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Does a large-scale continuous algal production system provide a stable supply of fatty acids to bivalve hatcheries?

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Abstract The variation of fatty acid (FA) content and composition of the microalgal production (*Isochrysis* sp., *Pavlova lutheri* and *Chaetoceros muelleri*) in a continuous large-scale production system (500-L bags) used in hatcheries were analysed. The variation of the FAs was analysed in replicate bags over time for the different species. Total FA content (pg cell^{-1}) increased significantly ($p<0.05$) in the *P. lutheri* and *C. muelleri* bags over time. The content of the essential FAs (arachidonic acid (ARA), eicosapentaenoic acid (EPA), *n*-6 docosapentaenoic acid (*n*-6 DPA) and docosahexaenoic acid (DHA)) increased over time in all of the species, except for DHA in *Isochrysis* sp. The content of EPA and ARA were highest in *C. muelleri*, whilst *n*-6 DPA and DHA were highest in *Isochrysis* sp. The FA composition in the *C. muelleri* bags showed large variability between bags at the beginning of the experiment, but decreased over time. In contrast, the FA composition of *Isochrysis* sp. and *P. lutheri* did not vary much over time, but larger variability was observed between the replicate bags. The results indicate that, even though the essential polyunsaturated FAs (PUFAs) varied between the different species, the total microalgal production secured a stable and increased supply of all the essential PUFAs to the scallop larvae and spat.

Keywords Fatty acids · Continuous algal culture · Bivalve hatchery · *Isochrysis* sp. · *Pavlova lutheri* · *Chaetoceros muelleri*

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Introduction

Several studies have shown the importance of fatty acids (FAs), especially the polyunsaturated FAs (PUFAs) for bivalve nutrition and health (Brown et al. 1989; Delaunay et al. 1993; Soudant et al. 1996; Caers et al. 1998, 2000; Milke et al. 2004, 2006, 2008), in maintaining membrane fluidity and as precursors of bioactive molecules (Freas and Grollman 1980; Osada et al. 1989). Most bivalves are unable to synthesise significant amounts of long-chain PUFAs and are reliant on dietary sources to meet the FA requirements (e.g. Langdon and Waldock 1981; Delaunay et al. 1993). The importance of the FA composition in the diet for hatching, larval and juvenile growth and survival and settlement success of bivalves has been demonstrated by Marty et al. (1992), Delaunay et al. (1993), Thompson et al. (1993), Soudant et al. (1996) and Milke et al. (2004, 2006). Particularly the *n*-6 and *n*-3 PUFAs such as arachidonic acid (20:4n6, ARA), eicosapentaenoic acid (20:5n3, EPA), *n*-6 docosapentaenoic acid (22:5n6, DPA) and docosahexaenoic acid (22:6n3, DHA) are considered as essential FAs for bivalves (Marty et al. 1992; Soudant et al. 1996; Parrish et al. 1998; Milke et al. 2004, 2006, 2008). A relatively high and stable production of these FAs is therefore important in the live feed in scallop hatcheries.

Many studies have been undertaken in order to determine the nutritional value of microalgal species, their

biochemical composition and potential use as a food source (e.g. Volkman et al. 1981, 1989, 1991, 1993; Brown et al. 1989, 1993; Brown 1991; Brown and Jeffrey 1992; Brown and Miller 1992; Dunstan et al. 1992, 1994; de Roeck-Holtzhauer et al. 1993; Brown and Farmer 1994; Brown et al. 1997).

The total concentrations of protein, lipid and carbohydrate may vary substantially between species (e.g. Brown et al. 1989, 1997). The biochemical composition of microalgae is affected by the growth environment which can be manipulated by changing the culture medium (Wikfors et al. 1984; Ben-Amotz et al. 1985), temperature (Redalje and Laws 1983; James et al. 1989), stage of harvest (Chu et al. 1982; Whyte 1987) and light conditions (Caron et al. 1988; Sicko-Goad et al. 1988; Cohen et al. 1988; Thompson et al. 1990).

Optimum culture conditions in terms of high growth rates are, however, not necessarily the same as optimum conditions for nutritional quality (e.g. Sanchez et al. 2000). A compromise between the nutritional quality and growth kinetics will often have to be considered. For instance, it has been shown that the essential FA, EPA, increased with decreasing light for *Nannochloropsis* sp. and *Chaetoceros gracilis* (=*Chaetoceros muelleri*; Sukenik et al. 1989; Thompson et al. 1990).

