± 0.05	*	3.21 ± 2.15 ^{b}	1.48 ± 2.23 ^{a}	4.70 ± 6.67 ^{c}	0.46 ± 0.01 ^{a}
16:2	*	2.15 ± 0.09 ^{c}	0.70 ± 0.16 ^{a}	1.82 ± 0.10 ^{b}	0.72 ± 0.02 ^{a}	*	0.98 ± 0.61 ^{c}	0.71 ± 0.30 ^{c}	8.86 ± 7.75 ^{b}	1.28 ± 0.02 ^{a}
16:3	*	2.04 ± 0.18 ^{c}	2.57 ± 0.03 ^{c}	1.56 ± 0.09 ^{b}	0.35 ± 0.01 ^{a}	*	0.52 ± 0.90 ^{a}	2.34 ± 0.37 ^{b}	3.18 ± 1.92 ^{b}	0.34 ± 0.01 ^{a}
18:0	**	8.78 ± 1.30 ^{c}	1.67 ± 1.14 ^{b}	8.75 ± 0.91 ^{c}	0.19 ± 0.00 ^{a}		4.94 ± 7.23	3.15 ± 0.03	9.11 ± 12.67	0.36 ± 0.01
18:1 n-9	*	4.45 ± 0.88 ^{b}	0.45 ± 0.52 ^{a}	3.52 ± 0.41 ^{b}	0.63 ± 0.98 ^{a}	**	1.01 ± 1.75 ^{a}	15.65 ± 0.01 ^{c}	7.33 ± 1.89 ^{b}	6.65 ± 0.23 ^{b}
18:2 n-6		12.77 ± 0.68	14.48 ± 1.95	10.52 ± 0.57	15.91 ± 0.12	*	18.77 ± 2.16 ^{b}	13.59 ± 1.33 ^{b}	6.48 ± 4.18 ^{a}	11.61 ± 0.08 ^{b}
18:3 n-3	**	4.65 ± 0.30 ^{c}	1.73 ± 1.51 ^{b}	0.94 ± 0.13 ^{a}	3.66 ± 0.21 ^{c}	**	ND ^{a}	2.83 ± 0.59 ^{b}	3.57 ± 3.43 ^{b}	3.14 ± 0.06 ^{b}
18:4 n-3	*	2.02 ± 0.38 ^{c}	0.38 ± 0.05 ^{a}	1.06 ± 0.17 ^{b}	1.07 ± 0.19 ^{b}		3.83 ± 3.32	0.83 ± 0.08	2.68 ± 4.01	1.15 ± 0.01
20:4 n-6	**	0.55 ± 0.62 ^{a}	2.52 ± 0.19 ^{b}	2.06 ± 0.07 ^{b}	0.14 ± 0.01 ^{a}	*	3.82 ± 1.35 ^{b}	1.90 ± 0.55 ^{b}	6.51 ± 0.85 ^{c}	0.70 ± 0.44 ^{a}
20:4 n-3	**	1.97 ± 0.65 ^{b}	0.54 ± 0.07 ^{a}	4.14 ± 0.18 ^{c}	0.17 ± 0.00 ^{a}		2.62 ± 0.76	1.08 ± 0.75	2.88 ± 0.33	0.94 ± 0.72
20:5 n-3	**	13.63 ± 1.10 ^{c}	0.36 ± 0.02 ^{a}	42.27 ± 2.44 ^{d}	3.91 ± 0.06 ^{b}	**	0.28 ± 0.49 ^{a}	0.16 ± 0.64 ^{a}	34.14 ± 5.03 ^{c}	1.62 ± 0.10 ^{b}
22:0	**	ND ^{a}	0.80 ± 0.30 ^{b}	ND ^{a}	19.59 ± 1.54 ^{c}	**	0.29 ± 0.50 ^{b}	0.37 ± 0.15 ^{b}	ND ^{a}	5.55 ± 9.24 ^{c}
22:5 n-6	**	ND ^{a}	2.32 ± 0.71 ^{c}	ND ^{a}	0.74 ± 0.32 ^{b}	**	ND ^{a}	3.27 ± 0.07 ^{c}	ND ^{a}	0.82 ± 0.70 ^{b}
22:6 n-3	**	ND ^{a}	13.97 ± 0.40 ^{b}	ND ^{a}	15.22 ± 0.32 ^{b}	**	ND ^{a}	3.94 ± 0.55 ^{b}	ND ^{a}	26.14 ± 0.36 ^{c}
Contents (mg/g dw)										
ArA	*	0.09 ± 0.06 ^{a}	0.32 ± 0.07 ^{b}	0.22 ± 0.03 ^{b}	0.16 ± 0.02 ^{b}		0.25 ± 0.07	0.17 ± 0.04	0.20 ± 0.02	1.06 ± 0.70
EPA	**	1.44 ± 1.94 ^{a}	0.05 ± 0.02 ^{a}	2.44 ± 0.70 ^{b}	4.36 ± 0.27 ^{c}	*	0.03 ± 0.05 ^{a}	0.01 ± 0.06 ^{a}	1.06 ± 0.01 ^{b}	2.44 ± 0.23 ^{c}
DHA	**	ND ^{a}	1.77 ± 0.62 ^{b}	ND ^{a}	16.96 ± 1.18 ^{c}	**	ND ^{a}	0.37 ± 0.05 ^{b}	ND ^{a}	39.37 ± 1.19 ^{c}
ΣSFA[1]	**	1.34 ± 0.43 ^{a}	2.62 ± 1.63 ^{a}	1.18 ± 0.42 ^{a}	36.61 ± 2.27 ^{b}	*	3.20 ± 1.12 ^{b}	1.98 ± 0.32 ^{b}	0.42 ± 0.88 ^{a}	35.94 ± 14.41 ^{c}
Σn-3	**	2.44 ± 1.11 ^{a}	2.10 ± 0.81 ^{a}	2.79 ± 0.81 ^{a}	26.75 ± 1.86 ^{b}	**	0.75 ± 0.51 ^{a}	1.10 ± 0.36 ^{b}	1.30 ± 0.48 ^{b}	49.68 ± 2.67 ^{c}
Σn-6	**	1.55 ± 0.25 ^{b}	2.43 ± 1.00 ^{b}	0.72 ± 0.21 ^{a}	18.71 ± 1.35 ^{c}	**	2.04 ± 0.27 ^{b}	1.87 ± 0.07 ^{b}	0.45 ± 0.56 ^{a}	57.91 ± 2.38 ^{c}
DHA/EPA	**	ND ^{a}	37.72 ± 30.23 ^{c}	ND ^{a}	3.89 ± 4.43 ^{b}	**	ND ^{a}	27.20 ± 0.73 ^{c}	ND ^{a}	16.16 ± 5.15 ^{b}
ArA/EPA	*	0.04 ± 0.03 ^{b}	6.91 ± 7.10 ^{b}	0.05 ± 0.05 ^{b}	0.04 ± 0.07 ^{b}	**	8.64 1.48 ^{b}	12.85 ± 0.87 ^{b}	0.19 ± 0.22 ^{a}	0.43 ± 1.05 ^{a}
n-3/n-6	*	1.58 ± 0.55 ^{b}	0.86 ± 0.81 ^{a}	3.85 ± 2.91 ^{b}	1.43 ± 1.38 ^{a}	*	0.37 ± 0.88 ^{a}	0.59 ± 0.14 ^{a}	2.91 ± 0.87 ^{b}	0.86 ± 0.12 ^{a}

