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Cod Larviculture Using High-density Rotifer Production with Different Enrichments

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Abstract

High-density (HD) rotifer culture systems have been recently commercialized, but are not commonly used by the aquaculture industry. The aim of this study was to determine if HD systems could be used in cod hatcheries. An enrichment strategy using the commercial products, ArteMac and Protein Selco Plus (Com), was compared with the manufacturer's suggested enrichment, using Pavlova-DHA (Pav). The Pav enrichment increased the eicosapentaenoic acid (EPA) levels in rotifers, but reduced the docosahexaenoic acid (DHA) and 22:5n-6 (n-6DPA, docosapentaenoic acid) levels. Larvae EPA levels in both polar and neutral lipids were relatively stable in the larvae fed with Com-rotifers; while they were higher in early stages, they were progressively reduced through ontogeny in the Pav-rotifers fed-larvae. DHA levels in polar lipids decreased in larvae, particularly when fed with HD-Pav rotifers. In all larvae, arachidonic acid (ARA) levels increased in the polar and neutral lipids, regardless of treatments. In both lipid fractions, the levels of ARA were quite stable in time, but still higher in larvae fed with Com-rotifers. Bacterial load was lower in larvae fed with Pav-rotifers. Denaturing gradient gel electrophoresis (DGGE) bacterial profiles of larvae and rotifers were all similar. This study shows the potential of using HD systems to produce rotifers, but highlights the necessity of adjusting the nutritional composition of rotifers prior to being fed to larvae.

Rotifers, *Brachionus rotundiformis* and *Brachionus plicatilis*, are widely used as first feed in various aquacultured species and are often a limiting factor in the production of finfish or crustacean species (Bentley et al. 2008). Rotifers exhibit high population growth rates and wide tolerance to culture variations, and their energy contents and nutritional values can be controlled with commercial enrichment products (Conceicao et al. 2010).

Culture systems for producing rotifers are classified as batch, semi-continuous, and high-density (HD) cultures (Dhert et al. 2001). Batch cultivation, due to its simplicity, is probably the most common type of rotifer production in marine fish hatcheries. However, these cultures are subject to highly variable conditions that include variations among batches, unstable physico-chemical water parameters, and low efficiency in terms of labor and utilization of infrastructure (Dhert et al. 2001). These problems contribute to unstable and unpredictable culture conditions and relatively low production at high cost (Dhert et al. 2001). Use of HD culture enables higher production of rotifers compared with that of a batch culture system. However, the high food supply necessary to support HD cultures can cause accumulation of organic

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wastes in the culture water (Yoshimura et al. 1997). This problem can generally be resolved by using physical and biochemical filters that clean the water and limit the organic waste accumulation in the culture media, which in return allows for the upkeep of rotifers as dense as 5000 rotifers/mL (Yoshimura et al. 1997).

Rotifers are generally enriched with oils or commercial products to increase their nutritional value, but their quality is not optimized mainly due to their low hygienic condition (Dhert et al. 2001). Live feed are the main vectors causing bacterial contamination (Munro et al. 1993; Munro et al. 1994) and several studies have shown that microbiota associated with live feed play a major role in the instability and low viability of the live feed cultures (Hirayama 1987; Yu et al. 1989; Yu et al. 1990; Skjermo and Vadstein 1993; Harzevili et al. 1997).

In addition to microbiological factors, the success of the larval culture of marine fish also depends on the nutritional quality of the rotifers. This is more so, as the lipid composition of the feed is one of the most important aspects in fish nutrition (Sargent et al. 2002). Indeed, growth and survival of fish larvae are dependent upon the acquisition of essential fatty acids (EFAs) such as docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and arachidonic acid (ARA, 20:4n-6), for incorporation into membrane phospholipids and metabolism (Watanabe 1982; Watanabe et al. 1983; Mourente and Tocher 1993; Watanabe and Kiron 1994; Ghioni et al. 1999; Sargent et al. 1999a; Park et al. 2006; Garcia et al. 2008). Most marine fish species require preformed ARA, EPA, and DHA in their diet to sustain growth and increase their survival due to limitations in early life stages to elongate and/or desaturate 18-carbon polyunsaturated fatty acids (PUFAs). It was recently reported that the microbial environment and bacterial load could be responsible for modifications of fatty acid (FA) composition in larvae. These FA modifications may serve as a biochemical indicator of the biological condition (e.g., stress) of the fish larvae (Sargent et al. 2002; Plante et al. 2007).

The aim of this study was to determine the effect of a commercial HD rotifer production system using two different enrichments on the survival, growth, lipids ontogeny, and bacterial profiles of Atlantic cod, *Gadus morhua*, larvae.

Materials and Methods

Fish Holding

Atlantic cod broodstock were reared at the Coastal Zones Research Institute, located in Shippagan, NB, Canada. Three hundred twenty-one fishes with an average mass of 3.25 kg were kept in a recirculating 45,000-L tank with a constant supply of new filtered seawater at a rate of 15–30 L/min which represented a daily exchange of 48–96%. Water temperature ranged between 8 and 10 C and salinity varied between 27 and 30‰. Dissolved oxygen levels were maintained between 100 and 110% saturation by diffusing O_2 directly into the tank. Fish were fed three to five times per week with a ration of smelt, *Osmerus mordax*, and shrimp, *Pandalus borealis*, corresponding to 2.25% of their biomass. The salinity was gradually raised to 34‰ during the spawning season to increase egg floatability for their ease of collection on the water surface with an egg collector.

Eggs were incubated at a maximum density of five eggs per mL in 250-L tanks. The water temperature in the tanks was maintained at 8 C. The photoperiod was 18:6 of light : darkness with an intensity of 60 lx supplied by incandescent bulb for egg incubation. Hatching occurred after 12 d and continued over a period of 2 d. One day post hatch (1 dph) was assigned when approximately 50% of larvae were visible. The evaluations were performed by three samplings of at least 100 eggs per larvae to establish the percentage of hatch.

Rotifers

HD Production System. An ALD-STD50K HD rotifer production system (Aquatic Eco-systems, Apopka, FL, USA) was used following the recommendations specified by the manufacturer. This HD continuous culture system consisted of a 150-L culture tank, equipped with a 75-L biofilter and a protein fractionator. Rotifers were

continuously fed with 1 mL of algal paste *Nannochloropsis* Premium 3600 (Reed Mariculture Inc., Campbell, CA, USA) per million rotifers. The algae paste was first diluted in 20 L of seawater and then continuously pumped with a peristaltic pump into the rotifer culture. The rotifers were harvested every day and enriched for a 24-h period before being fed to cod larvae. Rotifer density in the system was readjusted every day at 3000 rotifers/mL, corresponding to a population of 0.45 billion rotifers. Once a week, the rotifers were removed from the system to allow for a cleanup of the tank holding the rotifers with soap and fresh water. The biofilter was not cleaned to maintain bacterial activity. The rotifer population growth rate (r) was calculated as $r = -\ln(1 - D)$ where D represent the dilution rate and was calculated as $D = \Delta V/V$, where V represents the total of rotifers and ΔV the variation in the number of rotifers from day^{-1} and Day 0 (Navarro and Yufera 1998).

