Sprague M, Walton J, Campbell P, Strachan F, Dick JR & Bell JG (2015) Replacement of fish oil with a DHA-rich algal meal derived from Schizochytrium sp. on the fatty acid and persistent organic pollutant levels in diets and flesh of Atlantic salmon (Salmo salar, L.) post-smolts, *Food Chemistry*, 185, pp. 413-421.

DOI: 10.1016/j.foodchem.2015.03.150

Replacement of Fish Oil with a DHA-rich Algal Meal Derived from *Schizochytrium* sp. on the Fatty Acid and Persistent Organic Pollutant Levels in Diets and Flesh of Atlantic Salmon (*Salmo salar,* L.) Post-Smolts

Sprague, M.[1*], Walton, J.[2], Campbell, P.[2], Strachan, F.[1], Dick, J.R.[1] and Bell, J.G.[1]

[1]*Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK*

[2]*BioMar Ltd., North Shore Road,Grangemouth Docks, Grangemouth FK3 8UL, Scotland, UK*

*Corresponding author. Tel.: +44 1786 467989

E-mail address: matthew.sprague@stir.ac.uk

Abstract

The replacement of fish oil (FO) with a DHA-rich *Schizochytrium* sp. algal meal (AM) at two inclusion levels (11% and 5.5% of diet) was tested in Atlantic salmon post-smolts compared to fish fed a FO diet of northern (NFO) or southern hemisphere (SFO) origin. Fish were preconditioned prior to the 19-week experimental feeding period to reduce long-chain polyunsaturated fatty acid (LC-PUFA) and persistent organic pollutant levels (POPs). Dietary POP levels differed significantly between treatments in the order of NFO>SFO>11AM/5.5AM and were subsequently reflected in the flesh. Fish fed the 11AM diet contained similar DHA levels ($g.100g^{-1}$ flesh) to FO-fed fish, despite percentage differences. However, the low levels of EPA in the diets and flesh of algal-fed fish compromised the overall nutritional value to the final consumer. Nevertheless, further developments in microalgae culture offer a promising alternative lipid source of LC-PUFA to FO in salmon feeds that warrants further investigation.

Key words: Atlantic salmon, *Salmo salar*, *Schizochytrium* sp. algal-meal, fish oil replacement, fatty acids, persistent organic pollutants (POPs), PCDD/Fs, DL-PCBs, PBDEs.

Chemical compounds studied in this article

Docosahexaenoic acid (PubChem CID: 445580); Eicosapentaenoic acid (PubChem CID: 446284)

1. Introduction

It is widely accepted that fish consumption is an excellent source of the beneficial omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), which have important roles in protecting against cardiovascular disease as well as neurological and inflammatory conditions among other health benefits (Calder & Yaqoob, 2009). Nevertheless, fish consumption, particularly oily fish, is also a major dietary exposure route for humans to persistent organic pollutants (POPs), including dioxins [polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)], dioxin-like polychlorinated biphenyls (DL-PCBs) and polybrominated diphenyl ethers (PBDEs). These lipophilic compounds are easily absorbed and rapidly distributed to lipid-rich organs and tissues which can result in their bioaccumulation. Consequently, the beneficial effects may be offset by the negative risks associated with fish intake.

In farmed fish, such as Atlantic salmon (*Salmo salar*), the traditional marine-derived components of fish feed, fish oil and fish meal, are considered to be the major sources of POPs (Berntssen, Julshamn & Lundebye, 2010a; Jacobs, Covaci & Schepens, 2002). Reducing the POP levels in fish oils, and ultimately the feeds and flesh of fish, without affecting the LC-PUFA content, has been achieved using decontamination techniques (Berntssen et al., 2010b; Sprague et al., 2010) or by utilising less polluted fish oils from the southern hemisphere (Sprague et al., 2010). However, the increased competition from the pharmaceutical and nutraceutical industries for n-3 LC-PUFA coupled with the stagnated global supply of wild catch fisheries has led to increased prices and supply pressures resulting in the aquafeed sector investigating alternative lipid sources.

The partial and complete replacement of marine ingredients with agricultural plant products, mainly of oilseed origin, has been performed in salmon without any detrimental effects on growth performance or fish health (*e.g.* Bell, Henderson, Tocher & Sargent, 2004; Bell, McGhee, Dick & Tocher, 2005). Such substitution reduces the levels of undesirable POPs (Bell et al., 2005; Bell, Dick, Strachan, Guy, Berntssen & Sprague, 2012; Berntssen et al., 2010a). Conversely, since the fatty acid composition of fish tissue reflects that of the diet (Sargent, Tocher and Bell, 2002), increasing the vegetable content in aquafeeds reduces the favourable LC-PUFA levels abundant in fish oils, thereby compromising the overall nutritional quality of the final product. Finishing diets can be employed to restore flesh n-3 LC-PUFA levels in vegetable fed fish, although this still relies upon the inclusion of fish oils (Bell et al., 2004; 2012), albeit at the risk of increasing POP levels in a previously low contaminated product (Bell et al., 2005, 2012). Since marine fish lack the conversion pathways to efficiently produce EPA and DHA at appreciable levels, these essential fatty acids must be obtained through the diet (Sargent et al., 2002).

Marine microalgae are primary producers of n-3 LC-PUFA, and are therefore a promising alternative to the traditional marine derived ingredients of fish feed. Several species have been identified as potential sources, among which the thraustochytrids have been preferred due to their ease for large-scale heterotrophic cultivation under controlled conditions to produce a high lipid product rich in n-3 LC-PUFA (Lewis, Nichols & McMeekin, 1999; Ratledge, 2005). Furthermore, unlike crude oils and oilseeds, algal biomass produced by fermentation is generally free from environmental contaminants and heavy metals (Ratledge, 2005). *Schizochytrium* sp. is a fast growing thraustochytrid microalgae, rich in DHA, with a relatively simple culture process compared to other single-cell microalgae (Ganuza, Benítez-Santana,

87 Atalah, Vega-Orellana, Ganga & Izquierdo, 2008; Lewis et al. 1999). Subsequently,
88 the aquaculture industry has investigated the potential of thraustochytrids, particularly
89 *Schizochytrium* sp. in either dried biomass or oil extracted form, as an alternative
90 lipid source in enriching zooplankton for feeding to finfish larvae (Barclay & Zeller,
91 1996), supplementing channel catfish diets, *Ictalurus punctatus*, (Li, Robinson,
92 Tucker, Manning & Khoo, 2009), or as replacement for fish oils in diets for sea
93 bream, *Sparus aurata* (Ganuza et al., 2008), and Atlantic salmon (Carter, Bransden,
94 Lewis & Nichols, 2003; Miller, Nichols & Carter, 2007). However, to date no such
95 studies have been performed in post-smolt salmon to assess *Schizochytrium* sp.
96 inclusion as a potential replacement for fish oil in grow-out feeds and its effects on
97 the nutritional quality of the final product.

98 The present study therefore evaluated the replacement of fish oil with a DHA-
99 rich algal meal, derived from *Schizochytrium* sp., at two different inclusion levels (11
100 and 5.5% of diet) on the fatty acid and POP compositions of Atlantic salmon diets
101 and flesh compared to fish fed a fish oil diet of either northern or southern
102 hemisphere origin.

103

104 **2. Materials and Methods**

105 *2.1. Experimental set-up and diets*

106 The trial was performed at Marine Harvest's (Scotland) Feed Trial Unit (Ardnish,
107 Inverness-shire, Scotland) using a commercial strain of 1,700 1+ Atlantic salmon
108 post-smolts. Fish (850 ± 100 g, mean ± SD) were initially stocked into four 125 m^3
109 sea pens and acclimatized for 10 weeks during which they were fed a high
110 rapeseed/fish oil (6:1, w/w) preconditioning diet in order to reduce flesh POPs and
111 LC-PUFA levels. Fish (1534 ± 400 g) were then split between twelve pens (130 fish

per pen) and acclimated for one-week prior to the enrichment phase. Triplicate pens were fed one of four diets for 19 weeks consisting of either (a) northern hemisphere fish oil (NFO) as control, (b) southern hemisphere fish oil (SFO), or (c/d) a DHA-rich drum dried *Schizochytrium* sp. algal meal (AquaGrow Gold®; Advanced BioNutrition, Columbia, MD, USA) at two dietary inclusion levels, 5.5% (5.5AM) or 11% (11AM) with 25% and 21% added rapeseed oil respectively. All diets were formulated (Table 1) and produced by BioMar UK (Grangemouth, Scotland). Fish were fed twice daily using automatic feeders with uneaten food collected via waste uplift systems to monitor feed intake. Feed fed, waste feed and the resulting net feed intake were registered daily, as were any mortalities. Fish were reared under natural photoperiod and temperature (range 6.5-13.8^{o}C) for the duration of the trial. At the start and end of the enrichment phase, all fish in each pen were bulk weighed. The specific growth rate (SGR) was calculated as: SGR (%bw.day^{-1}) = $100\times[\ln(W_F/W_I)/d]$, where W_F and W_I are the final and initial weights (g) respectively, and d is the number of days. Thermal growth coefficient (TGC) was calculated as TGC = $(W_F^{1/3}-W_I^{1/3}) \times (1000/DD)$, where W_F and W_I are as previously addressed for SGR, and DD is the cumulative daily water temperature (^{o}C) in SW.

