

**The assessment of omega 3 oil
sources for use in aquaculture –
alternatives to the unsustainable
harvest of wild fish stocks**

By
Matthew R. Miller B Sc. (Hons)
University of Tasmania

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the requirements of the degree of
Doctor of Philosophy
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DECLARATION

This thesis contains no material which has been accepted for a degree or diploma by any tertiary institution. To the best of my knowledge the thesis does not contain any material written or published by another person, except where due reference is made.

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ABSTRACT

Worldwide harvest of wild marine fisheries for fish oil cannot increase. However, the demand for fish oil is increasing due to a rapidly expanding aquaculture industry and is further increased by nutraceutical/biomedical and agricultural companies. Aquaculture uses fish oil as a source for essential fatty acids in particular omega-3 long chain-polyunsaturated fatty acids (ω 3 LC-PUFA) and for energy. Other novel sources of renewable, environmentally sustainable oil that provide these nutritional requirements for Atlantic salmon (*Salmo salar* L.) are needed. This research looked at alternate sources of oil containing the ω 3 LC-PUFA that are associated with the many health benefits of eating Atlantic salmon. This thesis also contributed to the development of three techniques for use in aquaculture lipid nutrition research: 1) advanced chromatography and mass spectroscopy to examine intact molecular membrane lipids; 2) nuclear magnetic resonance (^{13}C NMR) to assess the regiospecific distribution of ω 3 LC-PUFA in oil, and 3) molecular RT-PCR to investigate endogenous ω 3 LC-PUFA production.

Two ways of supplying the nutritional requirement for ω 3 LC-PUFA in aquafeeds for Atlantic salmon were studied in a series of feeding trials. A biosynthetic precursor of ω 3 LC-PUFA from Patterson's curse (*Echium plantagineum* L.) was fed in two trials to Atlantic salmon parr and to smolt. It was shown that feeding oil rich in the biosynthetic precursor, stearidonic acid (18:4 ω 3 SDA), maintained concentrations of ω 3 LC-PUFA in the flesh of salmon parr comparable to fish fed a traditional fish oil diet. In smolt, it was demonstrated that dietary SDA elevated the expression of the genes encoding the enzymes responsible for the desaturation and elongation steps involved in the ω 3 LC-PUFA biosynthetic pathway. However, with increased expression and bypassing the Δ^6 desaturation step through the provision of SDA, the smolt stage, unlike parr, did not

maintain concentrations of ω 3 LC-PUFA. The high concentrations of ω 3 LC-PUFA found in traditional fish oil fed adult salmon will likely not be provided by diets rich in SDA.

Single cell organisms such as microalgae, including thraustochytrids, diatoms and other micro-organisms de novo synthesis ω 3 LC-PUFA and are the original sources in the marine food web. Thraustochytrids are heterotrophic protists, commonly found in the marine environment and produce high levels of ω 3 LC-PUFA rich oils. Thraustochytrid oil was fed to Atlantic salmon parr to investigate the effect of feed containing high concentrations of ω 3 LC-PUFA, in particular docosahexaenoic acid (22:6 ω 3, DHA), on performance and how this important fatty acid is incorporated into cell membranes and stored in the fish. The thraustochytrid oil in the diet significantly increased the amount of DHA in Atlantic salmon muscle and therefore is a candidate for use in oil blends for salmon diets. Thraustochytrid oil also significantly increased the ability of salmon parr to undergo smoltification. Regiospecificity analyses of intact lipids can indicate how diet, in particular high dietary DHA, can affect the membrane structure of muscle tissues. However, in the gill and liver, adaptive changes due to smoltification were the major factors that contributed to differences in membrane structure. The incorporation of high concentrations of dietary DHA into the membrane structure and storage molecules is achieved by adaptation of molecular species. Regiospecific analysis of the storage lipid demonstrated that increased dietary DHA increased its bioavailability to the consumer.