Values indicate mean ± SD in that diet. ΣSFA[1]: Total Saturated Fatty Acid (14:0, 16:0, 18:0, 22:0). Asterisks indicate significant differences among treatments in that fatty acid (One-Way ANOVA or Kruskal-Wallis test, *: $p < 0.05$; **: $p < 0.01$). Alphabetical superscript indicates the result of a Tukey pairwise multiple comparison test among treatment ($p < 0.05$, $c > b > a$).

higher content observed in the PL than in the NL (Table 2). DHA-enriched *C. vulgaris* was observed to have the highest DHA/EPA ratio in both the NL (37.72 ± 3.23 mg/g) and PL (27.20 ± 0.73 mg/g) and also in ArA/EPA ratio (PL: 12.85 ± 0.87 mg/g).

In L-type rotifers, significant differences were found in proportions of twelve fatty acid species in the NL region (14:0, 16:2, 18:0, 18;1 *n*-9, 18:2 *n*-6, 18:3 *n*-3, 18:4 *n*-3, 20:4 *n*-6, 20:5 *n*-3, 22:0, 22:5 *n*-6, 22:6 *n*-3) and eight fatty acid species in PL (16:3, 18:0, 18:2 *n*-6, 18:3 *n*-3, 20:4 *n*-6, 20:4 *n*-3, 20:5 *n*-3, 22:5 *n*-6) (Table 3, Table 4). Of the *n*-3 fatty acids in the NL, proportions of alpha-linolenic acid (ALA, 18:3 *n*-3; $p < 0.05$), stearidonic acid (SDA, 18:4 *n*-3; $p < 0.05$) and EPA, (20:5 *n*-3; $p < 0.01$) were significantly higher in the frozen *N. oculata* treatment whilst docosahexaenoic acid (DHA, 22:6 *n*-3; $p < 0.01$) was significantly higher in DHA-enriched *C. vulgaris* and salmon roe emulsion treatments. Proportion of *n*-6 fatty acids such as linolenic acid (LA, 18:2 *n*-6; $p < 0.05$) were significantly higher in control, docosapentaenoic acid (DPA, 22:5 *n*-6; $p < 0.01$) in DHA-enriched *C. vulgaris* and EPA ($p < 0.05$) in frozen *N. oculata* treatment. DHA was not detected in the frozen *N. oculata* treatment. In the PL, the *n*-3 fatty acids ALA (18:3 *n*-3; $p < 0.05$), eicosatetraenoic acid (ETA, 20:4 *n*-3; $p < 0.05$) and EPA (20:5 *n*-3; $p < 0.01$) were significantly higher in frozen *N. oculata* treatment whilst DHA (22:6 *n*-3; $p < 0.01$) was significantly higher in DHA-enriched *C. vulgaris* and salmon roe emulsion treatment. Similar to NL, DHA was not detected in the frozen *N. oculata* treatment. Significant differences were also observed in *n*-6 fatty acid proportions in the PL in LA (18:2 *n*-6; $p < 0.05$) of the control treatment, ArA (20:4 *n*-6; $p < 0.05$) in frozen *N. oculata* and DPA (22:5 *n*-6; $p < 0.01$) in DHA-enriched *C. vulgaris*.

In SS-type rotifers, significant differences were found in proportions of nine fatty acid species in the NL region (16:2, 18:0, 18:2 *n*-6, 18:3

Table 3. Fatty acid compositions (area %) and contents (mg/g dry weight) of non-polar lipids of L and SS type rotifers according to various enrichment treatments