2.2. Sample collection

Samples of the precondition and experimental feeds were collected, wrapped in aluminium foil, before placing into sealable polythene bags and stored at -70^{o}C until analysis. Diets were analysed using standard methods to determine crude lipid (acid hydrolysis of soxhlet samples, Teactor Soxtec method); moisture (AOAC, 2000); crude protein (Kjeldahl, calculated as N×6.25); Ash (AOAC, 2000) and energy (bomb calorimeter: Gallenkamp Autobomb, calibrated with benzoic acid).

At the end of the preconditioning phase (initial), 4 fish were removed from the cages, anaesthetized and killed by a single blow to the head. Flesh from the Norwegian Quality Cut (NQC) region was removed, wrapped in aluminium foil and stored in sealable polythene bags at -70^{o}C until analysis. Following termination of the enrichment phase, 6 fish per dietary treatment were anaesthetized and killed by a single blow to the head and NQC flesh removed and stored as per initial fish. Fatty acid analysis was performed for individual fish, whereas flesh was pooled from each pen (2 initial, 3 per enrichment treatment) for POPs analysis. The experiment was subjected to ethical approval by the University of Stirling Ethics Committee and carried out in accordance with the UK Animals (Scientific Procedures) Act 1986.

2.3. Lipid content and total lipid fatty acid composition

Total lipid content was determined gravimetrically after extraction of ~1 g tissue or diet by homogenizing in 20 or 36 volumes of ice-cold chloroform/methanol (2:1 v/v) respectively, using an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, UK) according to Folch, Lees and Stanley (1957). Non-lipid impurities were isolated by washing with 0.88% (w/v) KCl and the upper aqueous layer removed by aspiration and the lower solvent layer containing the lipid extract dried under oxygen-free nitrogen.

Fatty acid methyl esters (FAMEs) from total lipid were prepared by acid-catalyzed transmethylation at 50^{o}C for 16h (Christie, 1993). FAME were extracted and purified as described previously (Tocher & Harvie, 1988) and separated and quantified by GC using a Fisons GC-8160 (Thermo Scientific, Milan, Italy) equipped with a 30 m x 0.32 mm i.d. x 0.25 µm ZB-wax column (Phenomenex, Cheshire, UK), 'on column' injection and flame ionization detection. Hydrogen was used as carrier

gas with initial oven thermal gradient from 50 to 150°C at 40°C.min^{-1} to a final temperature of 230°C at 2°C.min^{-1}. Individual FAME were identified by comparison to known standards (Supelco™ 37-FAME mix; Sigma-Aldrich Ltd., Poole, UK) and published data (Tocher & Harvie, 1988). Data were collected and processed using Chromcard for Windows (Version 1.19; Thermoquest Italia S.p.A., Milan, Italy). Fatty acid content per g of tissue was calculated using heptadecanoic acid (17:0) as internal standard.

2.4. Persistent organic pollution (POP) extraction and clean-up

The 29 PCDD/F and DL-PCB congeners with WHO-TEF values (Van den Berg et al., 2006) and nine PBDE congeners (IUPAC numbers 28, 47, 49, 66, 99, 100, 153, 154, 183) were targeted in samples as previously described (Sprague, Dick, Medina, Tocher, Bell & Mourente, 2012). Briefly, lipid was extracted from 25 g of diet or freeze-dried tissue (ca. 65 g wet weight) by accelerated solvent extraction (ASE™100; Dionex, Camberley, UK) following addition of 5 ng.ml^{-1} PBDE119 and 2 ng.ml^{-1} ^{13}C-labelled PCDD/F and PCB internal standards (Wellington Laboratories, Guelph, Ontario, Canada). Sample extracts were loaded for further clean-up and fractionation of analytes using the automated Power-Prep™ system (Fluid Management Systems Inc., Watertown, MA, USA) followed by conditioning of the disposable column series, consisting of multi-layered silica (4 g acid, 2 g base, 1.5 g neutral), basic alumina (8 g) and carbon (2 g). Total run time was 150 min followed by a 40 min decontamination programme. The mono-*ortho* PCB and PBDE fraction (F1) was eluted in 120 ml isohexane/dichloromethane (1:1, v/v) and the PCDD/F and non-*ortho* PCB fraction (F2) in 120 ml toluene. Fractions underwent further clean up with F1 transferred to silanized vials containing 150 µl nonane as keeper and

evaporated to 500 or 100 µl prior to analysis for PBDE and mono-*ortho* PCBs, respectively, and F2 transferred to conical GC autosampler vials containing 10 µl of nonane as keeper and evaporated to 50 or 10 µl prior to analysis for non-*ortho* PCB and PCDD/F, respectively.

2.5. Instrumental analysis

Mono-, non-*ortho* PCBs and PCDD/Fs extracts were analysed using a PolarisQ™ ion trap MS/MS coupled to a Trace GC 2000 (Thermo Finnigan, Bremen, Germany) equipped with a 30 m x 0.25 mm i.d. x 0.25 µm Rxi®-5ms (5% diphenyl, 95% dimethyl polysiloxane) fused-silica capillary column (Thames Restek Ltd., Saunderton, UK). Samples and standards (2 µl) were injected in splitless mode. The GC oven temperature programmes were as reported by Sprague et al. (2012). Helium was used as carrier gas at constant flow (0.8 $ml.min^{-1}$). Injector, transfer line and ion source temperatures were maintained at 250, 305 and 250^{o}C, respectively. The MS operated in positive electron ionisation (EI+) mode using automatic gain control with electron energy of 70 eV and emission current of 250 µA. Quantification was based on US Environmental Protection Agency isotopic dilution methods (EPA, 1994, 1999). Relative response factors (RRFs) for individual 2,3,7,8-chlorosubstituted PCDD/F and DL-PCB congeners were determined using calibration standards. Xcalibar™ version 1.3 was used for data acquisition and results processing.

PBDEs (1µl) were injected in splitless mode (225^{o}C, 1.5 min) with surge (240 kPa) on a Trace GC Ultra™ equipped with a 30 m x 0.25 mm i.d. x 0.25 µm ZB5-MS column (Phenomenex, Cheshire, UK) coupled to a Trace DSQ™ MS (Thermo Finnigan, Bremen, Germany) operating in negative chemical ion mode (CI^{-}). The GC temperature programme was as previously reported (Sprague et al., 2012). Helium

was used as carrier gas at constant flow (1.2 $ml.min^{-1}$) and methane as reagent gas (2.0 $ml.min^{-1}$). The MS operated in selective ion monitoring (SIM) mode by monitoring bromide isotope ions (*m*/*z* 81 and 79) with dwell time of 80 ms. Quantification of PBDE congeners was performed by congener-specific linear calibration curves (r^2 >0.99). Xcalibar™ version 1.4 was used for data acquisition and results processing.

2.6. Quality assurance (QA) and quality control (QC)

Samples were ran with a procedural blank, a duplicate sample and an 'in-house' reference material, consisting of pooled salmon flesh, cross referenced with an external laboratory. Limits of detection (LOD) were determined using a software option for estimating signal-to-noise (S/N) ratio, where limit of quantification (LOQ) was three times LOD (nine times S/N ratio). LOQs were in the range of 0.01-0.03 $pg.g^{-1}$ wet weight (ww) for PCDD/Fs, 0.1-0.5 $pg.g^{-1}$ ww for non-*ortho* PCBs, 2.1-3.9 $pg.g^{-1}$ ww for mono-*ortho* PCBs and 6-48 $pg.g^{-1}$ ww for PBDEs. Recovery values for PCDD/Fs and DL-PCBs, based on congener-specific response factors of ^{13}C internal surrogate standard relative to ^{13}C performance standards (EPA, 1994), were in the range of 76-114%. Percentage recoveries for PBDEs, based on spiked sample matrix with internal standards for all congeners, were in the range of 78-118%. Method performance was further assessed through satisfactory participation of 'Interlaboratory Comparison on Dioxins in Food' tests organized by the Norwegian Institute for Public Health. Results for PCDD/Fs and DL-PCBs are presented on a WHO-TEQ basis using 2006 TEFs (Van den Berg et al., 2006), although total PCDD/Fs and DL-PCB values are also expressed on 1998 TEFs (Van den Berg et al., 1998) for comparative purposes.