Rotifer Enrichment. Each day, rotifers produced from one HD system were harvested and transferred to enrichment tanks as described in Haché and Plante (2011). Rotifers were enriched 24 h prior to being fed to cod larvae with either commercial enrichment products (Com-rotifers) or an algae paste (Pav-rotifers). The commercial enrichment treatment adapted in our hatchery consisted of a mix of 0.024 g/L of AlgaMac 3000 (Aquafauna Bio-Marine Inc., Hawthorne, CA, USA) and 0.028 g/L of Protein Selco Plus (INVE, Americas, Inc. Mountain Green, VT, USA), which was given at 0900 and 2100 h. The suggested enrichment treatment from the manufacturer of the HD system was the algal paste Pavlova-DHA (Reed Mariculture Inc.) given at a ratio of 1.5 mL/L once a day at 0900 h.

Experimental Design

Newly hatched cod larvae were transferred to six 1200-L rearing tanks (1.52 × 0.86 m, diameter; depth) at a density of 45 larvae/L. The initial temperature in the larvae tanks was 8 C and was gradually increased to 11 C over 8 d. Dissolved oxygen levels were maintained with the injection of pure oxygen with a ceramic diffuser, between 95 and 105% saturation, and the water was renewed every 12 h. On the first day, 16 L of algae T-ISO, *Isochrysis galbana*, was added to the rearing tanks at a concentration of 10,000 cells/mL; 8 L was replenished to the rearing tank from 2 to 10 dph, 6 L from 11 to 20 dph, and 4 L from 20 to 24 dph to maintain the initial microalgae density. Cod larvae were fed with rotifers enriched with the commercial enrichment (Com-rotifers) or with rotifers enriched with the algal paste Pavlova-DHA (Pav-rotifers). Each treatment was applied to triplicate tanks (Fig. 1).

For all larvae tanks, approximately 0.2 g of cod larvae (wet weight) were sampled at 1 (hatching), 7, 14, and 21 dph, which represented about 360, 200, 125, and 60 larvae, respectively, for dry mass and lipid analysis. Additionally, 20 larvae per time point were sampled for bacterial community profiling. Larvae were sampled before the morning feeding (0900 h) and while the rotifers were sampled after the 24-h enrichment period.

Biochemical Analysis

Moisture and ash content were determined using AOAC methods (AOAC 2002). Total nitrogen was measured using a macro analyzer (model vario MACRO N, Elementar, Hanau, Germany) and nitrogen value was converted to crude protein ($N \times 6.25$). Total lipid content was determined according to Folch et al. (1957).

Lipids were extracted following a modified Folch et al. (1957) procedure as described by Parrish (1999). Lipids were later fractionated into neutral lipids (including mainly phospholipids and minor amounts of glycolipids) using column chromatography on silica gel hydrated with 6% water (Pernet et al. 2006). The FAs were determined as described in Pernet et al. (2006) using a SRI 8610C gaschromatograph equipped with a DB-Wax fused-silica capillary column (Bellfonte, PA, USA). The chromatographs were analyzed with Peak Simple software (version 3.2, SRI Inc.).

Bacterial Analysis – Sample Collection and DNA Extraction

The bacterial load of the cod larvae was established as described in Haché and Plante

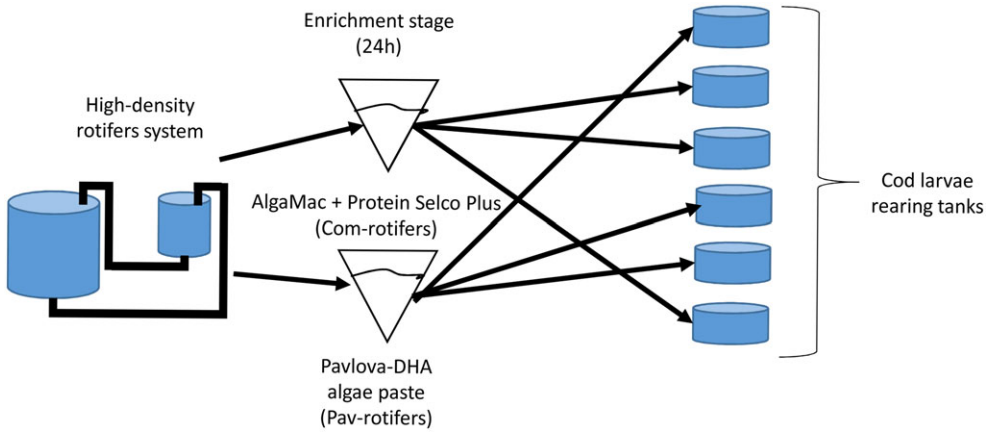

FIGURE 1. *Experimental scheme used in rotifers preparation and cod larvae,* Gadus morhua*, feeding.*

(2011). Larvae and rotifer samples were collected directly into RNALater (Ambion, Foster City, CA, USA) and stored frozen (−80 C), then shipped, on dry ice, to the Research and Productivity Council laboratory located in Fredericton NB, Canada. The denaturing gradient gel electrophoresis (DGGE) and the polymerase chain reaction (PCR) amplification of the 16S rDNA partial sequences were performed as described in Plante et al. (2007).

Sequence identification of the excised bands was performed using the Sequence Match search algorithm of the Ribosomal Database Project II (http://rdp.cme.msu.edu/), release 10.22 (Cole et al. 2007, 2009).

Statistical Analysis

Cod larvae survival was analyzed using a *t* test. Multivariate analyses of variance (MANOVA) were used to compare the proximate analysis of rotifers and the FA profiles of rotifers. MANOVAs were also performed on the polar and neutral FAs while a two-factor ANOVA was performed on the bacterial counts. Growth (mg) curves of cod larvae were compared among treatments using an analysis of covariance (ANCOVA). When significant differences occurred ($\alpha < 0.05$), Tukey-Kramer tests were used for pairwise comparisons (Day and Quinn 1989). Normal distribution of the error terms and homoskedasticity were checked with the Shapiro–Wilk test and Levene's test, respectively (Sokal and Rohlf 2011). FA profiles were analyzed with the arcsine transformation as the percentages of original data did not fall between 30 and 70% (Sokal and Rohlf 2011). Analyses were carried out using SPSS 17.0 (IBM SPSS, Armonk, NY, USA).