Rotifer species		L type					SS type			
Enrichment Treatment		*Chlorella vulgaris* (Control)	DHA-enriched *C. vulgaris*	Frozen *N. oculata*	Salmon roe emulsion oil		*Chlorella vulgaris* (Control)	DHA-enriched *C. vulgaris*	Frozen *N. oculata*	Salmon roe emulsion oil
Fatty acid (%)										
14:0	*	6.39 ± 1.91 ^{a}	4.37 ± 2.92 ^{a}	7.43 ± 0.72 $^{a+}$	14.06 ± 4.86 $^{b+}$		3.64 ± 1.08	4.08 ± 1.87	2.60 ± 1.53	3.62 ± 0.76
16:0		21.22 ± 5.28	14.79 ± 1.73	21.72 ± 1.93	14.01 ± 1.56		16.99 ± 0.07	16.51 ± 3.02	16.81 ± 8.92	14.73 ± 1.16
16:1		2.13 ± 0.15	2.81 ± 0.23 $^{+}$	3.76 ± 1.53	3.48 ± 0.27		2.43 ± 0.34 ^{a}	1.06 ± 0.88 ^{a}	3.39 ± 2.73 ^{b}	5.12 ± 1.68 ^{b}
16:2	*	3.93 ± 0.19 ^{b}	7.18 ± 2.35 $^{ab+}$	1.40 ± 0.29 ^{a}	1.64 ± 0.34 ^{a}	*	5.71 ± 1.77 ^{b}	2.02 ± 1.30 ^{ab}	0.24 ± 0.15 ^{a}	0.78 ± 0.04 ^{a}
16:3		0.51 ± 0.06	0.55 ± 0.47	1.29 ± 0.70	0.84 ± 0.25		0.35 ± 0.12	0.66 ± 0.55	2.46 ± 2.66	1.40 ± 0.44
18:0	*	8.45 ± 0.39 ^{b}	1.68 ± 2.34 ^{a}	4.57 ± 0.36 ^{ab}	4.73 ± 1.88 $^{ab+}$	**	5.70 ± 1.26 ^{b}	0.64 ± 0.24 ^{a}	3.28 ± 1.57 ^{ab}	0.70 ± 0.18 ^{a}
18:1 n-9	*	2.30 ± 0.25 ^{ab}	1.82 ± 1.14 ^{a}	3.58 ± 0.33 ^{ab}	5.23 ± 0.95 ^{b}		3.58 ± 0.77	1.13 ± 0.10	3.25 ± 2.08	3.75 ± 0.49
18:2 n-6	*	22.37 ± 1.25 ^{ab}	18.74 ± 3.63 ^{ab}	12.19 ± 2.30 ^{a}	20.24 ± 5.05 ^{ab}	*	28.21 ± 3.25 ^{b}	30.57 ± 6.61 $^{b+}$	19.48 ± 0.68 ^{a}	18.93 ± 1.97 ^{a}
18:3 n-3	*	2.55 ± 0.14 ^{a}	5.06 ± 0.71 ^{ab}	4.86 ± 1.07 ^{ab}	2.71 ± 1.30 ^{a}	*	7.33 ± 0.40 $^{b+}$	4.57 ± 1.97 ^{ab}	4.10 ± 0.77 ^{ab}	2.78 ± 1.13 ^{a}
18:4 n-3	*	0.59 ± 0.08 ^{a}	1.28 ± 0.62 ^{b}	2.91 ± 0.55 ^{c}	0.79 ± 0.38 ^{a}	*	0.21 ± 0.16 ^{a}	0.63 ± 0.78 ^{a}	1.59 ± 0.91 ^{b}	1.67 ± 0.07 $^{b+}$
20:4 n-6	*	0.30 ± 0.06 ^{a}	0.50 ± 0.03 ^{b}	3.49 ± 0.34 ^{ab}	2.21 ± 0.33 ^{ab}		1.87 ± 0.74 $^{a+}$	1.98 ± 0.70 $^{a+}$	3.74 ± 0.40 ^{b}	2.40 ± 0.82 ^{b}
20:4 n-3		1.50 ± 0.23 $^{+}$	0.71 ± 0.31	0.76 ± 0.17	0.88 ± 0.37	**	ND ^{a}	0.61 ± 0.26 ^{a}	1.62 ± 0.46 ^{b}	0.78 ± 0.12 ^{a}
20:5 n-3	**	0.31 ± 0.53 ^{a}	0.81 ± 0.46 ^{a}	8.74 ± 3.15 ^{b}	1.50 ± 0.39 ^{a}	**	ND ^{a}	0.50 ± 0.36 ^{a}	6.11 ± 1.74 ^{c}	1.39 ± 0.18 ^{b}
22:0	**	0.26 ± 0.05 $^{a+}$	5.03 ± 0.56 ^{b}	1.36 ± 0.48 ^{a}	1.61 ± 0.43 ^{b}	**	ND ^{a}	4.35 ± 1.10 ^{bc}	1.46 ± 0.75 ^{b}	2.26 ± 0.11 ^{b}
22:5 n-6	**	0.15 ± 0.04 ^{a}	3.11 ± 0.52 ^{c}	1.63 ± 0.49 ^{ab}	1.76 ± 0.95 ^{bc}		0.24 ± 0.41 ^{a}	2.93 ± 1.68 ^{b}	1.40 ± 1.07 ^{a}	3.96 ± 0.40 $^{b+}$
22:6 n-3	**	0.10 ± 0.17 ^{a}	7.70 ± 0.60 $^{b+}$	ND ^{a}	5.80 ± 1.94 ^{a}	**	0.27 ± 0.48 ^{a}	8.87 ± 0.93 ^{b}	ND ^{a}	10.73 ± 2.45 $^{b+}$
Contents (mg/g dw)										
ArA	*	0.13 ± 0.01 ^{a}	0.59 ± 0.19 ^{ab}	3.97 ± 2.80 $^{c+}$	1.00 ± 0.05 $^{b+}$	*	0.04 ± 0.02 ^{a}	1.28 ± 0.12 $^{b+}$	0.47 ± 0.18 ^{a}	0.33 ± 0.09 ^{a}
EPA	*	0.14 ± 0.07 ^{a}	1.39 ± 0.96 ^{ab}	4.74 ± 2.33 $^{c+}$	0.66 ± 0.05 ^{bc}	**	ND ^{a}	0.39 ± 0.09 ^{b}	1.22 ± 0.08 $^{d+}$	0.77 ± 0.08 ^{c}
DHA	**	0.04 ± 0.02 ^{a}	4.56 ± 1.53 $^{c+}$	ND ^{a}	3.07 ± 0.12 ^{b}	**	0.01 ± 0.02 ^{ab}	3.07 ± 1.29 ^{b}	ND ^{a}	2.74 ± 1.02 ^{b}
ΣSFA[1]		4.12 ± 1.48	6.59 ± 1.23 $^{+}$	1.55 ± 0.28	7.31 ± 1.39		2.52 ± 0.17	3.91 ± 1.51	4.67 ± 1.15 $^{+}$	4.63 ± 2.00
Σn-3	*	0.23 ± 0.08 ^{a}	7.59 ± 2.37 ^{c}	4.88 ± 2.30 $^{b+}$	3.75 ± 0.17 ^{b}	**	0.21 ± 0.19 ^{a}	5.66 ± 1.22 ^{b}	1.22 ± 0.08 ^{a}	3.72 ± 0.58 ^{b}
Σn-6	*	0.24 ± 0.19 ^{a}	2.74 ± 0.64 ^{ab}	3.99 ± 2.80 ^{b}	1.39 ± 0.26 ^{a}	**	0.82 ± 0.80 ^{a}	4.86 ± 0.49 ^{c}	0.58 ± 0.22 ^{a}	1.34 ± 0.44 ^{b}
DHA/EPA	**	0.32 ± 0.18 $^{a+}$	3.65 ± 1.39 $^{b+}$	ND ^{a}	4.63 ± 2.34 ^{b}	**	ND ^{a}	4.80 ± 1.05 ^{b}	ND ^{a}	3.63 ± 1.50 ^{ab}
ArA/EPA	*	2.52 ± 0.60 ^{b}	0.49 ± 0.21 ^{a}	1.06 ± 0.97 ^{a}	1.52 ± 0.95 ^{ab}	**	ND ^{a}	2.00 ± 0.14 ^{b}	0.39 ± 0.16 ^{a}	0.42 ± 0.08 ^{ab}
n-3/n-6		0.97 ± 0.44	2.86 ± 0.58	1.72 ± 1.10	2.69 ± 0.67	*	0.26 ± 0.04 ^{a}	1.16 ± 0.30 ^{a}	2.12 ± 0.33 ^{b}	3.07 ± 1.38 ^{b}