Many experiments (e.g. Emdadi and Berland 1989; Hodgson et al. 1991; Dunstan et al. 1993; Pernet et al. 2003) have shown that different culture techniques and stage of harvest have an impact on FA quality and content. It appears that lipid class and FA composition of microalgae are highly variable during culturing. Dunstan et al. (1993) investigated changes in lipid class and FA composition of *Pavlova lutheri* and *Isochrysis* sp. grown in 100-L bags, either as logarithmic and stationary phase batch cultures or as semi-continuous cultures, and found that they changed depending on the culture technique and growth phase. Pernet et al. (2003) found high variability in both lipid class and FA composition of *C. muelleri* and *Isochrysis* sp. in a semi-continuous system. However, at present, there are no data published on the variability of FAs in large-scale continuous bag cultures, which are well-used production systems in many bivalve hatcheries. In bivalve hatcheries where the quality of the live feed is an important factor for rearing success of the larvae and spat, it is important to know the variability of the FAs during the microalgal production over time.

The purpose of the present investigation was to see if the content and composition of FAs varied over time in large-scale continuous bag cultures (500 L) commonly used by hatcheries. Three species, *Isochrysis* sp., *P. lutheri* and *C. muelleri*, widely used as feed in bivalve hatcheries because of their nutritional profile, were grown in commercial scale (e.g. Magnesen et al. 2006). The variation of FAs in the algal production system were analysed between replicate bags over time. Implications for hatcheries are discussed.

Materials and methods

The microalgal cultures were grown and operated indoor at the Norwegian scallop hatchery *Scalpro* AS, located at Rong, outside Bergen, Norway.

Stock cultures of *Isochrysis* sp. (CCAP 927/14), *P. lutheri* (CCAP 931/1) and *C. muelleri* (CCAP 1010/3) were obtained from the Culture Collection of Algae and Protozoa (CCAP, UK). The stock cultures (30 mL) were grown and maintained under continuous white fluorescent light (Osram L 58W/965 Biolux) at 100±2 µmol photons m^{-2} s^{-1}, at 15±1°C and in Conway medium (Laing 1991). They were made axenic by antibiotic treatment and tested for sterility at each transfer (Droop 1967). Starter cultures (2 L) were inoculated with axenic stock cultures in good growth. The starter cultures were then grown in Conway medium at 20±1°C in continuous white fluorescent light (Osram L 58W/965 Biolux) at 110±3 µmol photons m^{-2} s^{-1} and with bubbling of CO_2-enriched air (0.5% of the volume of air). Dense starter cultures (20–30 mill. cells L^{-1}) were then used to inoculate 500-L continuous bag cultures (Seacaps Ltd., UK; www.seacaps.com). The bag cultures were operated according to the standard *Scalpro* AS hatchery protocol where about 10% of the bag volume was continuously harvested per day at late logarithmic phase. The large-scale bag cultures were grown under the same conditions as the starter cultures, except that the cultures were enriched with CO_2 released directly into the seawater at a flow rate of 6 L h^{-1}, giving a stable pH at 8.4±0.1. Three replicate bags were set up for each species.

Sampling

Three bags for each species (*Isochrysis* sp., *P. lutheri* and *C. muelleri*) were sampled three times over 6 weeks in May and June 2007 (7 May, 29 May and 18 June). When sampling started (7 May) all of the cultures were 1 week old. All of the bags were started at the same time and were therefore of the same age. The bags were operated as continuous cultures and sampled at late logarithmic phase. The duration life of cultures in this system is usually 1–2 months, although 3–4 months can be experienced. At each sampling time, three samples were collected from each bag for determination of microalgal cell numbers and FA content and composition.

Cell numbers (cells mL^{-1}) were determined by counting live cells in a Bürker counting chamber. A minimum of 400

cells were counted each time, giving a counting error of ±10% (Andersen and Throndsen 2003).

Samples for FA analyses (3×250 mL from each bag) were immediately brought to the laboratory at the University of Bergen after sampling for further processing.

Sample preparation for FA analysis

After gentle stirring to ensure homogeneous suspension in the flasks, six replicate subsamples were poured into 15 mL thick-walled glass tubes with Teflon-lined screw caps containing 8.26 μg of the internal standard FA 19:0. The internal standard had been added in advance to the tubes in a chloroform solution of accurately known concentration, and the solvent evaporated. Samples were concentrated by centrifugation at 4,500 rpm for 15 min. The supernatant was gently sucked off and the remaining water was evaporated under a stream of N_2 gas.

For *C. muelleri*, the algal material was not completely precipitated by the centrifugation, so parallels of 50 mL of the algal suspension were filtered by suction through a paper filter, 2-cm diameter, which had been thoroughly rinsed with chloroform and methanol. The filter paper with the algal material was transferred to 15-mL thick-walled glass tubes for further treatment as the other samples.