Results

Rotifers

Growth rate of rotifers in the HD systems, prior to the enrichment step, was 0.5 ± 0.1. Total lipids were higher in the Com-rotifers compared with Pav-rotifers (3.5 vs. 2.4%). No statistical difference was observed in moisture levels, crude protein, and ash between both treatments (Table 1). The FA composition of rotifers varied as a function of the enrichment used (Table 1). The Com-rotifers showed lower levels of monosaturated fatty acids (MUFAs, 16.4 vs. 22.4%) and higher levels of PUFA (64.5 vs. 56.3%). It is noteworthy that the levels of the EFA ARA (2.7 vs. 1.8%) and DHA (30.0 vs. 24.7%) were higher in the Com-rotifers while the level of EPA was two times lower (7.2 vs. 14.2%) (Table 1). The sum of the omega-6 in the Com-rotifers (21.9%) was much higher than in the Pav-rotifers (8.0%).

The bacterial microfloras of rotifers as revealed by DGGE analyses were similar, irrespective of rearing conditions and time (Fig. 2). Only minor variations were observed in band intensity. Species or genera from most major bands are presented in Table 2. The PCR

TABLE 1. *Proximate analysis (%) and fatty acid (mass % of total fatty acid) composition of rotifers enriched with AlgaMac 3000 and Protein Selco Plus (Com-rotifers) or with Pavlova-DHA (Pav-rotifers).*[1,2,3]

	Com-rotifers	Pav-rotifers
Proximate analysis		
Crude proteins	8.9 ± 0.6	8.7 ± 0.6
Total lipids	3.5 ± 0.3[a]	2.4 ± 0.4[b]
Moisture	84.6 ± 0.9	86.2 ± 2.1
Ash	2.0 ± 0.1	2.1 ± 0.1
Fatty acids		
14:0	3.8 ± 0.2[b]	5.9 ± 0.2[a]
16:0	12.8 ± 0.7	12.5 ± 0.7
18:0	1.7 ± 0.1[b]	1.9 ± 0.1[a]
ΣSFA (saturated fatty acids)	19.1 ± 0.8	21.3 ± 1.0
16:1n-7	4.7 ± 0.5[b]	8.2 ± 0.7[a]
16:1n-5	0.1 ± 0.1[b]	1.0 ± 0.1[a]
18:1n-9	5.9 ± 0.2	5.7 ± 0.3
18:1n-7	1.9 ± 0.1[b]	2.8 ± 0.1[a]
20:1n-9	1.2 ± 0.1[b]	1.5 ± 0.1[a]
ΣMUFA	16.4 ± 0.7[b]	22.4 ± 0.6[a]
18:2n-6	7.5 ± 0.6[a]	3.5 ± 0.4[b]
18:3n-3	1.5 ± 0.2[a]	0.8 ± 0.2[b]
18:4n-3	1.0 ± 0.1[b]	2.9 ± 0.5[a]
20:4n-3	1.4 ± 0.1	1.5 ± 0.2
20:4n-6 (ARA)	2.7 ± 0.1[a]	1.8 ± 0.1[b]
20:5n-3 (EPA)	7.2 ± 0.3[a]	14.2 ± 1.0[b]
22:5n-6 (n-6DPA)	9.8 ± 0.9[a]	1.4 ± 0.2[b]
22:5n-3	1.0 ± 1.7[b]	3.0 ± 2.4[a]
22:6n-3 (DHA)	30.0 ± 1.8[b]	24.7 ± 1.6[a]
ΣPUFA	64.5 ± 1.4[a]	56.3 ± 0.8[b]
Σn-3	42.5 ± 1.4[b]	47.5 ± 0.7[a]
Σn-6	21.9 ± 0.8[a]	8.0 ± 0.7[b]
n-3/n-6	2.0 ± 0.1[b]	5.9 ± 0.5[a]
DHA/EPA	4.2 ± 0.4[a]	1.8 ± 0.2[b]
EPA/ARA	2.6 ± 0.2[b]	7.8 ± 0.3[a]

ARA = arachidonic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; MUFA = monosaturated fatty acid; DPA = docosapentaenoic acid.

[1]Only fatty acids contributing ≥1.0% in at least one treatment are reported.

[2]All values represent the mean ± SD ($n = 3$).

[3]Different letters indicate significant differences ($P < 0.05$).

amplification and DNA sequencing of some darker bands was not successful (e.g., R6–R8).

Cod Larvae

Survival rate of cod larvae fed Com-rotifers (9.6 ± 0.5%) was statistically different ($P = 0.0253$) when compared with larvae fed with Pav-rotifers (5.7 ± 1.4%). Growth rate was, however, not significantly influenced by the type of rotifers fed to the larvae ($P = 0.194$; Fig. 3).

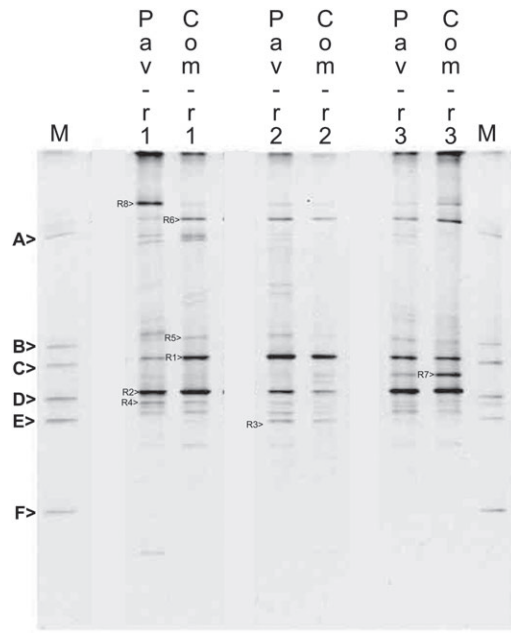

FIGURE 2. *Denaturing gradient gel electrophoresis profiles of the two types of rotifers used to feed cod larvae,* Gadus morhua*. The two types of rotifers were as follows: (1) Enriched with AlgaMac 3000 + Protein Selco Plus (Com-rotifers) and (2) enriched with Pavlova-DHA (Pav-rotifers). Samples from three separate batches of rotifers sampled on October 10 (Sample 12–13), October 16 (Sample 24–25), and October 21 (Sample 27–28) were examined. M = marker controls; A =* Pseudomonas *sp.; B =* Marinomonas *sp.; C =* Sulfitobacter *sp.; D =* Listonella anguillarum*; E =* Vibrio logei*; F =* Microbacterium *sp. Bands R1 to R8 were excised for DNA sequence.*

FA compositions of polar and neutral lipids of the cod larvae fed with the different rotifers are presented in Tables 3 and 4, respectively.