2.7. Statistical analysis

Results are presented as mean and relative standard deviation (%RSD), unless otherwise specified. Statistical analyses were performed using Minitab® v.16.1.0 statistical software (Minitab Inc.). Data were assessed for normality with Kolmogorov-Smirnov test and for homogeneity of variances by Bartlett's test and examination of residual plots and, where necessary, transformed using the natural logarithm or arcsine transformation. Data were compared by a one-way analysis of variance (ANOVA), with replicate cages nested within their dietary treatment groups. Post hoc comparisons were made using Tukey's test (Zar, 1999). A significance of $P<0.05$ was applied to all statistical tests performed.

3. Results and Discussion

3.1. Dietary fatty acid and POP compositions

The fatty acid compositions of the precondition and enrichment diets are presented in Table 1. Since the aim of the present study was to investigate the effects of the algal-feeds on LC-PUFA levels, fish were first fed a preconditioning feed consisting mainly of rapeseed oil to decrease n-3 LC-PUFA levels. Previous studies using vegetable-based diets have shown a decrease in the levels of flesh EPA and DHA due to the absence of these particular fatty acids in plant-based ingredients (Bell et al., 2004, 2005, 2012; Berntssen et al., 2010a). Thus, the precondition diet was largely comprised of oleic (18:1n-9), linoleic (18:2n-6), palmitic (16:0) and α-linolenic (18:3n-3) acids accounting for 46, 18, 8 and 7% of total fatty acids, respectively, with relatively low levels of EPA (3.6%) and DHA (2.3%) derived from the minor inclusion of fish oil and meal to maintain basic fish requirements.

Fatty acid compositions for both algal feeds were similar to the precondition diet due to the high inclusion of rapeseed oil. Commercial aquafeeds often incorporate blends of vegetable and fish or other oils to meet the nutritional requirements of the fish being farmed. Furthermore, the extraction of oil from the single cell biomass greatly increases the overall production costs (Miller et al., 2007; Ratledge, 2005), subsequently limiting its use as a sole oil source replacement for fish oil in feeds. Therefore, the present study combined the dried algal biomass with rapeseed oil to increase the essential n-3 LC-PUFA content resulting in a DHA content of 8.1% (11AM) and 5.3% (5.5AM). Additionally, the algal inclusion resulted in higher levels of the n-6 docosapentaenoic acid isomer (DPA; 22:5n-6) than the other feeds. This is a common feature of *Schizochytrium* sp. production, where approximately 20% of the DHA produced is DPA, a metabolically neutral fatty acid that has no overall effect on DHA uptake (Ratledge, 2005). Dietary lipid of the fish oil treatments, NFO and SFO, on the other hand contained 3-7 times lower levels of 18:1n-9, 18:2n-6 and 18:3n-3 than the precon and algal diets. Both diets resembled the nutritional composition of their natural diets being largely characterized by 16:0, 18:1n-9, cetoleic (22:1n-11) and gondoic (20:1n-9) acids. The major PUFA were DHA and EPA with the NFO diet containing a lower EPA (8.0%) and a higher DHA (10.2%) level than the SFO diet (12.3 and 8.4%, respectively), as is commonly observed between oils sourced from the northern and southern hemispheres (Sargent et al., 2002).

In addition to decreasing LC-PUFA levels, vegetable feeds have also been shown to contain lower POP levels than their fish oil counterparts (Bell et al., 2005, 2012; Berntssen et al., 2010a). Consequently, the vegetable-based precon diet contained lower POP levels (0.267 pgWHO-TEQ.g^{-1} ΣPCDD/Fs + DL-PCBs, 0.273 ng.g^{-1} ww ΣPBDEs) than both fish oil based feeds, irrespective of origin (Table 2). Furthermore,

287 the SFO diet contained approximately half the level of POPs than the NFO diet
288 (0.973 against 1.824 pgWHO-TEQ.g^{-1} ww ΣPCDD/Fs + DL-PCBs and 1.086 against
289 2.395 ng.g^{-1} ww ΣPBDEs, respectively). Southern hemisphere fish oils typically
290 contain lower POP levels than those from the northern hemisphere (Brevik et al.,
291 1990), reflecting the differences in pollution levels of marine waters between
292 hemispheres (Fowler, 1990). The NFO control feed however, is in the range reported
293 by Berntssen et al. (2010b) for a fish oil control diet, 2.31 against 2.18 pgWHO-
294 $TEQ_{1998}.g^{-1}$ ww ΣPCDD/Fs + DL-PCBs reported in this study, and is lower than
295 levels reported by Bell et al. (2005) for both a high- and low-fish oil diet (4.1 and 2.3
296 pgWHO-$TEQ_{1998}.g^{-1}$ ww ΣPCDD/Fs + DL-PCBs respectively). This highlights the
297 awareness and efforts taken by the aquafeed industry in recent years in reducing
298 dietary POP levels. Nevertheless, the lowest POP levels were measured in the two
299 algal feeds (0.080 and 0.120 pgWHO-TEQ.g^{-1} ww ΣPCDD/Fs + DL-PCBs and 0.154
300 and 0.059 ng.g^{-1} ww ΣPBDEs, 11AM and 5.5AM respectively) yielding a reduction
301 of >94% compared to the NFO diet and >85% relative to SFO, similar to reductions
302 seen where fish oil based feeds have been decontaminated (Berntssen et al., 2010b;
303 Sprague et al., 2010). Since the algal biomass is produced under controlled conditions
304 the potential for contamination from environmental pollutants and heavy metals is
305 greatly reduced (Ratledge, 2005). The residual amounts of POPs measured in the
306 algal feeds from the present study are therefore most likely contributable to other feed
307 ingredients, most notably fish meal, since this along with fish oil is known to be a
308 major POP contributor in fish feeds (Berntssen et al., 2010a; Jacobs et al., 2002).
309 Equally, the utilization of rapeseed oil in both the precon and algal-based feeds may
310 have resulted in the increase of another lipophilic POP not measured in this study.
311 Polycyclic aromatic hydrocarbons (PAHs) were found to increase in fish flesh when

vegetable oils replaced fish oils, although organochlorine pesticides along with PCDD/Fs, PCBs and PBDEs decreased (Berntssen et al., 2010a).

3.2. Growth and feed intake

The replacement of fish oil with the algal biomass had no significant effect on the overall weight gain of fish (Table 3). However, fish fed the 11AM diet exhibited a minor but significantly lower growth rate than both fish oil treatments but not 5.5AM fed fish. This is in contrast to Carter et al. (2003) and Miller et al. (2007) who found no growth detriment when algal biomass or oil replaced fish oil in the diets of Atlantic salmon parr-smolts. In addition, the food conversion ratio (FCR) for both algal-fed treatments was significantly higher than NFO fed fish but similar to SFO. One possible explanation, particularly at the higher inclusion of algal meal, may be related to digestibility. In a similar study, Reitan, Erikson, Galloway, Berge and Kjørsvik (2012) replaced fish oil with other microalgae species (*Nannochloropsis* sp., *Phaeodactylum tricornutum* and *Isochrysis galbana*) in the diets of salmon and Atlantic cod, *Gadus morhua*. They found that inclusion levels up to 6% gave good digestibility whereas 12% microalgae inclusion resulted in a reduced digestibility, although appetite remained unchanged.

No differences in the health status and/or immune function were recorded (data not presented), consistent with the results of several previous trials where partial or full replacement of fish oils were employed when using fish meal based diets (Bell et al., 2004; Bell et al., 2005).

3.3. Flesh fatty acid and POP compositions

Flesh lipid levels of Atlantic salmon fed the enrichment diets significantly increased from initial levels of 6.7 to 10.1-11.2%, with no significant differences observed between the four dietary treatments (Table 4). Fatty acid compositions of the flesh accurately reflected that of the diet fed, in accordance with results normally found for salmon and other fish species (Sargent et al., 2002). The algal dietary treatments shared similar fatty acid profiles with elevated fillet flesh levels of 18:2n-6 and 18:1n-9 compared to both fish oil treatments consistent with results where salmon have been fed diets based on plant oils due to the use of rapeseed oil in the diet (Bell et al., 2012; Miller et al., 2007). The major difference between the algal-fed treatments was a significantly higher level of DHA in the flesh of 11AM fish (8.9%) compared to 5.5AM fish (7.4%), arising from the difference in algal biomass inclusion. However, there was no overall increase in DHA in fish fed 5.5AM relative to initial levels (7.6%). Furthermore, percentage levels of DHA in the 11AM-fed fish were still significantly lower than for fish fed a fish oil based diet (10.4% SFO, 11.7% NFO). This would suggest that an algal inclusion level greater than 11% is required to increase flesh DHA above basal levels and to establish similar levels to those found in fish oil based diets. In previous studies, Miller et al. (2007) and Carter et al. (2003) observed greater flesh DHA levels in salmon parr-smolts when *Schizochytrium* sp. replaced fish oil. However, caution should be applied when comparing results since the former authors utilised the richer oil extract as the sole oil source compared to a fish oil control whereas the latter authors elected to use 10% algal biomass and rapeseed, as in the present study, with a control diet consisting of a blend of vegetable and fish oils. While the latter control diet is more consistent with the formulation of commercial diets currently used by the industry, the present study sought to evaluate the dried algal biomass against a fish oil only diet to assess DHA

levels and is, to our knowledge, the first to trial *Schizochytrium* sp. as a potential fish oil replacement in grow-out feeds for Atlantic salmon.