Other factors involved in oil replacement were examined. These included the effect and accumulation of minor components, such as phytosterols in vegetable oil and the effect rising ocean temperature has on the membrane structure and lipid storage in salmon. Phytosterols have a beneficial effect in humans by reducing low density lipoprotein (LDL) cholesterol. The digestibility of natural abundances of phytosterols by

Atlantic salmon was poor compared to cholesterol. However, significantly increased concentrations of the phytosterols were observed in both the liver and white muscle of Atlantic salmon fed vegetable oils which ultimately may provide health benefits to the consumer. Salmon adapt their membrane structures due to an elevated water temperature of 19°C. This temperature now often occurs in Tasmanian waters in summer and autumn and is approaching the upper limit for Atlantic salmon to maintain health and performance. Adaptation of structural and storage lipids at elevated temperatures was shown by a reduction in PUFA, especially eicosapentaenoic acid (EPA 20:5 ω 3), and an increase of saturated fatty acids in the gill and white muscle. Salmon altered their membrane structure to compensate for elevated water temperature, which could affect dietary FA requirements.

TABLE OF CONTENT

DECLARATION	II
AURTHORITY OF ACCESS	II
ABSTRACT	III
TABLE OF CONTENT	VI
LIST OF TABLES	X
TABLE OF FIGURES	XII
TABLE OF FIGURES	XII
ACKNOWLEDGEMENTS	XIII
CO-AUTHOURSHIP	XV
LIST OF ABBREVIATIONS	XVII
CHAPTER 1	1
General Introduction	2
1.1 Introduction.....	2
1.2 Factors driving replacement oil research	2
1.2.1 Sustainability of wild fish stocks	2
1.2.2 Sustainable Atlantic salmon farming.....	3
1.2.3 Lipid content and nutrition of aquafeeds	4
1.2.4 Omega 3/omega 6 PUFA.....	6
1.2.5 Biosynthetic pathway of omega 3 and omega 6 PUFA	8
1.3 Challenges and benefits of replacement oils.....	10
1.3.1 Advantages/disadvantages with vegetable/plant based oils.....	10
1.3.2 Phytosterols.....	12
1.3.3 Regiospecificity of fatty acids	13
1.4 Environmental influences on lipids	14
1.4.1 Temperature	14
1.5 Potential sources of ω 3 LC-PUFA for aquaculture	15
1.5.1 Biosynthetic precursors of ω 3 LC-PUFA.....	15
1.5.2 Single cell oils (SCO).....	16
1.5.3 Genetic modification (GM) of oils	17
1.6 The aims and hypothesis of thesis	18
1.7 References.....	20
CHAPTER 2	26
Replacement of dietary fish oil for Atlantic salmon parr (<i>Salmo salar</i> L.) with a stearidonic acid containing oil has no effect on omega 3 long chain polyunsaturated fatty acid concentrations.	26
2.1 Abstract.....	27
2.2 Introduction.....	28
2.3 Materials and methods	30
2.3.1 Experimental diets	30
2.3.2 Growth experiment	31
2.3.3 Lipid extraction and isolation	34
2.3.4 Chemical analysis	36
2.3.5 Statistical analysis.....	37
2.4 Results.....	37
2.4.1 Growth results.....	37
2.4.2 Lipid class composition	37
2.5.3 Fatty acid (FA) composition	38