The age of the larvae (dph) and the type of rotifers had a significant effect on the level of all three major EFAs (ARA, DHA, and EPA) on both neutral (Table 3) and polar lipids (Table 4). Overall, proportions of PUFA were higher in polar lipids than in neutral lipids during the entire larval ontogeny. The FAs ARA, EPA, and DHA collectively contributed 58 and 75% of the total PUFA in neutral and polar lipids, respectively. The proportions of these EFAs in the cod larvae neutral lipids (Table 3) reflected

TABLE 2. *Identity of bands from denaturing gradient gel electrophoresis gels of rotifers and cod larvae.*

Band	Identity
Controls	
A	*Pseudomonas* sp.
B	*Marinomonas* sp.
C	*Sulfitobacter* sp.
D	*Listonella anguillarum*
E	*Vibrio logei*
F	*Microbacterium* sp.
Rotifer samples	
R1	*Pseudomonas* sp.
R2	*Pseudoaltermonas* sp.
R3	*Vibrio* sp.
R4	*Methylophaga* sp.
R5	*Marinomonas* sp.
R6	Unknown
R7	Unknown
R8	Unknown
Larval samples	
L1	*Acinetobacter* sp.
L2	*Pseudomonas* sp.
L3	*Oceanospirillum* sp.
L4	*Pseudoalteromonas* sp.
L5	*Comamonas* sp.

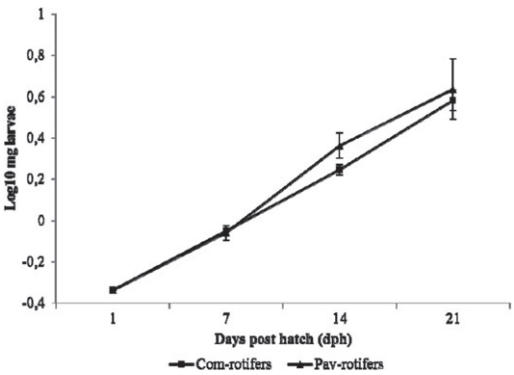

FIGURE 3. *Dry mass trajectories of cod larvae,* Gadus morhua*, fed with either of these methods: rotifers enriched with AlgaMac 3000 + Protein Selco Plus (Com-rotifers) or with Pavlova-DHA (Pav-rotifers) as a function of development time (vertical bar = SD,* n = *3).*

those found in the rotifers used to feed these larvae. Indeed, cod larvae fed Com-rotifers showed a higher level of PUFA and lower level of MUFA compared with those fed Pav-rotifers. The proportion of EFAs in cod larvae polar lipids showed far less variation due to rotifer type than was observed in neutral lipids (Table 4).

The ARA levels in cod larvae were similar in the neutral and polar lipids with higher levels found in the larvae feed with Com-rotifers. The levels of ARA reached 4.9% in cod larvae fed Com-rotifers and 4.0% in larvae fed Pav-rotifers. The levels of ARA observed in cod larvae were higher than those found in the rotifers (2.7 and 1.8%, Com-rotifers and Pav-rotifers, respectively).

At 21 dph, EPA levels of cod larvae neutral lipids became similar irrespective of the type of rotifers (about 7.5% of total FA; Table 3). In the polar lipids, EPA levels decreased markedly during larval ontogeny from 13.3% to 5.7 and 7.6% in cod fed Com-rotifers and Pav-rotifers, respectively (Table 4).

From 1 to 21 dph, the levels of DHA in cod neutral lipids decreased from an original level of 22.2% to 11.2 in cod larvae fed with Pav-rotifers and 15.6% in larvae fed with Com-rotifers (Table 3). In the polar lipids, DHA levels at 21 dph were much higher (25.1 and 29.2%; Table 4) than those observed in the neutral lipids (11.2 and 15.6%). At 21 dph, DHA proportions in cod larvae polar lipids decreased slightly, particularly in those fed with Pav-rotifers where they attained the level of 25.1% (Table 4).

In both treatments, levels of n-6DPA (docosapentaenoic acid) in cod neutral lipids increased markedly from 0.0% at hatching to 4.8% in cod larvae fed with Pav-rotifers and 7.1% in larvae fed with Com-rotifers at 14 dph (Table 3). In cod larvae fed with Pav-rotifers, polar lipids n-6DPA increased constantly from 0.3% at hatching to 3.3% at 21 dph, while the level of n-6DPA reached 5.9% at 14 dph and remained the same at 21 dph in cod larvae fed with Com-rotifers (Table 4).

Cod larvae bacterial loads were similar during the first 7 d of rearing. Thereafter, bacterial loads of fish larvae fed Pav-rotifers were much lower (Fig. 4). Indeed, bacterial loads of fish fed Com-rotifers increased markedly until the end of the study period. Therefore, it seemed that the enrichment mix of AlgaMac 3000 and Protein Selco Plus had a major effect on the bacterial load of cod larvae. Also, a significant interaction (treatment × dph, $P = 0.001$) was observed between the time and the type of

TABLE 3. *Fatty acid composition of neutral lipids for cod,* Gadus morhua*, larvae fed with rotifer produced in a high-density system then enriched with AlgaMac 3000 and Protein Selco Plus (Com-rotifers) or with Pavlova-DHA (Pav-rotifers) (mass % of total fatty acids).*[1,2,3]