The most significant, but not unexpected, difference in flesh fatty acid content between dietary treatments is the lack of EPA in fish fed the algal diets (2.6 and 2.9%, 11AM and 5.5AM respectively) compared to NFO (5.9%) and SFO (8.1%). This corresponds with results reported in earlier studies where a DHA-rich microalgal sp. replaced fish oil (Carter et al. 2003; Eryalçin et al., 2013; Ganuza et al., 2012; Miller et al., 2007). Moreover, EPA levels in the flesh of both algal treatments significantly decreased as compared to initial levels (4.9%), possibly a result of a dilution effect as flesh lipid increased or a depletion of this essential fatty acid. In general, n-3 LC-PUFA are important for the somatic growth of marine fish with DHA the most highly retained PUFA in a variety of species (Sargent et al., 2002). Ganuza et al. (2012) noted that growth, survival and disease resistance of sea bream larvae was unaffected when fish oil was substituted with *Schizochytrium* sp. but was altered when all dietary lipid (i.e. fish oil and fish meal) was replaced by algal biomass, a result of a dietary imbalance of fatty acids which was rectified by EPA supplementation. However, the dietary requirements of EPA and DHA in fish are more essential at early life stages (Sargent et al., 2002) and the inclusion of fish meal in the algal feeds would most likely have contained sufficient quantities of EPA for basic metabolic processes. Fish oil is still the main source of EPA, as the complex characteristics of EPA-producing algal species involve high-energy, high-costs and are time consuming (Ratledge, 2005). Nevertheless, since the trial was performed a *Schizochytrium* sp. algae with a minimum EPA and DHA content of no less than 10 and 22%, respectively, has come on to the market (Gray, 2010), primarily targeting the feed/infant formulation sectors. More recently, Ruiz-Lopez, Haslam, Napier and

386 Sayanova (2014) have successfully produced an alternative transgenic plant source of
387 n-3 LC-PUFA using an oilseed crop *Camelina sativa* to achieve levels of 12% EPA
388 and 14% DHA, similar to levels found in fish oils which may have potential in
389 aquafeeds (Betancor et al., 2015).

390 The POP concentrations from the flesh of fish fed the experimental diets for 19-
391 weeks are presented in Table 5. Berntssen et al. (2010b) stress that feeding trials of
392 short duration (e.g. 1-3 months) yield relatively lower levels of POPs compared to the
393 typical time taken to farm salmon to harvest size, due to flesh POP accumulation over
394 time. At the end of the present study significant differences in flesh POP levels
395 between treatments were observed being in the order of NFO>SFO>11AM/5.5AM
396 (0.803, 0.532, 0.247 and 0.263 pg.WHO-TEQ_{2005}.g^{-1} ww ΣPCDD/F + DL-PCBs
397 respectively, and 1.233, 0.702, 0.268 and 0.267 ng.g^{-1} ww ΣPBDEs respectively),
398 reflecting the differences in dietary POP levels. Over the course of a full production
399 cycle the fillet POP levels would further increase, although differences between
400 dietary treatments would be expected to be maintained. Furthermore, the current costs
401 for the algal biomass are similar to or even higher than those for fish meal and fish oil
402 meaning that, at present, it is not practicable to feed the algal-based feeds over a full
403 production cycle. Instead, it may be more economical to include algal diets as a
404 finishing feed by first feeding a vegetable-based feed followed by a short-period of
405 feeding the algal feeds, as performed in the present study. This would help alleviate
406 costs as well as further reduce the POP levels, in contrast to fish oil finishing feeds
407 (Bell et al., 2005; 2012).

408 The lower levels of POPs, specifically PCDD/Fs and DL-PCBs, in the flesh of
409 initial fish (0.156 pg.WHO-TEQ_{2005}.g^{-1} ww) compared to algal-fed fish, despite the
410 preconditioning diet containing higher POPs levels (0.267, 0.069 and 0.098 pgWHO-

411 $TEQ_{2005}.g^{-1}$ ww, Precon, 11AM and 5.5AM, respectively), is most likely a result of
412 differences in flesh lipid levels between initial (6.7%) and algal-fed fish (10.9 and
413 11.2%, 11AM and 5.5AM, respectively) affecting the uptake of these lipophilic
414 compounds. Thus, lipid normalized values were 2.33 (initial), 7.95 (NFO), 5.27
415 (SFO), 2.27 (11AM) and 2.35 (5.5AM) pgWHO-$TEQ_{2005}.g^{-1}$ lw.
416
417 *3.4. Nutritional value from salmon consumption: risk-benefit*
418 One of the major selling points cited for consuming oily fish, such as salmon, is
419 their unique source of n-3 LC PUFA, EPA and DHA, known to benefit human health
420 (Calder & Yaqoob, 2009). Replacing fish oil with *Schizochytrium* sp. results in a 130
421 g portion, as advised by the European Food Safety Authority (EFSA, 2005),
422 providing 1.4 and 1.3 g of EPA+DHA, 11AM and 5.5AM respectively (Figure 1),
423 equivalent to 40% (11AM) or 36% (5.5AM) of the suggested 3.5 g weekly intake of
424 EPA+DHA recommended by the International Society for the Study of Fatty Acids
425 and Lipids for optimal cardiac health in adults (ISSFAL, 2004). Nevertheless, this is
426 still significantly less than for fish oil fed salmon, supplying 2.0 g (NFO) and 1.9 g
427 (SFO) EPA+DHA or 57 and 53% of the recommended weekly intake respectively.
428 This difference is attributable to differences in EPA levels, since the *Schizochytrium*
429 sp. used was a DHA-rich only microalgae product and, as previously discussed, fish
430 oil is still the main source of EPA. Despite this, algal-fed fish still remain a rich
431 source of DHA when fed at the higher inclusion level, with no significant differences
432 between absolute amounts of DHA in 11AM fish (1.1 ± 0.1 $g.100g^{-1}$) compared to
433 both NFO (1.2 ± 0.2) and SFO (1.1 ± 0.1 $g.100g^{-1}$), despite differences in percentage
434 terms.

Fish consumption also represents a major dietary exposure risk to humans through the accumulation of POPs. Accordingly, the European Scientific Committee on Food (SCF) has set a tolerable weekly intake (TWI) level of 14 pg.WHO-TEQ.kg^{-1} body wt for PCDD/Fs + DL-PCBs (SCF, 2001), equivalent to 980 pg.WHO-TEQ for an adult of 70 kg. Based on the same 130 g servings, NFO-fed fish contribute 104.3 ± 8.1 pg.WHO-TEQ, significantly higher than SFO-fed fish (69.1 ± 3.0 pg.WHO-TEQ) both of which are significantly higher than algal-fed fish (32.1 ± 2.4 and 34.4 ± 3.1 pg.WHO-TEQ, 11AM and 5.5AM respectively). This represents just 10.6% (NFO), 7.1% (SFO), 3.3% (11AM) and 3.5% (5.5AM) of the TWI for an adult of 70 kg. Food health authorities typically recommend consuming two portions of fish per week, one of which is oily (EFSA, 2005). From the present study, consuming two portions of either fish oil- or algal-fed fish would contribute 120-130 or 80-90% of the recommended EPA+DHA weekly intake and only 15-20 or 7% of the TWI for PCDD/Fs and DL-PCBs, respectively. These results indicate that previously identified high-risk groups such as young girls, women of child bearing age, and those pregnant and/or breast-feeding can safely consume more than two portions per week without exceeding their TWI, although this does not take into consideration POP intake from other dietary sources.

At present, PBDE levels in foods are not limited by legislation although EFSA have issued an advisory TWI of 0.7 μg.kg^{-1} body wt.week^{-1}, equivalent to 49.0 μg PBDE.week^{-1} for a 70 kg adult (EFSA, 2005). From the present study, a 130 g portion corresponds to 0.16 or 0.09 μg PBDEs for the NFO and SFO treatments respectively and 0.03 μg PBDEs for both algal treatments, all of which are less than 1% of the advisory TWI for PBDEs.

4. Conclusion

The replacement of fish oil with a DHA-rich *Schizochytrium* sp. microalgae significantly decreases both dietary and flesh fillet POP levels compared to fish oil based treatments. Moreover, flesh fillet DHA levels can be tailored to similar levels in fish oil fed fish when algal biomass is included at 11% of the diet. However, the absence of EPA in algal-based diets significantly impairs the overall nutritional value, in terms of g EPA+DHA per serving, to the final human consumer. Current and future developments in algal culture technology may provide a final affordable product of nutritional quality in terms of LC-PUFA content as an alternative to fish oil in aquafeeds.