One half millilitre anhydrous methanol, containing hydrogen chloride (HCl) at a concentration of 2 mol L^{-1}, was added; the tubes were sealed with Teflon-lined screw caps and subjected to methanolysis for 2 h at 90°C. After cooling, approximately half of the methanol was evaporated with nitrogen gas and replaced by 0.5 mL distilled water. The water/methanol phase containing the FA methyl esters (FAME) was extracted twice with 1 mL hexane by thorough shaking followed by centrifugation and withdrawal of the hexane phase by a Pasteur pipette. The concentration of the FAME in the combined extracts was adjusted by the addition of hexane to obtain levels suitable for gas chromatography.

Gas chromatography

One microlitre of the hexane extracts was injected splitless and chromatographed on a 25-m×0.25-mm fused silica column with polyethyleneglycol (CP-WAX 52 CB Chrompack) with 0.2-μm thickness as stationary phase and helium at 20 psi as mobile phase. The column was mounted in a Hewlett-Packard 5892 Series II gas chromatograph equipped with a Hewlett-Packard 7673 autosampler and a flame ionisation detector. Injector and detector temperatures were 260°C and 330°C, respectively. The oven temperature was 90°C at injection, and this temperature was maintained for 4 min before being raised to 165°C at 30°C min^{-1}, thereafter being raised to 225°C at 3°C min^{-1} and maintained there for 10.5 min. A standard solution (GLC-68D from Nu-Check-Prep, Elysian, USA) containing 20 FAME was chromatographed after every eight samples. The detector signal was digitalised and sent to the lab data system Chromeleon. Peaks were identified by means of the standard mixture, previous experience of relative retention times of FAME and mass spectrometry. The peak areas of 31 selected FAME between 14:0 and 22:6n3 were integrated and corrected by response factors. These empirical response factors, relative to 18:0, were calculated for the 20 FAME present in known amounts in the standard mixture. The response factors for each of the five FAME, for which we had no standards, were estimated by comparison with the standard FAME with the closest retention time that resembled each of those most closely in terms of chain length and number of double bonds. These corrected areas were then used to calculate FA content and for multivariate analysis.

Statistical methods

The relative content of each of the 31 FAs in the samples was calculated as percentage of the total. To detect possible differences in the FA composition among the different species and bags, the data were subjected to multivariate principal component analysis (PCA) using the software package Sirius 7.0 (Kvalheim and Karstang 1987). The relative values of the 14 most abundant FAs were log-transformed. The samples were positioned in a 14-dimensional space, one dimension for each FA, and principal components (PCs) were then placed as vectors through the centroid of the samples in such a way that the first PC fell in the direction of the largest spread of the samples and the second PC, orthogonal to the first, in the direction of the second largest spread of the samples. The samples were projected onto the plane made of these two coordinates. The systematic variation among the samples embedded in the original 14 variables, FAs, was thus displayed in two dimensions.

An ANOVA for each of the 31 FAs was performed to detect significant difference ($p<0.05$) between replicate bags.

Results

The microalgal production system was operated as continuous cultures and harvested at late logarithmic phase. Cell numbers between replicate bags were relatively stable during the experiment (Table 1). At the start of the experiment (7 May), the cell numbers were 2.2±0.3, 2.1±0.1 and 1.4±0.1 $\times 10^6$ cells mL^{-1} for *Isochrysis* sp., *P. lutheri* and *C. muelleri* bags, respectively. After 6 weeks

Table 1 Cell numbers (CN, 10^6 cells mL^{-1}), total fatty acid content (TFA, pg cell^{-1}), content of ARA (pg cell^{-1}), EPA (pg cell^{-1}), *n*-6 DPA (pg cell^{-1}) and DHA (pg. cell^{-1}) in replicate bags (1, 2, 3) of *Isochrysis* sp. (Tiso), *P. lutheri* (Pav) and *C. muelleri* (CHM)