		Larvae 7 d		Larvae 14 d		Larvae 21 d	
	Larvae 1 d	Com-rotifers	Pav-rotifers	Com-rotifers	Pav-rotifers	Com-rotifers	Pav-rotifers
14:0	1.8 ± 0.1^{d}	2.9 ± 0.2^{ab}	3.7 ± 0.3^{a}	2.8 ± 0.5^{bc}	3.7 ± 0.4^{a}	2.1 ± 0.4^{cd}	2.4 ± 0.1^{bcd}
16:0	13.0 ± 0.6	13.9 ± 0.7	15.0 ± 0.6	13.2 ± 2.2	11.9 ± 0.6	11.7 ± 1.7	12.8 ± 0.8
18:0	5.2 ± 0.2^{ab}	5.1 ± 0.3^{b}	6.3 ± 0.2^{ab}	4.7 ± 1.0^{b}	5.3 ± 0.3^{ab}	6.8 ± 2.0^{ab}	7.3 ± 0.5^{a}
ΣSFA	21.0 ± 1.2	23.5 ± 1.6	25.9 ± 0.7	22.1 ± 2.7	23.4 ± 1.0	21.7 ± 3.4	24.1 ± 1.3
16:1n-7	3.6 ± 0.1	3.9 ± 0.2	3.5 ± 2.4	3.7 ± 0.8	5.2 ± 0.6	2.1 ± 0.7	2.9 ± 0.3
18:1n-9	21.6 ± 0.9^{a}	8.2 ± 0.6^{d}	10.4 ± 2.4^{cd}	8.0 ± 0.3^{d}	9.1 ± 0.9^{d}	14.2 ± 2.5^{bc}	16.7 ± 1.3^{b}
18:1n-7	4.2 ± 0.3^{ab}	2.6 ± 0.1^{c}	3.4 ± 0.2^{bc}	2.5 ± 0.2^{c}	2.9 ± 0.2^{c}	4.5 ± 1.3^{ab}	5.3 ± 0.4^{a}
20:1n-11	0.6 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.6 ± 0.7	0.4 ± 0.7
20:1n-9	2.6 ± 0.0^{a}	1.5 ± 0.1^{ab}	1.9 ± 0.1^{ab}	1.3 ± 0.2^{ab}	1.6 ± 0.2^{ab}	0.6 ± 0.8^{b}	0.9 ± 0.8^{ab}
24:1n-7	0.8 ± 0.0	0.9 ± 0.1	1.2 ± 0.4	1.2 ± 0.3	1.0 ± 0.5	0.7 ± 0.4	0.3 ± 0.5
ΣMUFA	36.7 ± 1.2^{a}	20.5 ± 0.2^{cd}	24.8 ± 3.0^{bc}	19.0 ± 0.4^{d}	22.9 ± 0.9^{cd}	23.8 ± 3.6^{bc}	27.8 ± 1.2^{b}
18:2n-6	4.1 ± 0.4^{b}	6.1 ± 0.3^{a}	4.1 ± 0.9^{a}	6.1 ± 1.0^{a}	4.3 ± 0.2^{ab}	5.6 ± 1.3^{ab}	4.7 ± 0.3^{ab}
18:3n-3	0.8 ± 0.1^{b}	1.3 ± 0.0^{b}	1.3 ± 0.2^{b}	1.7 ± 0.3^{b}	1.9 ± 0.4^{b}	8.1 ± 3.0^{a}	9.1 ± 1.2^{a}
18:4n-3	3.7 ± 0.9^{a}	1.0 ± 0.1^{c}	2.9 ± 1.7^{ab}	1.2 ± 0.4^{bc}	2.2 ± 0.4^{abc}	2.2 ± 0.5^{abc}	2.6 ± 0.1^{abc}
20:4n-6 (ARA)	1.7 ± 0.1^{c}	4.0 ± 0.1^{a}	3.0 ± 0.3^{b}	4.6 ± 0.0^{a}	2.7 ± 0.4^{b}	4.6 ± 0.2^{a}	3.2 ± 0.1^{b}
20:4n-3	1.0 ± 0.0^{ab}	1.1 ± 0.1^{ab}	1.3 ± 0.2^{a}	1.1 ± 0.2^{ab}	1.3 ± 0.2^{a}	0.9 ± 0.2^{b}	0.9 ± 0.1^{ab}
20:5n-3 (EPA)	6.9 ± 0.3^{c}	7.9 ± 0.1^{c}	11.4 ± 1.5^{ab}	8.6 ± 1.3^{bc}	12.0 ± 1.7^{a}	7.5 ± 0.8^{c}	8.1 ± 1.1^{c}
22:5n-6 (n-6DPA)	0.0 ± 0.0^{d}	6.3 ± 0.2^{a}	0.8 ± 0.1^{c}	7.1 ± 0.8^{a}	4.8 ± 2.3^{ab}	5.9 ± 1.5^{a}	2.8 ± 0.4^{bc}
22:5n-3	1.2 ± 0.0^{ab}	2.8 ± 0.2^{ab}	3.4 ± 0.7^{a}	3.0 ± 0.2^{a}	3.6 ± 0.5^{a}	1.1 ± 1.6^{b}	1.7 ± 0.5^{ab}
22:6n-3 (DHA)	22.2 ± 1.4^{ab}	22.9 ± 1.2^{a}	18.7 ± 2.5^{abc}	22.9 ± 2.0^{a}	16.0 ± 2.3^{bcd}	15.6 ± 4.8^{cd}	11.2 ± 1.1^{d}
ΣPUFA	42.4 ± 1.3^{d}	56.0 ± 1.5^{ab}	49.3 ± 2.5^{bcd}	58.9 ± 2.6^{a}	53.7 ± 0.8^{abc}	54.5 ± 6.9^{abc}	48.0 ± 2.1^{cd}
Σn-3	34.9 ± 1.0	37.0 ± 1.0	39.2 ± 3.2	38.6 ± 0.4	37.0 ± 5.3	35.8 ± 3.7	34.3 ± 1.6
Σn-6	5.8 ± 0.8^{c}	18.0 ± 0.7^{a}	8.9 ± 0.3^{bc}	19.3 ± 1.8^{a}	15.4 ± 6.0^{ab}	18.1 ± 2.8^{a}	12.6 ± 0.7^{ab}
n-3/n-6	6.0 ± 0.4^{a}	2.1 ± 0.1^{c}	4.4 ± 0.5^{ab}	2.0 ± 0.3c	2.8 ± 1.4^{bc}	2.0 ± 0.1^{c}	2.7 ± 0.0^{bc}
DHA/EPA	3.2 ± 0.2^{a}	2.9 ± 0.2^{ab}	1.6 ± 0.0^{c}	2.7 ± 0.7^{ab}	1.3 ± 0.1^{c}	2.1 ± 0.4^{bc}	1.4 ± 0.1^{c}
EPA/ARA	4.0 ± 0.1^{a}	2.0 ± 0.0^{bc}	3.8 ± 0.2^{a}	1.9 ± 0.3^{c}	4.5 ± 0.3^{a}	1.6 ± 0.1^{c}	2.5 ± 0.3^{b}

ARA = arachidonic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; MUFA = monosaturated fatty acid; PUFA = polyunsaturated fatty acid.

[1] Only fatty acids contributing ≥1% in at least one treatment are reported.

[2] All values, except for larvae 1 d, represent the mean ± SD of three experimental tanks.

[3] Different letters indicate significant differences ($P < 0.05$).

rotifers concerning the cod larvae bacterial load (Fig. 4). The total bacterial load in cod larvae had a clear tendency to increase with the age of the larvae. After reaching a summit at 7 dph, the bacterial load decreased with the age of the larvae fed with Pav-rotifers.