Conflict of Interest Statement

None of the authors have a conflict of interest.

References

AOAC, 2000. *Official methods of analysis of AOAC International*. 17th ed. AOAC International, Gaithersburg, Maryland, USA.

Barclay, W., Zeller, S. 1996. Nutritional enhancement of n-3 and n-6 fatty acid in rotifers and Artemia nauplii by feeding spray-dried *Schizochytrium* sp. *Journal of the World Aquaculture Society*, 314-322.

Bell, J.G., Dick, J.R., Strachan, F., Guy, D.R., Berntssen, M.H.G., Sprague, M. 2012. Complete replacement of fish oil with a blend of vegetable oils affects dioxin, dioxin-like polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in 3 Atlantic salmon (*Salmo salar*) families differing in flesh adiposity. *Aquaculture*, 324-325, 118-126.

485 Bell, J.G., Henderson, R.J., Tocher, D.R., Sargent, J.R. 2004. Replacement of dietary
486 fish oil with increasing levels of linseed oil: modification of flesh fatty acid
487 compositions in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet.
488 *Lipids*, 39, 223-232.
489 Bell, J.G., McGhee, F., Dick, J.R., Tocher, D.R. 2005. Dioxin and dioxin-like
490 polychlorinated biphenyls (PCBs) in Scottish farmed salmon (*Salmo salar*):
491 effects of replacement of dietary marine fish oil with vegetable oils. *Aquaculture*,
492 243, 308-314.
493 Berntssen, M.H.G., Julshamn, K., Lundebye, A.-K., 2010a. Chemical contaminants in
494 aquafeeds and Atlantic salmon (*Salmo salar*) following the use of traditional-
495 versus alternative feed ingredients. *Chemosphere*, 78, 637-646.
496 Berntssen, M.H.G., Olsvik, P.A., Torstensen, B.E., Julshamn, K., Midtun, T.,
497 Goksøyr, A., Johansen, J., Sigholt, T., Joerum, N., Jakobsen, J.-K., Lundebye,
498 A.-K., Lock, E.-J. 2010b. Reducing persistent organic pollutants while
499 maintaining long chain omega-3 fatty acid in farmed Atlantic salmon using
500 decontaminated fish oils for an entire production cycle. *Chemosphere*, 81, 242-
501 252.
502 Betancor, M.B., Sprague, M., Usher, S., Sayanova, O., Campbell, P.J., Napier, J.A.,
503 Tocher, D.R. 2015. A nutritionally-enhanced oil from transgenic *Camelina sativa*
504 effectively replaces fish oil as a source of eicosapentaenoic acid for fish.
505 *Scientific Reports*, 5, 8104.
506 Brevik, E.M., Biseth, A., Oehme, M. 1990. Levels of polychlorinated dibenzofurans
507 and dibenzo-p-dioxins in crude and processed fish oils in relation to origin and
508 cleaning method. *Organohalogen Compounds*, 1, 467-470.

509 Calder, P.C., Yaqoob, P. 2009. Omega-3 polyunsaturated fatty acids and human
510 health outcomes. *Biofactors*, 35, 266-272.
511 Carter, C.G., Bransden, M.P., Lewis, T.E., Nichols, P.D. 2003. Potential of
512 Thraustochytrids to partially replace fish oil in Atlantic salmon feeds. *Marine*
513 *Biotechnology*, 5, 480-492.
514 Christie, W.W., 1993. Preparation of derivatives of fatty acids for chromatographic
515 analysis, in: Christie, W.W. (Ed.), Advances in Lipid Methodology Two, The
516 Oily Press, Dundee, pp. 69-111.
517 EFSA, 2005. Opinion of the scientific panel on contaminants in the food chain on a
518 request from the European Parliament related to the safety assessment of wild
519 and farmed fish. EFSA J., 236, 1-118.
520 EPA, 1994. Method 1613: Tetra-through octa-chlorinated dioxins and furans by
521 isotope dilution HRGC/HRMS, EPA no. 821B94005, October 1994. Washington
522 DC: Environmental Protection Agency.
523 EPA, 1999. Method 1668: Chlorinated biphenyl congeners in water, soil, sediment,
524 biosolids and tissue by HRGC/HRMS, EPA no. 821R00002, December 1999.
525 Washington DC: Environmental Protection Agency.
526 Eryalçin, K.M., Roo, J., Saleh, R., Atalah, E., Benítez, T., Betancor, M., Hernandez-
527 Cruz, M.C., Izquierdo, M. 2013. Fish oil replacement by different microalgal
528 products in microdiets for early weaning of gilthead sea bream (*Sparus aurata*,
529 L.). *Aquaculture Research*, 44, 819-828.
530 Folch, J., Lees, M., Sloane Stanley, G.H. 1957. A simple method for the isolation and
531 purification of total lipids from animal tissues. *J. Biol. Chem.*, 226, 497-509.

532 Fowler, S.W., 1990. Critical review of selected heavy metal and chlorinated
533 hydrocarbon concentrations in the marine environment. *Mar. Environ. Res.*, 29,
534 1-64.
535 Ganuza, E., Benítez-Santana, T., Atalah, E., Vega-Orellana, O., Ganga, R., Izquierdo,
536 M.S. 2008. *Cryptocodinium cohnii* and *Schizochytrium* sp. as potential
537 substitutes to fisheries-derived oils from seabream (*Sparus aurata*) microdiets.
538 *Aquaculture*, 277, 109-116.
539 Gray, R.J.H. 2010. Application for the authorization of DHA and EPA-rich algal oil
540 from *Schizochytrium* sp.
541 http://multimedia.food.gov.uk/multimedia/pdfs/dhaoapplicdossier.pdf
542 ISSFAL (2004). International Society for the Study of Fatty Acids and Lipids
543 (ISSFAL) report of the sub-committee on: Recommendations for intake of
544 polyunsaturated fatty acids in healthy adults. http://www.issfal.org/news-
545 links/resources/publications/PUFAIntakeReccomdFinalReport.pdf
546 Jacobs, M.N., Covaci, A., Schepens, P. 2002. Investigation of selected persistent
547 organic pollutants in farmed Atlantic salmon (*Salmo salar*), salmon aquaculture
548 feed, and fish oil components of the feed. *Environmental Science and*
549 *Technology*, 36, 2797-2805.
550 Lewis, T.E., Nichols, P.D., McMeekin, T.A. 1999. The biotechnological potential of
551 Thraustochytrids. *Marine Biotechnology*, 1, 580-587.
552 Li, M.H., Robinson, E.H., Tucker, C.S., Manning, B.B., Khoo, L.2009. Effects of
553 dried algae *Schizochytrium* sp., a rich source of docosahexaenoic acid, on
554 growth, fatty acid composition, and sensory quality of channel catfish *Ictalurus*
555 *punctatus*. *Aquaculture*, 292, 232-236.

556 Miller, M.R., Nichols, P.D., Carter, C.G. 2007. Replacement of fish oil with
557 thraustochytrid *Schizochytrium* sp. L oil in Atlantic salmon parr (*Salmo salar* L)
558 diets. *Comparative Biochemistry and Physiology Part A*, 148, 382-392.
559 Ratledge, C. 2005. Single cell oils for the 21st century In: Cohen, Z. and Ratledge, C.
560 (Eds), *Single Cell Oils*, AOCS Press, Illinois, pp. 1-20.
561 Reitan, K.I., Erikson, T., Galloway, T., Berge, G. and Kjørsvik, E. (2012). Can fish
562 meal and fish oil be replaced by microalgae in feed for Atlantic salmon and
563 Atlantic cod? Aqua 2012, European Aquaculture Society p.91.
564 Ruiz-Lopez, N., Haslam, R.P., Napier, J.A., Sayanova, O. 2014. Successful high-
565 level accumulation of fish oil omega-3 long-chain polyunsaturated fatty acids in
566 a transgenic oilseed crop. *The Plant Journal*, 77, 198-208.
567 Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The lipids In: Halver, J.E., Hardy, R.W.
568 (Eds), Fish Nutrition, Academic Press, San Diego, pp. 182-246.
569 SACN/COT, 2004. Scientific Advisory Committee on Nutrition (SACN) and
570 Committee on Toxicity (COT). Advice on Fish Consumption: Benefits and
571 Risks. The Stationary Office, Norwich, UK.
572 SCF, 2001. European Scientific Committee on Food (SCF), Opinion of the Scientific
573 Committee on Food on the risk assessment of dioxins and dioxin-like PCBs in
574 food. http://ec.europa.eu/food/fs/sc/scf/out90_en.pdf
575 Sprague, M., Bendiksen, E.Å., Dick, J.R., Strachan, F., Pratoomyot, J., Berntssen,
576 M.H.G., Tocher, D.R., Bell, J.G. 2010. Effects of decontaminated fish oil or a
577 fish and vegetable oil blend on persistent organic pollutant and fatty acid
578 compositions in diet and flesh of Atlantic salmon (*Salmo salar*). *British Journal*
579 *of Nutrition*, 103, 1442-1451.