Values indicate mean ± SD in that treatment. ΣSFA[1]: Total Saturated Fatty Acid (14:0, 16:0, 18:0, 22:0). Asterisks indicate significant differences among treatments in that fatty acid (One-Way ANOVA or Kruskal-Wallis test, *: $p < 0.05$; **: $p < 0.01$). Crosses indicate significant differences between same treatments among rotifer type in that fatty acid (Unpaired *T*-test or Mann-Whitney *U*-Test, +: $p < 0.05$). Alphabetical superscript indicates the result of a Tukey pairwise multiple comparison test among treatment ($p < 0.05$, $c > b > a$).

n-3, 18:4 *n*-3, 20:4 *n*-3, 20:5 *n*-3, 22:0, 22:6 *n*-3) and five fatty acid species in PL (18:1 *n*-9, 20:4 *n*-3, 20:5 *n*-3, 22:0, 22:6 *n*-3) (Table 3, Table 4). Of the *n*-3 fatty acids in the NL, there were significantly higher proportions of ALA (18:3 *n*-3; $p < 0.05$) observed in control treatment, ETA (20:4 *n*-3; $p < 0.01$) and EPA (20:5 *n*-3; $p < 0.01$) in frozen *N. oculata* treatment and SDA (18:4 *n*-3; $p < 0.05$) and DHA (22:6 *n*-3; $p < 0.01$) in salmon roe emulsion and DHA-enriched chlorella. ArA, ETA, EPA were not detected in the control whilst DHA was not detected in the frozen *N. oculata* treatment. LA (18:2 *n*-6; $p < 0.05$) was the only *n*-6 fatty acid species that had significantly higher proportion in the NL of the *C. vulgaris* based control treatment and DHA-enriched *C. vulgaris*. In the PL region, *n*-3 fatty acids were significantly higher in proportion in ETA (20:4 *n*-3; $p < 0.01$) in DHA-enriched *C. vulgaris*, EPA (20:5 *n*-3; $p < 0.01$) in frozen *N. oculata* and DHA (22:6 *n*-3; $p < 0.01$) in both DHA-enriched *C. vulgaris* and salmon roe emulsion treatment. DHA was not detected in the frozen *N. oculata* treatment whilst ETA, EPA and DHA were not detected in the control treatment. There were no significant differences observed in *n*-6 fatty acid proportions in PL region of the SS-type morphotype.

Of the essential fatty acids important for larviculture in the NL region of L-type rotifers; ArA (3.97 ± 2.80 mg/g) and EPA (4.74 ± 2.33 mg/g) were significantly higher in the frozen *N. oculata* treatment. Whilst DHA was significantly higher in DHA-enriched *C. vulgaris* (4.56 ± 1.53 mg/g) and salmon roe emulsion (3.07 ± 0.12 mg/g) treatments along with significantly higher DHA/EPA ratios 3.65 ± 1.39 mg/g (DHA-enriched *C. vulgaris*) and 4.63 ± 2.34 mg/g (salmon roe emulsion) (Table 3). Similar trends were observed in the PL region, however the content was slightly lower than observed in the NL, furthermore significantly high levels of total Saturated Fatty Acids (14:0, 16:0, 18:0, 22:0) was observed (Table 4). Of the essential

Table 4. Fatty acid compositions (area %) and contents (mg/g dry weight) of polar lipids of L and SS type rotifers according to various enrichment treatments