Even if the bacterial load of cod larvae varied with rotifer type, the bacterial microfloras as revealed by DGGE analyses were similar (Fig. 5).

The bacterial microfloras of cod larvae collected at 1, 14, and 21 dph were remarkably similar (Fig. 5). Only cod larvae fed Com-rotifers collected at 14 dph (Sample 17) showed unusual bands, not present or present at very low levels in the other samples (Fig. 5 in the vicinity of Control A and B and L3).

Discussion

Effect of the HD Culture System

Plante et al. (2007) described another type of continuous rotifer culture that did not include a biofilter, was not fed continuously, and where the rotifer density was maintained around 200–250 rotifers/mL. In this rotifer culture, 20% of the water culture was replaced daily by fresh algae (T-iso) and rotifers were fed with yeast three times a day. When comparing Plante et al. (2007) techniques to the present HD system, the time

TABLE 4. *Fatty acid composition of polar lipids for cod,* Gadus morhua*, larvae fed with rotifers produced in a high-density system then enriched with AlgaMac 3000 and Protein Selco Plus (Com-rotifers) or with Pavlova-DHA (Pav-rotifers) (mass % of total fatty acids).*[1,2,3]

		Larvae 7 d		Larvae 14 d		Larvae 21 d	
	Larvae 1 d	Com-rotifers	Pav-rotifers	Com-rotifers	Pav-rotifers	Com-rotifers	Pav-rotifers
14:0	1.1 ± 0.1^{bc}	1.1 ± 0.1^{bc}	1.3 ± 0.0^{b}	1.3 ± 0.1^{bc}	1.9 ± 0.1^{a}	1.0 ± 0.2^{c}	1.3 ± 0.1^{bc}
16:0	18.6 ± 0.2^{a}	16.8 ± 0.3^{bc}	16.5 ± 0.1^{bc}	16.3 ± 0.3^{c}	15.7 ± 0.3^{d}	17.0 ± 0.1^{b}	16.4 ± 0.1^{c}
18:0	4.7 ± 0.3^{c}	6.4 ± 0.3^{b}	6.8 ± 0.1^{ab}	6.4 ± 0.4^{b}	6.5 ± 0.0^{b}	7.1 ± 0.8^{ab}	7.4 ± 0.3^{a}
ΣSFA	25.1 ± 0.8	24.9 ± 0.7	25.4 ± 0.5	24.8 ± 0.4	25.4 ± 0.4	25.9 ± 1.2	25.6 ± 0.4
16:1n-7	2.3 ± 0.1^{a}	1.8 ± 0.1^{b}	2.3 ± 0.1^{a}	1.7 ± 0.2^{b}	2.6 ± 0.1^{a}	1.0 ± 0.3^{bc}	1.4 ± 0.2^{c}
18:1n-9	10.9 ± 0.6^{a}	6.6 ± 0.1^{cd}	7.2 ± 0.5^{cd}	6.7 ± 1.5^{d}	6.4 ± 0.4^{d}	8.6 ± 0.5^{bc}	10.4 ± 1.3^{ab}
18:1n-7	3.4 ± 0.2^{c}	3.0 ± 0.1^{d}	3.3 ± 0.0^{c}	2.9 ± 0.0^{d}	3.4 ± 0.0^{c}	3.8 ± 0.4^{b}	4.7 ± 0.2^{a}
20:1n-9	1.9 ± 0.1	1.1 ± 0.0	0.9 ± 0.7	0.9 ± 0.1	1.1 ± 0.1	0.6 ± 0.0	0.8 ± 0.0
ΣMUFA	22.3 ± 0.5^{a}	14.9 ± 0.3^{d}	17.2 ± 0.5^{bc}	14.5 ± 1.8^{d}	16.6 ± 0.8^{cd}	15.5 ± 0.7^{cd}	19.5 ± 1.1^{b}
18:2n-6	1.6 ± 0.1^{c}	2.9 ± 0.2^{ab}	1.8 ± 0.1^{c}	3.6 ± 0.8^{a}	2.4 ± 0.1^{bc}	2.9 ± 0.7^{ab}	2.6 ± 0.3^{ab}
18:3n-3	0.2 ± 0.0^{d}	0.6 ± 0.1^{cd}	0.5 ± 0.0^{cd}	0.7 ± 0.1^{c}	0.8 ± 0.0^{c}	3.8 ± 1.3^{b}	5.1 ± 0.6^{a}
20:4n-6 (ARA)	2.7 ± 0.0^{e}	3.9 ± 0.1^{c}	3.0 ± 0.0^{d}	4.5 ± 0.1^{b}	2.8 ± 0.1^{de}	4.9 ± 0.3^{a}	4.0 ± 0.2^{c}
20:4n-3	0.2 ± 0.0^{c}	0.8 ± 0.1^{b}	0.9 ± 0.0^{b}	0.8 ± 0.1^{b}	1.2 ± 0.0^{c}	0.7 ± 0.2^{b}	0.9 ± 0.1^{b}
20:5n-3 (EPA)	13.3 ± 0.8^{a}	7.5 ± 0.2^{d}	9.8 ± 0.3^{b}	5.4 ± 0.3^{e}	8.6 ± 0.2^{c}	5.7 ± 0.8^{e}	7.6 ± 0.1^{d}
22:5n-6 (n-6DPA)	0.3 ± 0.1^{c}	2.8 ± 2.3^{bc}	0.9 ± 0.0^{bc}	5.9 ± 0.4^{a}	1.7 ± 0.1^{bc}	5.9 ± 0.4^{a}	3.3 ± 0.1^{ab}
22:5n-3	1.3 ± 0.1^{d}	2.9 ± 0.3^{b}	3.6 ± 0.2^{a}	2.8 ± 0.2^{b}	4.3 ± 0.1^{a}	1.8 ± 0.5^{c}	2.4 ± 0.3^{b}
22:6n-3 (DHA)	29.9 ± 1.1^{b}	36.4 ± 0.8^{a}	34.7 ± 0.7^{a}	34.2 ± 2.3^{a}	32.8 ± 0.8^{ab}	29.2 ± 3.2^{b}	25.1 ± 1.8^{c}
ΣPUFA	52.6 ± 1.3^{d}	60.1 ± 1.0^{ab}	57.4 ± 0.8^{bc}	60.7 ± 1.8^{a}	58.0 ± 1.1^{ab}	58.6 ± 1.9^{ab}	54.9 ± 1.3^{cd}
Σn-3	46.6 ± 1.2^{bc}	48.7 ± 1.3^{ab}	50.2 ± 1.3^{a}	44.6 ± 2.2^{cd}	48.9 ± 0.8^{ab}	42.3 ± 1.6^{d}	42.9 ± 1.7^{d}
Σn-6	5.3 ± 0.4^{d}	11.0 ± 1.9^{b}	6.6 ± 0.1^{cd}	15.6 ± 0.7^{a}	8.2 ± 0.2^{c}	15.8 ± 0.0^{a}	11.8 ± 0.2^{b}
n-3/n-6	8.8 ± 0.8^{a}	4.5 ± 1.0^{c}	7.6 ± 0.3^{a}	2.9 ± 0.2^{de}	6.0 ± 0.1^{b}	2.7 ± 0.1^{e}	3.6 ± 0.2^{cd}
DHA/EPA	2.3 ± 0.2^{d}	4.9 ± 0.0^{b}	3.5 ± 0.1^{c}	6.3 ± 0.5^{a}	3.8 ± 0.1^{c}	5.2 ± 1.3^{b}	3.3 ± 0.2^{b}
EPA/ARA	5.0 ± 0.0^{a}	1.9 ± 0.0^{d}	3.3 ± 0.1^{b}	1.2 ± 0.1^{e}	3.0 ± 0.1^{e}	1.2 ± 0.1^{e}	1.9 ± 0.1^{d}