	7 May 2007						29 May 2007						18 June 2007					
	CN $\times10^6$mL^{-1}	TFA pg cell^{-1}	ARA pg cell^{-1}	EPA pg cell^{-1}	*n*-6 DPA pg cell^{-1}	DHA pg cell^{-1}	CN $\times10^6$mL^{-1}	TFA pg cell^{-1}	ARA pg cell^{-1}	EPA pg cell^{-1}	*n*-6 DPA pg cell^{-1}	DHA pg cell^{-1}	CN $\times10^6$mL^{-1}	TFA pg cell^{-1}	ARA pg cell^{-1}	EPA pg cell^{-1}	*n*-6 DPA pg cell^{-1}	DHA pg cell^{-1}
Tiso—1	2.50	2.11	0.00	0.02	0.03	0.30	2.40	3.06	0.01	0.02	0.05	0.42	2.00	2.20	0.01	0.02	0.04	0.27
Tiso—2	2.00	2.47	0.01	0.02	0.04	0.34	2.20	3.33	0.01	0.02	0.06	0.47	1.60	2.54	0.01	0.02	0.04	0.32
Tiso—3	2.20	2.12	0.00	0.02	0.03	0.29	1.90	2.56	0.01	0.02	0.04	0.37	2.10	–[a]	–[a]	–[a]	–[a]	–[a]
Pav—1	2.20	0.67	0.00	0.17	0.01	0.07	2.10	1.33	0.00	0.35	0.01	0.14	2.00	1.17*	0.01	0.28	0.01	0.10
Pav—2	2.10	0.76	0.00	0.20	0.01	0.09	2.20	1.21	0.00	0.33	0.01	0.13	2.10	1.37*	0.00	0.32	0.01	0.12
Pav—3	2.10	0.64	0.00	0.17	0.01	0.07	2.50	0.88	0.00	0.23	0.01	0.08	1.60	1.83*	0.01	0.43	0.02	0.15
CHM—1	1.30	0.97	0.02	0.17	0.00	0.01	0.90	2.14	0.05	0.34	0.00	0.03	1.50	3.44*	0.11	0.50	0.01	0.03
CHM—2	1.30	1.35	0.03	0.25	0.00	0.01	0.80	2.23	0.05	0.32	0.00	0.02	1.50	2.62*	0.08	0.33	0.00	0.03
CHM—3	1.50	1.29	0.03	0.25	0.00	0.02	1.10	2.49	0.04	0.39	0.00	0.03	1.50	2.33*	0.07	0.36	0.00	0.03

[a] Indicates missing data

*$p<0.05$ (significant increase)

(18 June), the cell numbers were 1.9±0.3 mill. cells mL^{-1} in the *Isochrysis* sp. and *P. lutheri* bags and 1.5±0.0 ×10^6 cells mL^{-1} in the *C. muelleri* bags (Table 1).

At the start of the experiment (7 May), total FA content were 2.2±0.2 pg cell^{-1} for *Isochrysis* sp., 0.7±0.1 pg cell^{-1} for *P. lutheri* and 1.2±0.2 pg cell^{-1} for *C. muelleri* (Table 1). Maximum total FA concentration registered was 3.44 pg cell^{-1} in one of the *C. muelleri* bags (18 June, Table 1). The concentration of total FAs increased significantly ($p<0.05$) in the *P. lutheri* and *C. muelleri* bags during the experiment, but no statistical significant increase ($p=0.06$) was observed for *Isochrysis* sp. The content of the essential FAs (ARA, EPA, *n*-6 DPA, DHA) increased over time in all of the species, except for DHA in *Isochrysis* sp. (Table 2). The content (pg cell^{-1}) of EPA and ARA were highest in *C. muelleri*, whilst *n*-6 DPA and DHA were highest in *Isochrysis* sp. (Tables 1 and 2)

The sum of saturated FA (SFA), monounsaturated FA (MUFA) and polyunsaturated FA (PUFA) changed between replicates and species over time (Fig. 1). The content of SFAs was in general higher in the *C. muelleri* bags (42.6±3.6%) than in the *Isochrysis* sp. (25.5±1.8%) and *P. lutheri* (31.2±3.0%) bags (Fig. 1, Electronic supplementary material (ESM) Tables 1, 2 and 3). However, the content of SFA decreased over time in the *C. muelleri* bags from a mean initial content of 44.3±2.1% to a mean content of 38.2±0.4% on the last sampling date. An increase of SFA was observed in the *P. lutheri* and *Isochrysis* sp. bags (Fig. 1, ESM Tables 1, 2, 3), corresponding to a mean increase from 29.2±0.9% to 34.6±2.7% in the *P. lutheri* bags and 25.6±0.8% to 27.6±0.7% in the *Isochrysis* sp. bags. The dominating SFAs in all of the three species were 14:0 and 16:0. In *C. muelleri*, it was particularly 14:0 that decreased over time, whilst 15:0 and iso-17:0 increased in *Isochrysis* sp. and *P. lutheri*, respectively (ESM Tables 1, 2 and 3). The content of MUFAs in the *Isochrysis* sp. and *P. lutheri* bags were relatively stabile over time, whilst the MUFAs 16:1n7 and 20:1n9 increased in the *C. muelleri* bags (Fig. 1, ESM Table 3). The content of PUFAs was higher in the *Isochrysis* sp. bags (56.5±2.9%) compared to the *P. lutheri* (40.6±2.4%) and *C. muelleri* (27.3±2.2%) bags (Fig. 1, ESM Tables 1, 2 and 3). The dominating PUFAs in *Isochrysis* sp. were 18:3n3, 18:4n3 and DHA; in *P. lutheri* 18:4n3, EPA; and DHA and in *C. muelleri* EPA. A minor decrease in proportion was, however, detected in all of the species over time (Fig. 1, ESM Tables 1, 2 and 3).