Rotifer species		L type					SS type			
Enrichment Treatment		*Chlorella vulgaris* (Control)	DHA-enriched *C. vulgaris*	Frozen *N. oculata*	Salmon roe emulsion oil		*Chlorella vulgaris* (Control)	DHA-enriched *C. vulgaris*	Frozen *N. oculata*	Salmon roe emulsion oil
Fatty acid (%)										
14:0		3.49 ± 0.51	9.74 ± 2.35 $^{+}$	3.08 ± 0.40	7.34 ± 4.90 $^{+}$		4.17 ± 2.98	2.98 ± 0.37	1.49 ± 0.44	1.95 ± 0.83
16:0		16.69 ± 1.06	18.64 ± 1.61	15.70 ± 2.41	14.47 ± 4.92		16.08 ± 5.07	15.41 ± 2.11	16.59 ± 1.80	17.86 ± 1.50
16:1		1.73 ± 0.34	0.68 ± 0.16	3.04 ± 1.68	2.55 ± 1.26		3.41 ± 0.16 ^{b}	2.25 ± 0.44 $^{a+}$	3.98 ± 0.51 ^{b}	3.30 ± 0.10 ^{b}
16:2		1.53 ± 0.27	1.41 ± 0.27	2.30 ± 0.26	0.50 ± 0.33		1.10 ± 0.59	1.32 ± 0.99	1.39 ± 0.35	1.29 ± 0.52 $^{+}$
16:3	*	1.36 ± 0.25 ^{a}	2.91 ± 0.58 ^{a}	1.78 ± 0.30 ^{a}	6.29 ± 1.89 $^{b+}$		3.26 ± 1.99 $^{+}$	1.80 ± 0.50	1.18 ± 0.27	1.22 ± 0.37
18:0	*	6.65 ± 0.46 $^{c+}$	3.48 ± 0.47 $^{ab+}$	2.73 ± 1.11 ^{a}	5.01 ± 1.62 $^{bc+}$		2.37 ± 0.97 ^{b}	1.73 ± 0.75 ^{a}	1.47 ± 0.41 ^{a}	4.52 ± 1.54 ^{c}
18:1 n-9		5.67 ± 0.79	3.41 ± 0.22 $^{+}$	5.12 ± 1.38	6.35 ± 1.20	*	5.22 ± 1.40 ^{b}	1.66 ± 0.59 ^{a}	5.21 ± 0.07 ^{b}	4.96 ± 0.54 ^{b}
18:2 n-6		30.02 ± 3.77 ^{c}	22.74 ± 2.72 ^{ab}	17.04 ± 0.54 ^{a}	21.76 ± 5.43 ^{bc}		22.91 ± 3.19 ^{b}	25.11 ± 1.88 ^{b}	19.89 ± 3.61 ^{a}	18.45 ± 2.50 ^{ab}
18:3 n-3	**	3.18 ± 0.15 ^{b}	1.16 ± 0.12 ^{a}	3.52 ± 0.99 ^{b}	3.31 ± 0.77 ^{b}		3.02 ± 0.75	4.36 ± 0.73 $^{+}$	2.68 ± 0.18	2.32 ± 0.34
18:4 n-3		1.43 ± 0.29	1.78 ± 0.09	1.58 ± 0.08	2.27 ± 0.81 $^{+}$		2.99 ± 1.11	4.64 ± 0.40 $^{+}$	2.20 ± 0.26	2.28 ± 0.14
20:4 n-6		1.59 ± 0.18	1.51 ± 0.18	3.47 ± 0.21	2.42 ± 0.74		4.55 ± 2.61 $^{+}$	2.50 ± 0.16	3.50 ± 0.22	3.71 ± 1.34
20:4 n-3	*	2.24 ± 0.12 $^{b+}$	1.32 ± 0.29 ^{a}	2.02 ± 0.58 ^{b}	0.75 ± 0.24 $^{a+}$	**	ND ^{a}	3.11 ± 0.60 $^{c+}$	2.52 ± 0.43 ^{c}	0.15 ± 0.04 ^{b}
20:5 n-3	**	0.46 ± 0.22 $^{a+}$	0.36 ± 0.02 ^{a}	6.26 ± 0.85 ^{c}	2.46 ± 0.82 $^{b+}$	**	ND ^{a}	0.26 ± 0.21 ^{b}	5.11 ± 1.45 ^{c}	0.53 ± 0.22 ^{b}
22:0	**	ND ^{a}	4.11 ± 0.43 $^{d+}$	0.44 ± 0.16 ^{b}	2.25 ± 0.72 ^{c}	**	ND ^{a}	1.22 ± 0.78 ^{b}	1.64 ± 0.46 ^{b}	2.87 ± 0.05 ^{c}
22:5 n-6	**	ND ^{a}	3.40 ± 0.89 ^{b}	2.57 ± 0.11 ^{b}	3.13 ± 0.94 ^{b}		2.47 ± 0.81 $^{+}$	2.11 ± 1.07	2.41 ± 0.27	3.26 ± 0.25
22:6 n-3	**	ND ^{a}	4.25 ± 0.42 ^{b}	ND ^{a}	3.80 ± 1.16 ^{b}	**	ND ^{a}	3.54 ± 0.42 ^{b}	ND ^{a}	4.00 ± 0.52 ^{b}
Contents (mg/g dw)										
ArA	*	0.16 ± 0.03 ^{a}	0.19 ± 0.06 ^{a}	1.22 ± 0.07 $^{b+}$	0.78 ± 0.19 $^{b+}$	*	0.02 ± 0.01 ^{a}	0.45 ± 0.24 ^{b}	0.67 ± 0.47 ^{b}	0.22 ± 0.10 ^{a}
EPA	**	0.06 ± 0.01 $^{a+}$	0.50 ± 0.01 ^{ab}	3.62 ± 1.75 ^{c}	0.97 ± 0.06 $^{ab+}$	**	ND ^{a}	0.60 ± 0.08 ^{c}	1.55 ± 0.51 ^{c}	0.48 ± 0.27 ^{b}
DHA	**	ND ^{a}	2.08 ± 0.02 ^{c}	ND ^{a}	1.05 ± 0.03 $^{b+}$	**	ND ^{a}	1.03 ± 0.02 ^{b}	ND ^{a}	0.64 ± 0.50 ^{b}
ΣSFA[1]	*	4.45 ± 0.93 $^{b+}$	11.60 ± 4.97 $^{c+}$	1.50 ± 1.05 ^{a}	5.78 ± 2.54 ^{b}		3.97 ± 1.50 ^{a}	4.21 ± 1.56 ^{ab}	4.14 ± 1.48 $^{ab+}$	6.24 ± 1.73 ^{b}
Σn-3	*	0.08 ± 0.01 $^{a+}$	2.38 ± 0.03 $^{c+}$	0.71 ± 0.08 ^{b}	2.11 ± 1.07 ^{c}	*	0.14 ± 0.04 $^{a+}$	1.66 ± 0.07 ^{c}	0.93 ± 0.09 ^{b}	1.18 ± 0.53 ^{b}
Σn-6		1.53 ± 0.04 $^{+}$	1.20 ± 0.18	0.24 ± 0.08	1.58 ± 0.13 $^{+}$	*	0.19 ± 0.05 ^{a}	1.68 ± 0.25 ^{b}	0.12 ± 0.02 ^{b}	0.52 ± 0.43 ^{a}
DHA/EPA	*	ND ^{a}	4.19 ± 0.16 $^{c+}$	ND ^{a}	1.08 ± 0.09 ^{b}	**	ND ^{a}	1.73 ± 0.25 ^{b}	ND ^{a}	1.34 ± 1.49 ^{b}
ArA/EPA		0.63 ± 0.07 $^{+}$	0.39 ± 0.12	0.37 ± 0.13	0.80 ± 0.21	*	ND ^{a}	0.74 ± 0.38 $^{b+}$	0.48 ± 0.25 ^{b}	0.46 ± 0.14 ^{b}
n-3/n-6	*	0.20 ± 0.02 $^{a+}$	2.02 ± 0.31 $^{c+}$	3.28 ± 1.09 ^{c}	1.33 ± 0.07 ^{b}	**	0.13 ± 0.13 ^{a}	1.00 ± 0.14 ^{b}	8.09 ± 1.01 $^{c+}$	2.27 ± 2.06 ^{a}

Values indicate mean ± SD in that treatment. ΣSFA[1]: Total Saturated Fatty Acid (14:0, 16:0, 18:0, 22:0). Asterisks indicate significant differences among treatments in that fatty acid (One-Way ANOVA or Kruskal-Wallis test, *: $p < 0.05$; **: $p < 0.01$). Crosses indicate significant differences between same treatments among rotifer type in that fatty acid (Unpaired *T*-test or Mann-Whitney *U*-Test, +: $p < 0.05$). Alphabetical superscript indicates the result of a Tukey pairwise multiple comparison test among treatment ($p < 0.05$, $c > b > a$).

fatty acids in the NL region of SS-type rotifers; ArA (1.28 ± 0.12 mg/g) was significantly higher in DHA-enriched *C. vulgaris*, whilst EPA (1.22 ± 0.08 mg/g) was high in frozen *N. oculata* and DHA in both DHA-enriched *C. vulgaris* (3.07 ± 1.29 mg/g) and salmon roe emulsion (2.74 ± 1.02 mg/g) treatment (Table 3). Significantly high DHA/EPA (4.80 ± 1.05 mg/g) and ArA/EPA (2.00 ± 0.14 mg/g) ratio was observed in DHA-enriched *C. vulgaris*. In the PL region of SS-type, ArA (0.67 ± 0.47 mg/g) and EPA (1.55 ± 0.51 mg/g) levels were significantly higher in the frozen *N. oculata* treatment whilst DHA (1.68 ± 0.25 mg/g) was highest in the DHA-enriched *C. vulgaris*. DHA/EPA (1.73 ± 0.25 mg/g) and ArA/EPA (0.74 ± 0.38 mg/g) ratio was significantly higher in the DHA-enriched *C. vulgaris* treatment.