ARA = arachidonic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; MUFA = monosaturated fatty acid; PUFA = polyunsaturated fatty acid.

[1]Only fatty acids contributing ≥1% in at least one treatment are reported.

[2]All values represent the mean ± SD of three experimental tanks.

[3]Different letters indicate significant differences ($P < 0.05$).

and space requirements for cultivating rotifers in the HD system were reduced by 60 and 50%, respectively, as reported in other studies (Suantika et al. 2003; Yoshimura et al. 2003; Bentley et al. 2008). In addition, the growth rate was much higher and more stable when using a HD system. However, a slight decrease in growth rate was observed during tank cleaning, but this was also reported by Suantika et al. (2000). Growth rate in this study was 0.5 ± 0.1, which was more than six times higher (0.08 ± 0.11) when compared with a continuous culture system previously used in our laboratory (Plante et al. 2007).

Both types of rotifers produced in this experiment showed a high level of EPA in their tissues (Table 1) when compared with previous studies (Haché and Plante 2011; Plante et al. 2007; Park et al. 2006; Garcia et al. 2008). The high level of EPA could be attributed to the algae paste consisting of *Nannochloropsis* sp., an algae rich in EPA and fairly poor in DHA (Roncarati et al. 2004). The sum of the omega-6 FAs was higher in Com-rotifers and was attributed in part to the FA 22:5n-6 (DPA) which was close to nine times higher than seen in the Pav-rotifers (Table 1). Despite the difference in the higher level of EPA and lower level of DHA, the overall FA profiles of rotifers, from both Com and Pav treatments, were similar to those reported in other previously mentioned studies.

Surprisingly, bacterial profiles of rotifers were remarkably similar regardless of the enrichment process (Fig. 2). However, it is noteworthy that bacterial species present at low levels may not have been detected by PCR-DGGE, and therefore we cannot discount the possibility

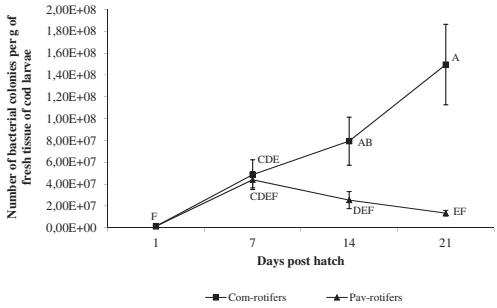

FIGURE 4. *Bacterial colony count trajectories per gram of fresh tissues of cod larvae,* Gadus morhua*, fed with either of the following: rotifers enriched with Alga-Mac 3000 + Protein Selco Plus (Com-rotifers) or rotifers enriched with Pavlova-DHA (Pav-rotifers) after 7 d of incubation at 20 C on marine agar in function of the development time. Different letters indicate significant differences (A > B > C > D > E > F, P < 0.05) (vertical bar = SD,* n = *3).*

that rearing conditions had induced subtle differences in the microflora composition of rotifers.

Effect of Rotifers on Cod Larvae

Cod larvae fed Com-rotifers had a higher larvae survival than the Pav-rotifers while the growth of cod larvae was similar, regardless of the type of rotifers (Fig. 2).

Our study showed that the FA composition of cod larvae (Tables 3 and 4) reflected that of rotifers (Table 1), which, in turn, varied as a function of the rotifer enrichment. Lower levels of PUFA found in cod larvae fed with Pav-rotifers were mainly due to the lower values of 18:2n-6, ARA, n-6DPA, and DHA compared with the Com-rotifers (Table 3). The higher level of MUFA observed in the larvae fed with Pav-rotifers was mostly attributed to the 18:1n-9. Fewer variations were observed in polar lipids (Table 4) than in neutral lipids (Table 3). The neutral lipids represent the organism's energy reserves while the polar lipids constitute the principal structural component of cellular membranes (Sargent et al. 2002). Therefore, polar lipids tend to be less variable than the neutral lipids.

The marked diminution of the DHA levels in the neutral lipids in both groups of cod larvae raises the possibility that DHA was selectively incorporated into membrane phospholipids at the expense of reserve lipids, most likely in response to the low dietary proportions. This hypothesis is supported by the fact that phospholipids in fish are generally higher in PUFA and more resistant to dietary changes than neutral lipids (Sargent et al. 2002). These results suggest that the dietary proportions of DHA (24.7 and 30.0% of total fatty acids) reported in this study were insufficient to sustain cod larval growth without utilizing the DHA located into the neutral lipid fraction.

The levels of EPA in neutral (Table 3) and polar lipids (Table 4) of cod larvae reflected dietary values and, therefore, varied markedly as a function of the type of rotifers fed to larvae. Levels of EPA in polar lipids of cod decreased markedly during larval ontogeny until reaching values similar or lower than those found in neutral lipids. Marked utilization or elimination of EPA during larval ontogeny of cod was also reported by Park et al. (2006) and may reflect an energetic role of this FA. It is also possible that the EPA could be elongated and desaturated as reported in previous studies (Mourente et al. 1993; Morais et al. 2012). This pathway could have been activated to compensate for the apparent lack of DHA suffered by the cod larvae.

Maintenance of high levels of DHA and reduction of EPA in the polar lipids during the larval ontogeny of cod larvae led to a marked increase in DHA/EPA ratio from 2.3 to values ranging from 3.3 in cod larvae fed with Pav-rotifers to 8.3 in cod larvae fed with Com-rotifers, depending on the type of rotifer. Although the requirement of marine fish larvae for DHA is species and stage specific (Sargent et al. 1999a), survival of cod larvae is generally improved in fish fed rotifers with DHA/EPA ratios higher than 2 (O'Brien-MacDonald et al. 2006a, 2006b; Park et al. 2006; Garcia et al. 2008). Our results support the hypothesis that higher DHA/EPA ratio lead to an increase in the survival of cod larvae.