The PCA (Figs. 2, 3 and 4) detected variation among the replicate bags of the different species over time. Each FA was in addition tested pairwise between the replicate bags of each species to detect difference on 95% level (ESM Tables 1, 2 and 3).

Table 2 Mean values ± STD of fatty acid content (n=3, pg cell^{-1}) of ARA, EPA, n-6 DPA and DHA at start and end of the experiment for *Isochrysis* sp., *P. lutheri* and *C. muelleri* bags

	Isochrysis sp.				*Pavlova lutheri*				*Chaetoceros muelleri*			
	Start		End		Start		End		Start		End	
ARA	0.004	±0.01	0.001	±0.00	0.002	±0.00	0.01	±0.00	0.03	±0.03	0.09	±0.02
EPA	0.02	±0.00	0.02	±0.00	0.18	±0.02	0.34	±0.08	0.22	±0.05	0.40	±0.09
n-6 DPA	0.03	±0.01	0.04	±0.00	0.01	±0.00	0.02	±0.01	0.001	±0.00	0.004	±0.00
DHA	0.31	±0.03	0.30	±0.03	0.08	±0.01	0.12	±0.03	0.01	±0.01	0.03	±0.00

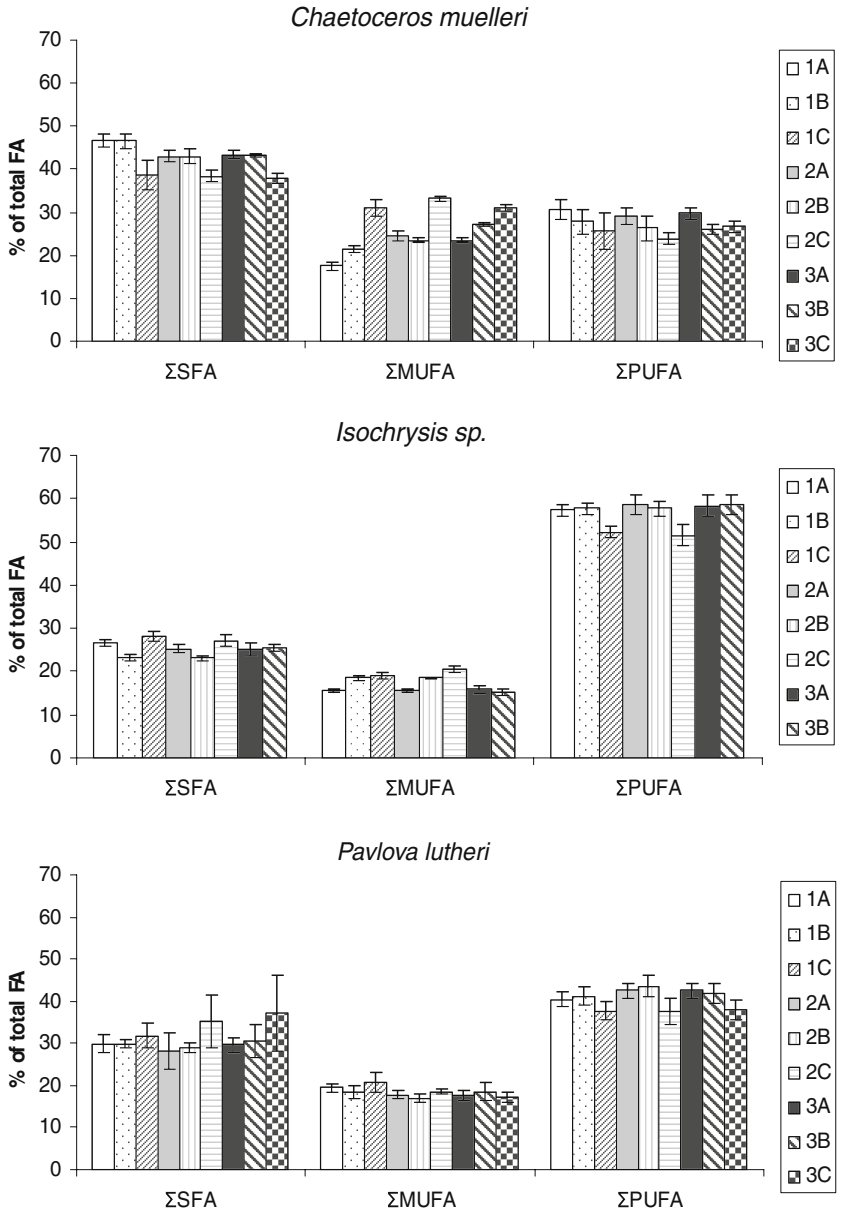

Fig. 1 Proportions (% of total fatty acid) of saturated (*SFA*), monounsaturated (*MUFA*) and polyunsaturated fatty acids (*PUFA*) of *C. muelleri*, *Isochrysis* sp. and *P. lutheri* in replicate bags (1, 2, 3) at 7 May (**a**), 29 May (**b**) and 18 June (**c**) 2007. *Error bars* indicate STD