Sprague, M., Dick, J.R., Medina, A., Tocher, D.R., Bell, J.G., Mourente, G. 2012. Lipid and fatty acid composition, and persistent organic pollutant levels in tissues of migrating Atlantic bluefin tuna (*Thunnus thynnus*, L.) broodstock. *Environmental Pollution*, 171, 61-71.

Tocher, D.R., Harvie, D.G., 1988. Fatty acid compositions of the major phosphoglycerides from fish neural tissues: (*n*-3) and (*n*-6) polyunsaturated fatty acids in rainbow trout (*Salmo gairdneri* L.) and cod (*Gadus morhua*) brains and retinas. *Fish Physiol. Biochem.*, 5, 229-239.

Van den Berg, M., Birnbaum, L., Bosveld, A.T., Brunström, B., Cook, P., Freeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., van Leeuwen, F.X., Liem, A.K., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. 1998. *Environmental Health Perspectives*, 106, 775-792.

Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E. 2006. The 2005 World Health Organisation reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicological Sciences*, 93, 223-241.

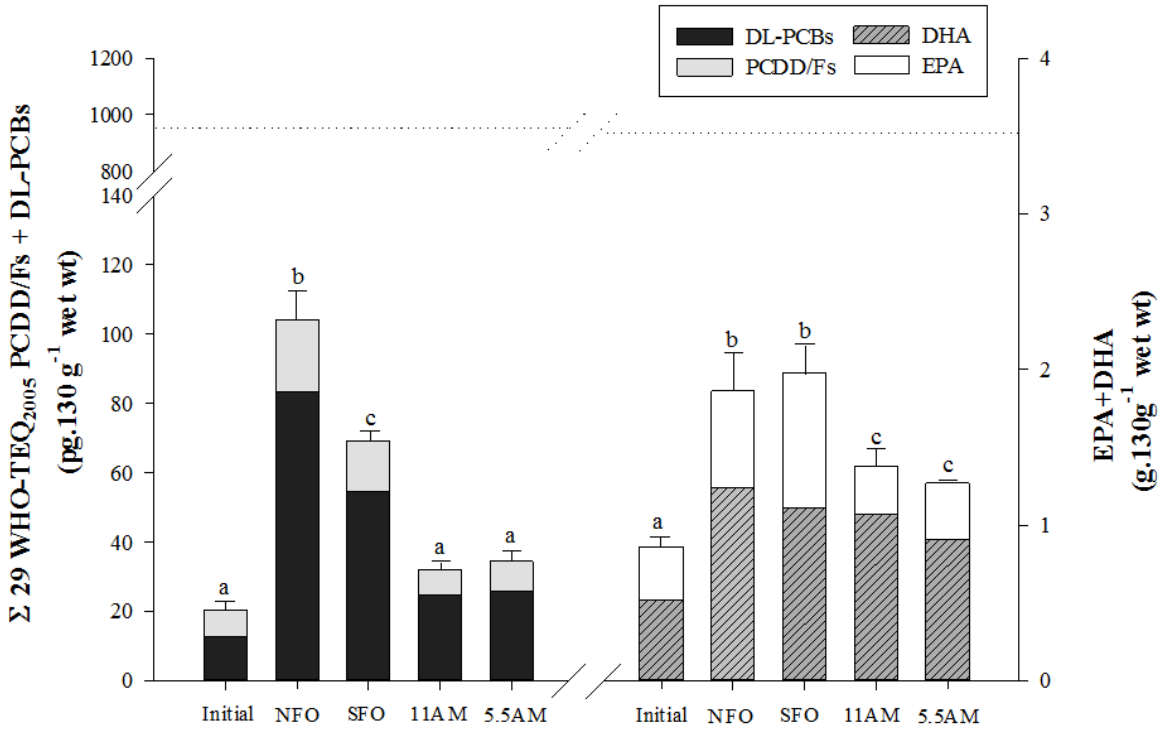
Zar, J.H. 1999. *Biostatistical Analysis*, 663 pp. Prentice-Hall International, New Jersey.

Legend to Figure

Figure 1.

Risk-benefit association, in terms of PCDD/F+DL-PCBs$_{WHO\text{-}TEQ2005}$ and EPA+DHA intake (mean ± SD), from 130 g consumption of Atlantic salmon fed one of four experimental diets. Dotted line represents the 980 pgWHO-TEQ TWI for an adult of 70 kg and the recommended 3.5 g EPA+DHA weekly intake for optimal cardiac health. Stacked bars represent contribution of DL-PCB and PCDD/Fs and EPA + DHA to respective total values. Initial data are presented for comparative purposes.

615 **Figure 1.**



616

617

Table 1. Ingredients, proximate composition (g.kg^{-1}), energy (MJ.kg^{-1}) and fatty acid content (% of total fatty acids) of the precondition and four experimental diets.

	Precon	Experimental			
		NFO	SFO	11%AM	5.5%AM
Component (g.kg^{-1})					
Fish meal	350	270	270	260	260
Vegetable protein concentrates	110	110	110	90	100
Oil seed meals	129	230	230	210	226
DDGS	40	-	-	-	-
Starch sources	151	130	130	120	120
Premixes / micronutrients	7	5	5	5	5
Northern fish oil	32	270	-	-	-
Southern fish oil	-	-	270	-	-
Rapeseed oil	185	-	-	213	246
ABN AquaGrow® Gold	-	-	-	110	55
Analysed Composition (as is)					
Lipid	252	300	303	318	313
Protein	393	342	349	336	347
Moisture	75	71	68	59	62
Ash	74	64	64	71	68
Gross energy (MJ.kg^{-1})	20.6	21.1	21.3	21.4	21.4
Fatty acid (% of total)					
14:0	1.7	5.3	6.4	2.6	1.7
16:0	8.4	14.1	16.0	10.3	8.2
18:0	2.2	2.6	2.8	2.0	1.9
Total saturates[1]	**13.2**	**22.7**	**25.9**	**15.9**	**12.7**
16:1n-7	2.1	4.8	6.8	0.7	1.1
18:1n-9	45.5	13.2	11.6	43.4	45.8
18:1n-7	3.5	2.6	2.8	2.5	3.1
20:1n-9	1.4	7.6	5.4	1.2	1.5
22:1n-11	0.5	12.1	8.2	0.1	0.5
Total monoenes[2]	**53.5**	**43.3**	**36.8**	**48.6**	**52.9**
18:2n-6	17.9	5.8	5.2	16.2	17.5
20:2n-6	0.1	0.3	0.2	0.1	0.1
20:4n-6	0.2	0.6	0.7	0.4	0.3
22:5n-6	0.1	0.2	0.2	2.5	1.3
Total n-6 PUFA[3]	**18.2**	**7.2**	**6.6**	**19.2**	**19.1**
18:3n-3	7.0	1.3	1.0	6.1	6.9
18:4n-3	0.5	3.1	2.9	0.2	0.3
20:5n-3	3.6	8.0	12.3	1.2	2.0
22:5n-3	0.4	1.7	1.6	0.2	0.3
22:6n-3	2.3	10.2	8.4	8.1	5.3
Total n-3 PUFA[4]	**13.9**	**25.4**	**27.0**	**16.0**	**14.8**
Total PUFA[5]	**33.3**	**34.0**	**37.3**	**35.5**	**34.4**
n-3:n-6	**0.8**	**3.5**	**4.1**	**0.8**	**0.8**

Values are presented as means based upon duplicate analyses

[1]includes 15:0, 20:0, 22:0, 24:0

[2]includes 16:1n-9, 20:1n-11, 20:1n-7, 22:1n-9, 24:1

[3]includes 18:3n-6, 20:2n-6, 20:3n-6, 22:4n-6

[4]includes 20:3n-3, 20:4n-3

[5]includes 16:2, 16:3, 16:4

628 **Table 2**. Concentrations of PCDD/Fs, DL-PCBs (pg.g^{-1} ww, upperbound WHO-
629 TEQ$_{2005}$) and PBDEs (ng.g^{-1} ww) in the precondition and four experimental diets fed
630 to Atlantic salmon. ΣWHO-TEQ$_{1998}$ are presented for comparative purposes.