Protein content

There were no significant differences observed between each enrichment treatments and between rotifer morphotype in crude protein (% dw) and crude lipid (% dw; % ww) (Table 5). Significant differences were observed in soluble protein levels of DHA-enriched *C. vulgaris* (184.1 ± 2.6 mg/g) and frozen *N. oculata* (144.7 ± 1.5 mg/g) whilst low soluble protein was observed in the salmon roe emulsion treatment (85.6 ± 4.4 mg/g). The fraction of soluble protein to crude protein was significantly high in DHA-enriched *Chlorella* and lowest in salmon roe emulsion treatment. Similar trends were observed in SS-type, however the levels of soluble protein were significantly higher in some treatments (Table 5). DHA-enriched *C. vulgaris* (306.8 ± 1.3 mg/g) and the control treatment (268.3 ± 1.3 mg/g) were observed to have significantly high soluble protein compared to the low levels detected in the other two treatments. Furthermore, significantly high fraction of soluble protein to crude protein was observed in DHA-enriched *C. vulgaris* (46.0 ± 2.9 mg/g) and the control treatment (38.5 ± 2.9 mg/g). These results allude to possible effects of *Chlorella vulgaris* based enrichment on the levels of soluble levels in both morphotypes.

Discussion

Early rotifer enrichment practices were quantitative using media such as Baker's yeast (Imada et al. 1979; Watanabe et al. 1983) with aquaculturists in the field of larval rearing having little problem with these methods (Kitajima et al. 1979). However, recent studies have greatly improved the technology of rotifer enrichment (Dhert et al. 2001; Hagiwara et al. 2007; Kotani et al. 2009, 2010) and have highlighted the importance of essential fatty acids in larviculture (Park et al. 2006; Hamre et al. 2013; Hamre 2016; Kotani et al. 2017). Due to insufficient levels of essential fatty acids and *n*-3 HUFA, these studies highlight that enrichment is an important requirement in optimizing the nutritional characteristics of euryhaline rotifers for larviculture (Watanabe et al. 1978; Tomoda et al. 2004). Information on the effect of different enrichment on different morphotypes within the commercially valuable *B. plicatilis* sp. complex is limited, with only a few studies focusing on varying reproduction and stress tolerance between morphotypes and strains

Table 5. Crude protein, soluble protein and crude lipid contents of enriched L and SS type rotifers

Rotifer Species	Culture Conditions							
	L-Type				SS-Type			
Enrichment Treatment	*C. vulgaris* (Control)	DHA-enriched *C. vulgaris*	Frozen *N. oculata*	Salmon roe emulsion oil	*C. vulgaris* (Control)	DHA-enriched *C. vulgaris*	Frozen *N. oculata*	Salmon roe emulsion oil
Crude protein (mg/g dw)	682.8 ± 16.7	694.5 ± 10.5	755.5 ± 7.8	677 ± 13.2	722.5 ± 6.1	696.3 ± 15.3	688.8 ± 73.7	628.9 ± 4.8
Soluble protein (mg/g dw)	* 105.3 ± 0.9 ^{a}	184.1 ± 2.6 ^{c}	144.7 ± 1.5 ^{ab}	85.6 ± 4.4 ^{a}	** 268.3 ± 1.3 $^{bc+}$	306.8 ± 1.3 $^{c+}$	127.1 ± 4.4 ^{a}	97.1 ± 1.9 ^{b}
Soluble protein (% of crude protein)	* 16.7 ± 1.4 ^{a}	27.3 ± 2.9 ^{b}	20.7 ± 2.5 ^{ab}	12.8 ± 6.7 ^{a}	* 38.5 ± 1.3 $^{b+}$	46.0 ± 2.9 $^{c+}$	19.9 ± 4.7 ^{a}	15.9 ± 3.7 ^{a}
Crude lipid (% dw)	17.3 ± 1.3	16.1 ± 3.2	16.0 ± 1.7	15.9 ± 3.0	14.3 ± 4.1	16.3 ± 2.2	16.2 ± 2.6	17.8 ± 2.3
Crude lipid (% ww)	2.4 ± 0.6	3.4 ± 0.7	2.6 ± 1.1	2.9 ± 0.7	3.4 ± 0.4	3.7 ± 1.5	3.5 ± 1.6	4.7 ± 1.6

Values indicate mean ± SD in that treatment. Asterisks indicate significant differences among treatments in that fatty acid (One-Way ANOVA, *: $P < 0.05$). Crosses indicate significant differences between same treatments among rotifer type in that fatty acid (Unpaired *T*-test, +: $P < 0.05$). Alphabetical superscript indicates result of Tukey pairwise multiple comparison test among treatment ($p < 0.05$, $c > b > a$).

(Hagiwara et al. 1995; Hagiwara and Hino 1989; Araujo and Hagiwara 2005).

In this study, DHA-enriched *C. vulgaris* is highlighted to be the best performing enrichment based on population growth, levels of fatty acids and levels of soluble protein observed. In both morphotypes, the DHA-enriched *C. vulgaris* treatment was observed to have significantly higher population growth (L-type: 55.24 ± 24.35%; SS-type: 25.34 ± 10.87%) and egg bearing (L-type: 7.31 ± 5.86%; SS-type: 7.83 ± 2.01%). L-type species displayed higher growth ratio compared to SS-type with DHA-enriched *C. vulgaris* treatment having significantly higher growth and egg ratio in both morphotypes (Table 1). Hirayama and Rumengan (1993) found that the fecundity patterns of S and L type rotifers were similar and that the difference in growth response between the two types stemmed from differences in net reproduction rates. Studies show that based on morphological features, reproduction and allozyme patterns, SS strains differed from L strains in mating behavior. As outlined in the methods of this study, this variability occurred despite rotifers being stocked equally (1300 ± 100 individuals) in each replicate and with enrichment conditions kept as uniform as possible (salinity: 20 ± 1 psu; temperature: 25 ± 1°C; dissolved oxygen: 7.5 ± 0.3 mg/*l*) to keep the effects of the culture environment minimal.

The DHA-enriched *C. vulgaris* diet was observed to have high DHA proportion in both the NL and PL (Table 2) and this was also reflected with high DHA observed in both morphotypes (Table 2, Table 3). This trend was also observed for ArA and EPA in the frozen *N. oculata* diet which had high proportions of ArA (NL: 2.06 ± 0.07%; PL: 6.51 ± 0.85%), elevated levels of EPA (NL: 42.27 ± 2.44%; PL: 34.14 ± 5.03%) and mirrored by high assimilation of ArA and EPA in the NL and PL region of both morphotypes (Table 2 and 3). This reciprocal relationship was also observed in fatty acid species in the diet that had high proportions such as 14:0, 16:0, 18:0 and 18:2 *n*-6, which was also observed to be high in both morphotypes. Therefore it can be said that the fatty acid composition of both morphotypes effectively reflect the fatty acid composition of their enrichment diets as seen in this study, which is in agreement with what has previously been assumed to be true for invertebrate animals (Sakamoto et al. 1982; Frolov et al. 1991). Furthermore a study by Seychelles et al. (2009) highlighted that the levels of ArA, EPA and DHA in diets and those in *B. plicatilis* rotifers are linearly correlated.