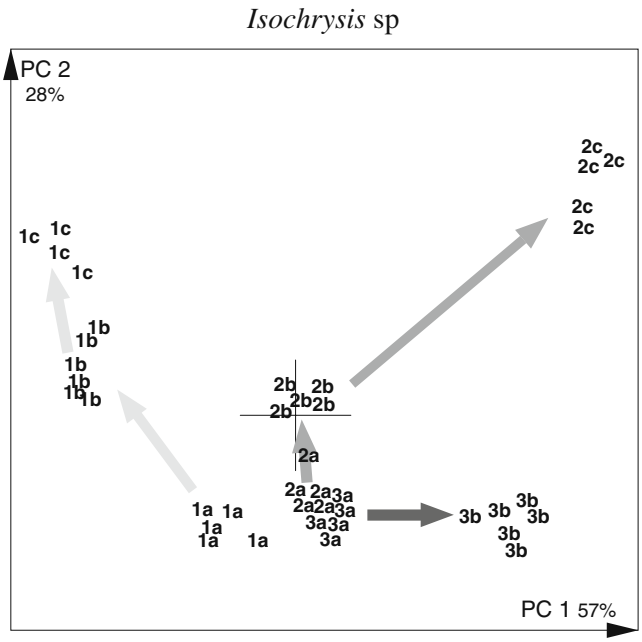

Fig. 2 Principal component analysis plot of *Isochrysis* sp. in replicate bags (1, 2, 3) at 7 May (**a**), 29 May (**b**) and 18 June (**c**) 2007

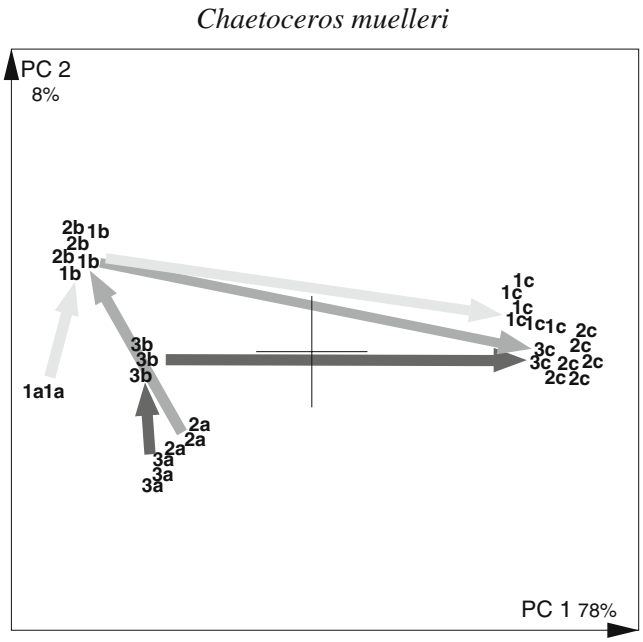

Fig. 4 Principal component analysis plot of *C. muelleri* in replicate bags (1, 2, 3) at 7 May (**a**), 29 May (**b**) and 18 June (**c**) 2007

The *Isochrysis* sp. bags were similar at the start of the experiment, but the replicate bags developed in different directions (Fig. 2). The first principal component (PC1) described 57% of the total variation between the bags. The FAs on the last sampling day (1c, 2c) are the furthest away from origo and are therefore more different. On the first sampling date (7 May), only three FAs (18:2n6, 18:3n6, 20:1n9) differed significantly ($p<0.05$, ESM Table 1) between the replicates. After 2 weeks (29 May), 13 FAs differed significantly ($p<0.05$) between the replicates, whilst 8 FAs were significant different on the last sampling date (ESM Table 1). Analyses showed that particularly the long-chained FAs (C20-24) were stable between the replicates throughout the experiment and were not differing significantly (ESM Table 1).