	Precon	Experimental			
		NFO	SFO	11%AM	5.5%AM
PCDD					
2378-TCDD	0.012	0.052	0.016	0.010	0.010
12378-PeCDD	0.059	0.113	0.051	0.010	0.010
123478-HxCDD	0.0015	0.0019	0.0010	0.0010	0.0010
123678-HxCDD	0.002	0.013	0.002	0.003	0.003
123789-HxCDD	0.0010	0.0011	0.0010	0.0010	0.0010
1234678-HpCDD	0.0003	0.0015	0.0004	0.0007	0.0007
OCDD	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
ΣPCDD	**0.076**	**0.183**	**0.071**	**0.026**	**0.026**
PCDF					
2378-TCDF	0.0053	0.1463	0.0470	0.0042	0.0071
12378-PeCDF	0.0005	0.0052	0.0040	0.0003	0.0003
23478-PeCDF	0.012	0.174	0.110	0.006	0.016
123478-HxCDF	0.001	0.001	0.002	0.001	0.001
123678-HxCDF	0.0010	0.0046	0.0032	0.0010	0.0010
234678-HxCDF	0.0024	0.0046	0.0013	0.0010	0.0010
123789-HxCDF	0.001	0.001	0.001	0.001	0.001
1234678-HpCDF	0.0008	0.0020	0.0009	0.0004	0.0006
1234789-HpCDF	<0.0001	0.0003	0.0001	0.0004	0.0002
OCDF	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
ΣPCDF	**0.024**	**0.339**	**0.169**	**0.015**	**0.028**
DL-PCBs					
Non-ortho PCBs					
PCB 77	0.0013	0.0048	0.0040	0.0004	0.0005
PCB 81	0.0001	0.0006	0.0007	0.0001	<0.0001
PCB 126	0.132	1.156	0.643	0.024	0.038
PCB 169	0.026	0.058	0.038	0.001	0.002
Σnon-ortho PCBs	**0.159**	**1.219**	**0.686**	**0.026**	**0.041**
Mono-ortho PCBs					
PCB 105	0.0022	0.0208	0.0111	0.0005	0.0009
PCB 114	<0.0001	0.0009	0.0006	<0.0001	<0.0001
PCB 118	0.005	0.051	0.030	0.001	0.002
PCB 123	0.0003	0.0010	0.0007	<0.0001	<0.0001
PCB 156	0.0003	0.0041	0.0020	<0.0001	0.0001
PCB 157	<0.0001	0.0017	0.0008	<0.0001	<0.0001
PCB 167	0.0002	0.0023	0.0011	<0.0001	<0.0001
PCB 189	<0.0001	0.0007	0.0003	<0.0001	<0.0001
Σmono-ortho PCBs	**0.008**	**0.083**	**0.047**	**0.002**	**0.003**
ΣPCDD/Fs$_{2005}$	**0.100**	**0.522**	**0.240**	**0.041**	**0.054**
ΣDL-PCBs$_{2005}$	**0.167**	**1.302**	**0.733**	**0.028**	**0.044**
ΣPCDD/Fs+DL-PCBs$_{2005}$[1]	**0.267**	**1.824**	**0.973**	**0.069**	**0.098**
ΣPCDD/Fs+DL-PCBs$_{1998}$[2]	**0.285**	**2.180**	**1.170**	**0.080**	**0.120**
Polybrominated diphenyl ethers					
BDE28	0.009	0.053	0.028	0.008	0.008
BDE47	0.141	1.126	0.526	0.006	0.006
BDE49	0.037	0.396	0.183	0.006	0.006
BDE66	nd	0.052	0.022	0.006	0.006
BDE99	0.040	0.214	0.086	0.093	0.015
BDE100	0.032	0.364	0.165	0.012	0.006
BDE 153	0.006	0.033	0.012	0.011	0.006
BDE 154	0.008	0.157	0.064	0.012	0.006
BDE 183	nd	nd	nd	nd	nd
Σ9 PBDEs	**0.273**	**2.395**	**1.086**	**0.154**	**0.059**

631 Values are presented as means based upon duplicate analyses

nd – not detected
[1]TEF_{2005}, Van den Berg et al. (2006)
[2]TEF_{1998}, Van den Berg et al. (1998)

636 **Table 3.** Growth performance of Atlantic salmon fed experimental diets for 19
637 weeks. Means (%RSD) bearing identical superscripts are not significantly different
638 (P>0.05).

	NFO	SFO	11%AM	5.5%AM
Initial mass (g)	1544 (1.0)	1527 (1.1)	1543 (1.2)	1522 (2.9)
Final mass (g)	3245 (2.0)	3220 (2.7)	3030 (4.9)	3170 (3.7)
Weight gain (g)	1701 (3.4)	1692 (4.2)	1487 (8.9)	1648 (4.7)
SGR (% bw.day^{-1})	0.59 (2.0)^{a}	0.59 (2.6)^{a}	0.53 (5.7)^{b}	0.58 (1.7)^{ab}
Total feed consumption (kg)	251.2 (2.1)	258.7 (2.5)	254.5 (1.3)	248.9 (0.8)
FCR	1.28 (3.9)^{a}	1.35 (1.9)^{ab}	1.42 (1.9)^{b}	1.40 (2.5)^{b}

639
640

Table 4. Lipid (%) and fatty acid composition (% of total lipid) from flesh of initial and Atlantic salmon fed one of four experimental enrichment diets. Means bearing identical superscripts within same row are not significantly different (P>0.05).

		Initial	Experimental			
			NFO	SFO	11%AM	5.5%AM
	n	4	6	6	6	6
Lipid (%)		6.7 (9.9)^{a}	10.1 (9.3)^{b}	10.1 (11.7)^{b}	10.9 (15.5)^{b}	11.2 (7.1)^{b}
Fatty acid						
14:0		3.0 (4.7)^{a}	3.8 (6.6)^{b}	4.3 (6.8)^{c}	2.3 (7.8)^{d}	2.3 (3.7)^{d}
16:0		13.2 (3.1)^{a}	13.5 (1.2)^{a}	14.6 (3.8)^{b}	10.7 (4.4)^{c}	10.8 (3.5)^{c}
18:0		3.4 (3.1)^{a}	3.1 (1.1)^{b}	3.3 (3.5)^{a}	2.7 (4.3)^{c}	2.8 (4.5)^{c}
20:0		0.3 (9.4)^{a}	0.2 (6.8)^{a}	0.2 (9.8)^{a}	0.3 (1.9)^{a}	0.3 (4.4)^{a}
Total saturates[1]		**20.2 (3.2)^{a}**	**20.9 (2.0)^{a}**	**22.7 (4.1)^{b}**	**16.4 (5.2)^{c}**	**16.5 (3.2)^{c}**
16:1n-7		4.0 (3.2)^{a}	4.3 (2.9)^{b}	5.6 (2.5)^{c}	2.0 (7.1)^{d}	2.4 (3.2)^{e}
18:1n-9		32.8 (1.9)^{a}	20.9 (2.7)^{b}	19.7 (3.2)^{c}	38.0 (1.0)^{d}	38.1 (1.3)^{d}
18:1n-7		3.4 (4.2)^{a}	3.1 (3.0)^{bc}	3.2 (1.5)^{abc}	3.0 (1.2)^{c}	3.3 (6.2)^{ab}
20:1n-9		2.7 (4.8)^{a}	6.2 (2.8)^{b}	4.8 (1.9)^{c}	2.6 (4.1)^{a}	3.1 (4.5)^{d}
22:1n-11		0.9 (7.6)^{a}	6.3 (5.3)^{b}	4.5 (4.2)^{c}	0.6 (18.8)^{a}	1.0 (12.3)^{d}
22:1n-9		0.4 (11.1)^{ad}	0.7 (8.9)^{b}	0.5 (4.8)^{c}	0.4 (7.1)^{d}	0.4 (9.3)^{d}
24:1n-9		0.4 (3.4)^{ad}	0.9 (6.5)^{b}	0.7 (13.6)^{c}	0.4 (11.8)^{a}	0.5 (9.8)^{d}
Total monoenes[2]		**45.0 (2.0)^{a}**	**42.9 (1.3)^{b}**	**39.7 (0.9)^{c}**	**47.3 (1.0)^{d}**	**49.1 (1.1)^{e}**
18:2n-6		11.4 (2.0)^{a}	7.7 (2.8)^{b}	7.2 (4.1)^{c}	13.4 (2.5)^{d}	13.3 (1.8)^{d}
20:2n-6		0.8 (2.1)^{a}	0.6 (3.8)^{b}	0.5 (3.7)^{c}	1.0 (4.1)^{d}	1.0 (4.0)^{d}
20:4n-6		0.5 (5.9)^{ad}	0.6 (3.1)^{ac}	0.6 (6.2)^{c}	0.6 (4.6)^{ac}	0.5 (4.5)^{d}
22:5n-6		0.1 (18.2)^{a}	0.2 (6.5)^{b}	0.2 (5.6)^{b}	1.5 (5.5)^{c}	0.9 (1.4)^{d}
Total n-6 PUFA[3]		**13.2 (1.6)^{a}**	**9.6 (2.6)^{b}**	**9.1 (3.8)^{b}**	**16.7 (2.6)^{c}**	**16.0 (1.5)^{d}**
18:3n-3		3.8 (4.0)^{a}	2.1 (5.3)^{b}	1.8 (7.7)^{c}	4.6 (3.8)^{d}	4.5 (2.1)^{d}
18:4n-3		0.8 (3.4)^{a}	1.6 (3.1)^{b}	1.6 (2.3)^{b}	0.5 (5.0)^{c}	0.5 (7.0)^{c}
20:4n-3		0.8 (6.7)^{a}	1.5 (2.2)^{b}	1.3 (1.0)^{c}	0.6 (3.2)^{d}	0.6 (4.8)^{d}
20:5n-3		4.9 (6.7)^{a}	5.9 (3.3)^{b}	8.1 (3.1)^{c}	2.6 (6.6)^{d}	2.9 (6.7)^{d}
22:5n-3		2.1 (4.4)^{a}	2.8 (1.8)^{b}	3.3 (1.9)^{c}	1.3 (4.5)^{d}	1.4 (6.5)^{d}
22:6n-3		7.6 (8.5)^{a}	11.7 (2.7)^{b}	10.4 (3.1)^{c}	8.9 (6.0)^{d}	7.4 (3.8)^{a}
Total n-3 PUFA[4]		**20.4 (6.0)^{a}**	**25.7 (1.9)^{b}**	**26.7 (2.4)^{b}**	**18.9 (4.4)^{c}**	**17.7 (3.4)^{c}**
Total PUFA[5]		**34.8 (3.8)^{ab}**	**36.2 (1.4)^{ac}**	**37.6 (2.5)^{c}**	**36.2 (3.4)^{ac}**	**34.4 (2.4)^{b}**
n-3:n-6		**1.5 (5.7)^{a}**	**2.7 (0.1)^{b}**	**2.9 (2.8)^{c}**	**1.1 (0.0)^{d}**	**1.1 (0.0)^{d}**