The proportion of ARA (Tables 3 and 4) observed in cod larvae regardless of the types of rotifers used was higher than those found in rotifers for both polar and neutral lipids. This suggests a selective incorporation of this FA in both membrane and reserve lipids. Similar

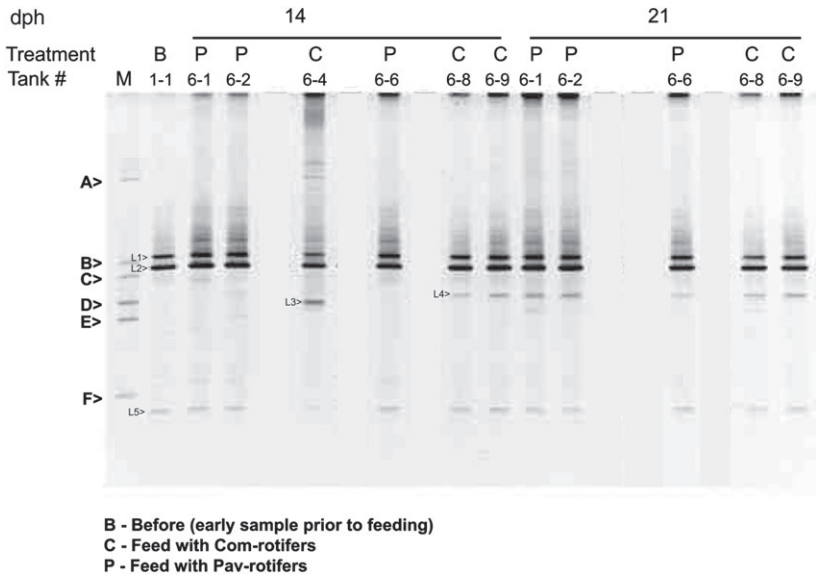

FIGURE 5. *Denaturing gradient gel electrophoresis profiling of cod larvae fed two types of rotifers. dph refers to the number of days post hatch at the time when the larvae sample was taken. The tank numbers and sample numbers are shown. Treatments indicated include B: prior to feeding; C: larvae fed with rotifers enriched with AlgaMac 3000 + Protein Selco Plus (Com-rotifers) and P: larvae fed with rotifers enriched with Pavlova-DHA (Pav-rotifers). M = marker controls; A =* Pseudomonas *sp.; B =* Marinomonas *sp; C =* Sulfitobacter *sp.; D =* Listonella anguillarum*; E =* Vibrio logei*; F =* Microbacterium *sp. Bands L1 to L5 were excised for DNA sequence.*

results were obtained by Park et al. (2006) and Garcia et al. (2008) in cod larvae and by Plante et al. (2007) in haddock larvae. Interestingly, EPA/ARA ratio in diets (Table 1) and in the neutral and polar lipids of cod larvae (Tables 3 and 4) varied inversely with survival. Both EPA and ARA represent precursors of eicosanoids, a group of highly biologically active hormones (Smith and Murphy 2003) associated with stressful situations or pathological conditions (Bell and Sargent 2003). However, eicosanoids produced from EPA are biologically less active than those produced from ARA (Sargent et al. 1999b). Thus, a comprehensive evaluation of a given diet for marine fish should take into account the DHA/EPA/ARA ratio, rather than only the DHA/EPA ratio or the concentrations of each EFA separately. Garcia et al. (2008) suggest that the optimal DHA/EPA/ARA ratio is closer to 11:1.5:1 for cod larvae. In this study, a better survival was associated with a ratio of 11.1:2.7:1 in cod larvae fed the Com-rotifers.

Marked accumulation of n-6DPA was observed during the larval ontogeny of cod larvae fed both types of rotifers (Tables 3 and 4). Likewise, accumulation of DPA was reported in the lipids of several bivalve species (Delaunay et al. 1993; Milke et al. 2004; Pernet et al. 2005). Moriguchi et al. (2000) showed that DPA could act as a substitute for DHA in the developing brain of mammals under DHA-deficient conditions. Replacement of DHA by DPA alters the properties of neural membranes that in turn alter the function of integral receptor proteins (Eldho et al. 2003). As membrane function and metabolic pathways in lower and higher animals are similar, DPA may serve as a homologous functional role to DHA in phospholipids of cod larvae with possible effects on transmembrane protein functions. In line with our conclusions, Garcia et al. (2008) showed that n-6DPA could play a crucial role in cod larvae development at an early stage.

The bacterial load of cod larvae fed Pav-rotifers was lower than cod larvae fed Com-rotifers (Fig. 4). Therefore, it seems that the bacterial load in cod larvae varied as a function of the rotifer enrichment product. Haché

and Plante (2011) showed that the enrichment affected the bacterial load in rotifers, which in return could explain the result obtained on cod larvae. The DGGE bacterial profiling of cod larvae (Fig. 5) was similar regardless of the type of rotifers, which was predictable, as the bacterial profiles of the rotifers were similar. As previously reported in rotifers, there was little variation in bacterial profiling of cod larvae among replicate tanks and development stages, likely reflecting microbiological similarity of the different type of rotifers and rotifer batches (Figs. 2 and 5). The number of bacterial species in rotifers was higher than that recorded in cod larvae, but they both exhibited *Pseudomonas* sp. and *Pseudoalteromonas* sp. It is possible that both species represent important components of the innate microflora important for the maintenance of health. It has been observed that numerous members of the *Pseudoalteromonas* sp. produce antagonistic compounds to aquaculture pathogens such as *Listonella anguillarum* and *Vibrio* spp. (B. Forward, unpublished results). Further, Gram et al (1999) successfully employed a *Pseudoalteromonas* sp. AH2 as a probiotic with protective effect in trout aquaculture. Hence, it is possible these two species have potential for the development of probiotics for use in improving the health and yield in cod and rotifer culture.

This study showed the potential of using a HD system in cod early larvae culture. The benefits of using such a system included lower capital costs, lower labor costs, higher and more stable growth rate, and less bacterial contamination in cod larvae. However, this study highlights the necessity of adjusting the nutritional composition of rotifers prior to being fed to cod larvae. Further work should be done to develop an enrichment procedure that will provide a more suitable nutritional profile of rotifers for cod larvae. Further studies are also necessary to include the enrichment of rotifers within the production step in HD culture systems.

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