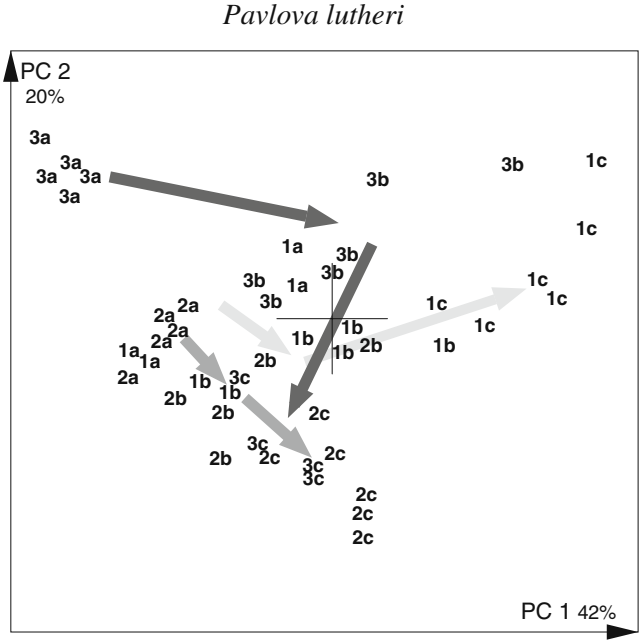

Fig. 3 Principal component analysis plot of *P. lutheri* in replicate bags (1, 2, 3) at 7 May (**a**), 29 May (**b**) and 18 June (**c**) 2007

The *P. lutheri* bags showed large variation and the replicates did not develop in the same direction (Fig. 3). PC1 described 42% of the total variation between the bags. At start, 17 of the FAs were significant different between the replicates, and after 2 weeks (29 May), only three FAs (17:0, 17:1nx, 22:6n3) differed, whilst 12 FAs were significantly different after 6 weeks (ESM Table 2).

All of the *C. muelleri* bags had a similar development over time (Fig. 4). The PC1 described 78% of the total variation. The development from the second to the third sampling dates (29 May–18 June) was largest compared to from the first to the second sampling dates (7–29 May, Fig. 4). The variation between the replicate bags was highest at the start of the experiment, but the replicates became more similar towards the end (Fig. 4). On the first and second sampling date, 22 and 20 FAs, respectively, differed significantly between the replicates, but only ten after 6 weeks (ESM Table 3). Of the essential PUFAs (ARA, EPA, *n*-6 DPA and DHA), EPA was stable between the replicates for all of the species (ESM Tables 1, 2 and 3), ARA was stable in the *P. lutheri* bags, DHA was stable in the *C. muelleri* bags, whilst *n*-6 DPA and DHA were stable for *Isochrysis* sp. bags.

Discussion

The results highlight species-specific variations of FAs between replicate bags and over time in a continuous large-scale production system (500-L bags) commonly used by many bivalve hatcheries. Variation of lipid class and FA composition has been found previously during the culture process of *Isochrysis* sp., *P. lutheri* and *C. muelleri* in semi-continuous systems and in batch systems (e.g. Dunstan et al. 1993; Pernet et al. 2003).

The FA composition of *C. muelleri* changed over time. The variation between replicate bags was largest at the start of the experiment, but the replicates became more similar over time. In these bags, FAs such as 22:6n3, 20:1n9 and 22:1n11 had high STD, indicating that these FAs may have contributed to the observed variability. In contrast, the FA composition of *Isochrysis* sp. and *P. lutheri* were relatively stable over time, but the variation between replicate bags was larger than for the *C. muelleri* bags, particularly towards the end of the experiment. In the *Isochrysis* sp. bags particularly 22:1n9 and 22:5n3 had high STD, whilst 20:0, 20:2n6, 20:3n3, 22:1n9 and 24:0 had high STD in the *P. lutheri* bags. The results also showed that even though the essential PUFAs varied between the different species, the total microalgal production secured a stable supply of all the essential PUFAs.