[1]includes 15:0, 22:0, 24:0
[2]includes 16:1n-9, 20:1n-11, 20:1n-7
[3]includes 18:3n-6, 20:3n-6, 22:4n-6
[4]includes 20:3n-3
[5]includes 16:2, 16:3, 16:4

Table 5. Concentrations of PCDD/Fs, DL-PCBs (pg.g^{-1} ww, upperbound WHO-TEQ$_{2005}$) and PBDEs (ng.g^{-1} ww) in fillet flesh of initial and experimental fed Atlantic salmon. Σmean values (%RSD) bearing identical superscript lettering within same row are not statistically different (P>0.05). ΣWHO-TEQ$_{1998}$ are presented for comparative purposes.

	Initial	Experimental			
		NFO	SFO	11%AM	5.5%AM
n	2	3	3	3	3
PCDD					
2378-TCDD	0.010 (0)	0.017 (19)	0.015 (26)	0.010 (0)	0.010 (0)
12378-PeCDD	0.010 (0)	0.032 (27)	0.029 (36)	0.011 (16)	0.012 (29)
123478-HxCDD	0.0015 (44)	0.0010 (0)	0.0010 (0)	0.0010 (0)	0.0010 (0)
123678-HxCDD	0.001 (0)	0.0013 (28)	0.001 (0)	0.0011 (16)	0.0013 (4)
123789-HxCDD	0.0010 (0)	0.0010 (0)	0.001 (0)	0.001 (0)	0.001 (0)
1234678-HpCDD	0.0005 (24)	0.0001 (17)	0.0001 (23)	0.0002 (54)	0.0001 (17)
OCDD	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
ΣPCDD	**0.024 (3)^{a}**	**0.052 (22)^{b}**	**0.047 (28)^{b}**	**0.024 (8)^{a}**	**0.025 (13)^{a}**
PCDF					
2378-TCDF	0.0015 (15)	0.049 (29)	0.025 (44)	0.015 (3)	0.018 (3)
12378-PeCDF	0.0011 (63)	0.0017 (14)	0.0009 (11)	0.0004 (44)	0.0006 (14)
23478-PeCDF	0.026 (29)	0.054 (10)	0.034 (21)	0.012 (22)	0.016 (12)
123478-HxCDF	0.001 (0)	0.001 (0)	0.001 (0)	0.001 (0)	0.001 (0)
123678-HxCDF	0.0012 (24)	0.0010 (0)	0.0010 (0)	0.0010 (0)	0.0011 (20)
234678-HxCDF	0.0010	0.0011 (16)	0.0011 (9)	0.0010 (0)	0.0010 (11)
123789-HxCDF	0.001 (0)	0.001 (0)	0.001 (0)	0.001 (0)	0.0012 (29)
1234678-HpCDF	0.0001	0.0002 (33)	0.0003 (13)	0.0007 (68)	0.0001 (9)
1234789-HpCDF	0.0002 (42)	0.0001 (0)	0.0001 (0)	0.0001 (0)	0.0001 (0)
OCDF	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
ΣPCDF	**0.033 (25)^{a}**	**0.109 (8)^{b}**	**0.064 (28)^{c}**	**0.032 (7)^{a}**	**0.039 (7)^{ac}**
DL-PCBs					
Non-ortho PCBs					
PCB 77	0.0007 (17)	0.0020 (4)	0.0017 (9)	0.0022 (76)	0.0008 (10)
PCB 81	<0.0001	0.0002 (12)	0.0003 (24)	0.0003 (46)	<0.0001
PCB 126	0.080 (37)	0.576 (9)	0.379 (5)	0.169 (7)	0.181 (9)
PCB 169	0.003 (25)	0.023 (3)	0.013 (27)	0.005 (31)	0.006 (18)
Σnon-ortho PCBs	**0.084 (36)^{a}**	**0.601 (8)^{b}**	**0.394 (5)^{c}**	**0.177 (8)^{d}**	**0.188 (9)^{d}**
Mono-ortho PCBs					
PCB 105	0.0034 (6)	0.0101(4)	0.0065 (11)	0.0035 (32)	0.0027 (13)
PCB 114	0.0002 (25)	0.0005 (3)	0.0003 (11)	0.0002 (47)	0.0001 (17)
PCB 118	0.0086 (10)	0.025 (3)	0.017 (7)	0.009 (28)	0.007 (12)
PCB 123	0.0004 (15)	0.0003 (14)	0.0002 (40)	0.0002 (76)	<0.0001
PCB 156	0.0011 (6)	0.0022 (9)	0.0015 (13)	0.0008 (8)	0.0007 (10)
PCB 157	0.0003 (7)	0.0009 (13)	0.0005 (8)	0.0003 (1)	0.0003 (20)
PCB 167	0.0005 (12)	0.0013 (10)	0.0008 (8)	0.0004 (9)	0.0004 (11)
PCB 189	0.0001 (2)	0.0004 (4)	0.0002 (9)	<0.0001	<0.0001
Σmono-ortho PCBs	**0.015 (8)^{a}**	**0.041 (4)^{b}**	**0.027 (8)^{c}**	**0.014 (28)^{a}**	**0.011 (11)^{a}**
ΣPCDD/Fs$_{2005}$	**0.057 (16)^{a}**	**0.161(8)^{b}**	**0.111 (13)^{c}**	**0.056 (4)^{ad}**	**0.064 (8)^{d}**
ΣDL-PCBs$_{2005}$	**0.099 (32)^{a}**	**0.642 (8)^{b}**	**0.421 (5)^{c}**	**0.191 (10)^{a}**	**0.199 (9)^{a}**
ΣPCDD/Fs+DL-PCBs$_{2005}$^{1}	**0.156 (14)^{a}**	**0.803(8)^{b}**	**0.532 (4)^{c}**	**0.247 (8)^{d}**	**0.263 (9)^{d}**
ΣPCDD/Fs+DL-PCBs$_{1998}$^{2}	**0.227 (9)^{a}**	**0.963 (7)^{b}**	**0.638 (4)^{c}**	**0.301 (9)^{d}**	**0.312 (9)^{d}**
Polybrominated diphenyl ethers					
BDE28	0.016 (14)	0.036 (5)	0.023 (5)	0.009 (11)	0.007 (8)
BDE47	0.250 (8)	0.610 (6)	0.365 (7)	0.141 (3)	0.144 (6)
BDE49	0.059 (10)	0.173 (5)	0.100 (9)	0.034 (5)	0.033 (3)
BDE66	0.010 (7)	0.030 (5)	0.015 (12)	0.007 (0)	0.006 (0)
BDE99	0.041 (14)	0.113 (5)	0.057 (11)	0.029 (7)	0.027 (4)
BDE100	0.047 (8)	0.176 (4)	0.090 (7)	0.027 (8)	0.028 (7)
BDE 153	0.008 (28)	0.017 (7)	0.009 (6)	0.006 (0)	0.007 (0)

BDE 154	0.025 (14)	0.078 (2)	0.043 (7)	0.015 (4)	0.015 (4)
BDE 183	nd	nd	nd	nd	nd
Σ9 PBDEs	**0.456 (10)[a]**	**1.233 (5)[b]**	**0.702 (8)[c]**	**0.268 (3)[d]**	**0.267 (4)[d]**

655 nd - not detected
656 [1]TEF_{2005}, Van den Berg et al. (2006)
657 [2]TEF_{1998}, Van den Berg et al. (1998)
658