DHA is known to have various positive effects on the development and survival of finfish larvae (Watanabe et al. 1978; Takeuchi et al. 1994) and ensure membrane absorbency, enzyme activation and production of prostaglandin among other functions (Sargent et al. 1989). Due to the fortification of DHA in DHA-enriched *C. vulgaris*, the results showed high DHA proportions and content in both morphotypes with the L-type having significantly higher DHA content in both NL and PL. In contrast, the EPA content was around fourfold less in both morphotypes in this treatment, this is perhaps due to the low detection of EPA in the diet (Table 2). Furthermore, previous studies have shown that *C. vulgaris* is a known poor source of EPA (Maruyama et al. 2006; Thépot et al. 2016). In both species, ArA/EPA ratio was high in DHA-enriched *C. vulgaris* with significantly high average content in the NL and PL of SS-type (Table 3, Table 4). Previous studies by Matsunari et al. 2013 and Thépot et al. 2016 highlighted that DHA-enriched *C. vulgaris* has low ArA which was not the case in this study. Kotani et al. (2013) reported that rotifers can naturally contain many fatty acids and there is a possibility that ArA can be naturally synthesized from linoleic acid (18:2 *n*-6), which had significantly higher proportion, up to 20-30% in both morphotypes in this study (Table 3, Table 4). The *Chlorella*-based enrichments may have supplemented this natural process. Furthermore, Lubzens et al. (1985) supports this hypothesis in that, while ArA production is low, rotifers have the ability to *de novo* synthesize polyunsaturated fats, though in small amounts, through the process of elongation and desaturation of

n-6 series as was reported by Robin (1995) and for *n*-3 series by Le Milinaire et al. (1983). The variability in the results of essential fatty acid proportions and content could also be due to differing growth states of the rotifer population. A study by Tomoda et al. (2004) highlighted that rotifers change their nutritional value depending on their growth state, where in spite of enrichment the dietary value of rotifers was poor during or just prior to the stationary growth phase of batch culture. However, the effects of such phenomenon were avoided in this study as the stock was harvested during periods of exponential growth from the continuous culture, as is an essential feature of the chemostat continuous culture system (Kotani et al. 2009).

Significant higher soluble protein levels detected in the DHA-enriched *Chlorella* and in control treatment for both morphotypes alludes to possible effects of *Chlorella vulgaris* based enrichment on soluble protein levels. *C. vulgaris* is one of the most exploited species due to its high protein content and favorable essential amino acid composition (Becker 2007; Bleakley and Hayes 2017). SS-type had significantly higher soluble protein content (306.8 ± 1.3 mg/g) with the soluble protein making up 46.0 ± 2.9% ratio of the crude protein. Only in the L-type rotifer was frozen *N. oculata* treatment observed to have high levels of soluble protein (184.1 ± 2.6 mg/g), second to DHA-enriched *C. vulgaris* (144.7 ± 1.5 mg/g). Srivastava et al. (2006) highlighted that variation in rotifer total soluble protein between species, stages, physiological status and variations according to culture conditions is to be expected. Previous studies have highlighted that live feed usually contain a large fraction of soluble intact proteins (Watanabe 1981; Govoni et al. 1986; Carvalho et al. 2003) and that soluble proteins are more available for larval digestion and absorption than insoluble proteins (Carvalho et al. 2004). Srivastava et al. (2006) stated that due to the drive for insoluble proteins to aggregate in insoluble inclusions, soluble proteins may be more available to intestinal proteases and also more available for uptake by pinocytosis. Soluble proteins have been suggested as having a higher importance in general larval dietary protein uptake (Watanabe 1981; Govoni et al. 1986) thus further highlighting the favorable effect of DHA-enriched *C. vulgaris* in both morphotypes observed in this study.

Compared to the low levels of EPA in the DHA-enriched *C. vulgaris*, salmon roe emulsion treatment was observed to contain EPA and near similar levels of DHA in the NL and PL of both morphotypes (Tables 2, Table 4). Thus this resulted in high DHA/EPA and ArA/EPA ratios. SS-type had higher proportion of DHA (NL: 10.73 ± 2.45; PL: 4.00 ± 0.52) compared to L-type (NL: 5.80 ± 1.94; PL: 3.80 ± 1.16) in both the NL and PL. Upon comparing the fatty acid constituents of the enrichment diet (Table 2) it can be said that the salmon roe emulsion treatment is an effective treatment due to its high lipid content and especially its fortification of essential fatty acids. However, both morphotypes were observed to experience significant drops in population growth (L-type: −41.07 ± 5.65%; SS-Type: −63.88 ± 6.68%) and egg ratio (L-type: −14.53 ± 4.95; SS-Type: −9.33 ± 3.43%) under the salmon roe emulsion treatment (Table 1). This is despite the relatively high HUFA composition in both NL and PL observed in both morphotypes under this treatment. These results suggest that this treatment was nutritionally effective but negatively affected population growth and reproductive capabilities. Salmon roe emulsion oil diet had much higher proportions ($p < 0.01$) of DHA (NL: 15.22 ± 0.32%; PL: 26.14 ± 0.36%) and DHA content (NL: 16.96 ± 1.18 mg/g; PL: 39.37 ± 1.19 mg/g) compared to DHA-enriched *C. vulgaris*, despite this both morphotypes had higher DHA content in the DHA-enriched *C. vulgaris*. These results would suggest that lipid rich diets do not necessarily mean increased uptake. The viscous nature of the oil-based salmon roe emulsion treatment may suggest that this is what is causing this effect. A study by Hagiwara et al. (1998) highlighted that high viscosity in culture water lowers ingestion rate, fecundity and population growth of *B. plicatilis* due to sub-lethal physiological stress. Larsen

et al. (2008) showed that viscosity in culture water affects cilia action and reduces swimming velocity in *B. plicatilis* by 26%. Araujo et al. (2000, 2001) suggested that an increase of culture water viscosity decreased enzyme activity in *B. plicatilis* and affected reproductive responses in *B. rotundiformis*. Several other studies (Foscarini 1988; Dhert et al. 1990) have also shown that rotifers enriched in oil emulsions often experience high mortality related to "sticking" at high densities, the production of lower quality rotifers with a too high lipid content and resultant reduction in water quality in fish culture tanks. As the environmental conditions were kept uniform, the dosage amount of oil enrichment media could prove to be the reason why there is a large discrepancy observed in population growth and reproduction between the salmon roe emulsion enrichment and the microalgae-based enrichment diets. The dosage of salmon roe emulsion oil used in this study was, 0.06 g/*l* of culture volume fed twice to both morphotypes over a 24 h period, as outlined by the manufacturer. Kotani et al. (2017) mentioned that generally, manufacturers of enrichment diets have determined the appropriate method and dosage for rotifers however, there is little information (Kotani et al. 2009, 2010) on the effects on rotifers of nutritional enrichment using methods not previously described by manufacturers. This is an area of research that requires important consideration, as dosage of enrichment will vary according to the scale of culture, environmental conditions and larval fish nutritional requirements.