Different studies have demonstrated that changes in light intensity (Thompson et al. 1990, 1993; Brown et al. 1993), culture media (Ben-Amotz et al. 1985), temperature (James et al. 1989; Thompson et al. 1992; Zhu et al. 1997), pH (Guckert and Cooksey 1990), stage of harvest and different culture techniques (e.g. Emdadi and Berland 1989; Hodgson et al. 1991; Dunstan et al. 1993; Brown et al. 1997; Pernet et al. 2003) have an impact on fatty acid content and composition.

In this experiment, both temperature, culture media and pH were kept stable and were not limiting. However, light conditions decreased over time due to growth of algal cells on the bag walls (personal obs.). This reduced light penetration into the bags by approximately 20–30% of initial light intensity (results not shown). Thompson et al. (1990) investigated the influence of irradiance on the FA composition of eight species commonly used in aquaculture, including *Isochrysis* sp., *P. lutheri* and *C. muelleri*, and found that the essential FAs increased with decreasing light intensities for *C. muelleri*, but decreased for *Isochrysis* sp. and *P. lutheri*. In this experiment, we found that the content of the essential FAs increased in *C. muelleri* and *P. lutheri*, but was rather stable (ARA, EPA, ng DPA) or decreased (DHA) for *Isochrysis* sp. *Isochrysis* sp. is in general low in EPA (e.g. Volkman et al. 1989), which was also the case in this experiment. The FA composition is highly dynamic and responds significantly to variation in light intensity (e.g. Thompson et al. 1990; Brown et al. 1993). The variable light conditions due to different growing of cells on the bag walls were probable causes to the observed variability between the replicates over time, but did not explain the variability between the replicates at the start of the experiment for the *P. lutheri* and *C. muelleri* bags. The starter cultures were all grown under the same conditions before they were used to initiate the bag cultures and should therefore in theory be quite similar. However, the different cultures might have been in different growth phases, causing the FAs to differ between the replicates (e.g. Brown et al. 1997). Hodgson et al. (1991) showed that the proportions of the lipid classes and their component FAs were subject to variation dependent upon growth phase of the culture. Changes were particularly prominent in early stages of a culture. This could also be the case in this experiment, resulting in the observed variability between the replicate bags.

The total fatty acid content increased significantly ($p < 0.05$) in the *P. lutheri* and *C. muelleri* bags over time. The total FA content was higher than Pernet et al. (2003) reported from 170-L semi-continuous cultures of *C. muelleri*. Although the proportions (% of total FA) of PUFAs decreased over time, an increase of the content of the essential PUFAs (picograms per cell) was observed. The content of the essential PUFAs was nearly doubled in the *C. muelleri* bags. An increase of SFAs in the *P. lutheri* bags and MUFAs (% of total FA) in the *C. muelleri* bags were also registered. The *C. muelleri* cultures contained higher proportions of PUFAs than reported by Volkman et al. (1989) from batch cultures, but similar to Milke et al. (2004) from semi-continuous cultures. In the *C. muelleri* bags, EPA dominated over the PUFAs, but ARA dominated in content (picograms per cell). The *Isochrysis* bags were dominated by C18 PUFAs and DHA, whilst the *P. lutheri* bags were dominated by C18 PUFAs, EPA and DHA.

Relatively high proportions of 14:0, 16:0, 16:1n7 in addition to *n*-6 PUFAs such as 18:2n6, 18:3n6 and ARA were also recorded in the *C. muelleri* bags, typical for diatoms in general (Volkman et al. 1989). The *n*-6 PUFAs such as ARA and *n*-6 DPA are considered as essential FAs for scallop larvae and spat and should be present in the diet (Parrish et al. 1998; Milke et al. 2004, 2006, 2008). For example, Milke et al. (2008) showed that *n*-6 DPA was important in early life stages of *Placopecten magellanicus*. Our results showed that the content of ARA and *n*-6 DPA was stable or increasing in all of the species and bags over time.

Variation of the FAs over time and also between replicate bags may have an impact on the rearing success of scallop larvae and juveniles. Although the results showed that many FAs varied between replicates and over time, the production of the essential PUFAs increased. Considering

the total microalgal production of the essential PUFAs in this experiment, a stable supply of all the essential PUFAs to the scallop larvae and spat was secured at a ratio of 1:1:1 of *Isochrysis* sp., *P. lutheri* and *C. muelleri*, similar to combinations used in scallop hatchery by Magnesen et al. (2006).

Improvements to be considered in order to increase PUFA production further are lower pH and increase light penetration, particularly in the *Isochrysis* sp. and *P. lutheri* bags, as these factors has been shown to increase PUFA content (e.g. Thompson et al. 1990; Roncarati et al. 2004), in addition to improved mixing to avoid growing on the bag walls.

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