In the frozen *N. oculata* treatment, there was high growth ratio and egg ratio in both morphotypes with no significant difference found between it and the higher DHA-enriched *Chlorella* treatment (Table 3). As expected, EPA proportion and content were significantly higher ($p < 0.01$) in the NL and PL of frozen *N. oculata*. *N. oculata* is known to support high rates of rotifer reproduction and contains significant amount of EPA (Hirayama et al. 1979; Lubzens et al. 1995; Kobayashi et al. 2008). ArA was also observed to have high proportion and contents in both NL and PL in both morphotypes with higher levels observed in L-type (Table 3, Table 4). During larviculture, Thépot et al. (2016) observed that larvae fed rotifers enriched with *N. oculata* diet had high ArA content and they hypothesized that this could be due to excess ArA supplied in the *N. oculata* diet. Furthermore, recent studies by Ferreira et al. (2008, 2009, 2018) highlighted the importance of nutritional quality of microalgae like *Nannochloropsis* sp. in influencing biochemical composition of rotifer cultures over the quantity and concentration of enrichment with further complication rising from differences in metabolism of enrichment, as was observed in this study in the two different morphotypes.

Comparing the fatty acid composition between the lipid regions, it can be observed that it is generally higher in the NL than in the PL in both morphotypes. In the salmon roe emulsion treatment, DHA assimilation in the diet is around double the proportion in PL region compared to NL (Table 2). A study by Kotani (2017) highlighted that the main constituent of fish eggs or yolk are phospholipids and our results also coincide with this. However, despite high PL content in the diet, we observed that in both morphotypes, fatty acid content is higher in the NL region, which suggests that there is selective assimilation of fatty acids by both morphotypes. These variations in fatty acid content in the lipid regions, within species and between morphotypes, possibly arises from effects of enrichment and also dissimilar bio-chemical roles played by these compounds in tissue metabolism as suggested by Frolov et al. (1991). In tissue metabolism, the PL is associated with regulation and catalysis in the cellular membrane and their fatty acids are known to produce a strong effect on biological functions (Frolov et al. 1991). Therefore its composition is usually not highly affected by abiotic and biotic factors, as the composition of its fatty acids must remain fairly invariable for the efficient functioning of membranes. On the other hand, NL functions are related to cellular energy and its fatty acid composition is influenced by several factors acting simultaneously.

These include the turnover of dietary lipids from PL as well as amino acid synthesis and as a result of impacts from these factors, a wide variability in its fatty acid composition is often observed (Frolov et al. 1991). There are limited studies that highlight the effect of lipid metabolism and population growth. However, a recent study by Lee et al. (2018) documented the phenomenon of aging extension and lipid metabolism modification in *B. koreanus*. Lee et al. (2018) highlighted that under reduced feeding conditions of *B. koreanus*, caloric restriction caused modulation of lipid metabolism, which led to a reduction of population growth and an extension of life span as a trade-off. In the present study, we highlight that DHA-enriched *C. vulgaris* and frozen *N. oculata* had the highest population growth and egg bearing individuals and both these treatments had high HUFA assimilation in the NL than in the PL. We can assume that the assimilation and possible *de novo* synthesis of fatty acids in the lipid regions is an adaptive mechanism of the L and SS morphotypes. This is possibly achieved by active reorganization of dietary lipids in accordance with its metabolic requirements for its proper function and where necessary population growth (Le Milinaire et al. 1983; Lubzens et al. 1985; Robin 1995; Lee et al. 2018). Our results highlight that not only do differences occur within the lipid regions of each species but also between rotifer morphotype and its diet which are a result of the peculiarities of metabolism and functional roles of these fractions in each morphotype.

In conclusion, this study highlights that according to enrichment media and rotifer morphotype, response to the enrichment in fatty acid content, population growth and soluble protein content varies. Using the batch culture system, DHA-enriched *Chlorella* and frozen *N. oculata* was found to have good population growth, fatty acid content and soluble protein. In contrast, using the same culture method and culture conditions for the salmon roe emulsion oil treatment, both morphotypes were affected negatively in population growth and low soluble protein content, despite having high HUFA composition in both the diet and in the rotifer. Natural synthesis of certain fatty acid species is possible via elongation and desaturation of precursor fatty acid species as was observed in this study for archidonic acid (ArA, 20:4 *n*-6) in normally ArA poor DHA-enriched *C. vulgaris*. Further studies are needed on culture methods and enrichment dosages and its impacts on different morphotypes due to the varying effects on fatty acid assimilation in the NL and PL and between rotifer morphotypes. The *Brachionus plicatilis* sp. complex, which includes a wide size range is an important first feed in the field of larviculture and therefore consideration of effects of enrichment on different morphotypes is also as important as different fish species have different qualitative and quantitative requirements for survival, growth and development.

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各種栄養強化剤を摂取した広塩性ツボワムシ *Brachionus plicatilis* 複合種2種の個体群増殖，脂肪酸組成およびタンパク質含量の比較

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Brachionus plicatilis 複合種 SS および L 型に及ぼす3種の栄養強化剤（DHA 強化クロレラ，冷凍ナンノクロロプシスおよび乳化筋子油）の餌料効果を比べた（対照区：濃縮淡水クロレラ）。両型ワムシの増殖速度と脂肪酸含量は試験区間で変化した。非極性脂質と極性脂質の含量はワムシ型間で変化した。可溶性タンパク質は両型で DHA 強化クロレラおよび生クロレラ給餌で多かった。冷凍ナンノクロロプシスを除いた全ての試験区で，SS 型の可溶性タンパク質含量は L 型よりも高かった。ワムシ型に応じて，培養方法，強化剤の投与量および脂肪酸要求量を考慮する必要がある。