2.5 Discussion	43
2.6 Conclusion	53
2.7 Acknowledgements.....	53
2.8 References.....	54
CHAPTER 3	58
Effect of stearidonic acid enriched diet on growth, fatty acid composition and elongase and desaturase gene expression in seawater Atlantic salmon (<i>Salmo salar</i> L.)	58
3.1 Abstract	59
3.2 Introduction.....	60
3.3 Materials and methods	63
3.3.1 Experimental diets	63
3.3.2 Growth experiment	65
3.3.3 Digestibility	67
3.3.4 Lipid extraction and isolation	67
3.3.5 Chemical analysis	69
3.3.6 RNA isolation and preparation	69
3.3.7 Reverse transcription	69
3.3.8 Quantitative PCR	70
3.3.9 Relative expression	71
3.3.10 Statistical analysis.....	71
3.4 Results.....	72
3.4.1 Growth results.....	72
3.4.2 Lipid class composition	72
3.4.3 Fatty acid (FA) composition	73
3.4.4 Omega 3 biosynthetic pathway.....	75
3.4.5 Omega 6 biosynthetic pathway.....	78
3.4.6 Digestibility	79
3.4.7 Gene expression	79
3.5 Discussion.....	81
3.5.1 Fatty acid profiles	82
3.5.2 Gene expression	84
3.6 Conclusion	89
3.7 Acknowledgements.....	89
3.8 References.....	90
CHAPTER 4	94
Replacement of fish oil with thraustochytrid <i>Schizochytrium sp. L</i> oil in Atlantic salmon parr (<i>Salmo salar</i> L) diets	94
4.1 Abstract	95
4.2 Introduction.....	96
4.3 Materials and Methods.....	99
4.3.1 Experimental diets	99
4.3.2 Growth experiment	101
4.3.3 Lipid extraction and isolation	103
4.3.4 Chemical analysis	105
4.3.5 Statistical analysis.....	105
4.4 Results.....	106
4.4.1 Growth results.....	106
4.4.2 Lipid class composition	107
4.4.3 Fatty acid (FA) composition.....	107

4.4.4 Digestibility	110
4.4.5 Saltwater challenge	112
4.5 Discussion	112
4.5.1 Digestion	116
4.5.2 Smoltification	117
4.5.3 ω 3/ ω 6 ratio	119
4.6 Conclusion	122
4.7 Acknowledgements	123
4.8 References	123
CHAPTER 5	127
The digestibility and accumulation of dietary phytosterols in Atlantic salmon (<i>Salmo salar</i> L) smolt fed diets with replacement plant oils.	127
5.1 Abstract	128
5.2 Introduction	129
5.3 Materials and methods	131
5.3.1 Experimental diets	131
5.3.2 Growth experiment	131
5.3.3 Sterol extraction and isolation	135
5.3.4 Statistical analysis	136
5.4 Results	137
5.4.1 Growth results	137
5.4.2 Sterol (ST) composition	137
5.4.3 Digestibility of phytosterols	139
5.5 Discussion	140
5.5.1 Digestibility of phytosterols in Atlantic salmon	141
5.5.2 Accumulation of phytosterols in Atlantic salmon	142
5.5.3 Environmental effects of phytosterols	145
5.6 Conclusions	146
5.7 Acknowledgements	146
5.8 References	147
CHAPTER 6	150
Regiospecificity profiles of storage and membrane lipids from the gill and muscle tissue of Atlantic salmon (<i>Salmo salar</i> L) grown at elevated temperature	150
6.1 Abstract	151
6.2 Introduction	152
6.3 Material and methods	154
6.3.1 Experimental system	154
6.3.2 Sampling	155
6.3.3 Lipid extraction, fractionation and fatty acid analysis	156
6.3.4 Membrane lipid analysis – ESI-RP-LCMS	158
6.3.5 Storage lipid analysis - ^{13}C NMR spectroscopy	158
6.3.6 Statistical analysis	159
6.4 Results	159
6.4.1 Lipid class	159
6.4.2 Fatty acid profiles of total lipid	160
6.4.3 15°C Fish	162
6.4.4 Lipid class composition of polar lipid	162
6.4.5 Fatty acid profiles of polar lipid	164
6.4.6 Regiospecific analysis of the membrane lipids	166
6.4.7 Regiospecific analysis of storage lipid - ^{13}C NMR	168

6.5 Discussion	170
6.5.1 Membrane Lipids	171
6.5.2 Storage lipids	174
6.5.3 Temperature effects	176
6.6 Conclusion	180
6.7 Acknowledgements.....	180
6.8 References.....	181
CHAPTER 7	185
The effect on the cell membrane structure and lipid storage of Atlantic salmon (<i>Salmo salar</i> L.) fed high levels of docosahexaenoic acid.	185
7.1 Abstract.....	186
7.2 Introduction.....	187
7.3 Materials and methods.....	190
7.3.1 Experimental diets	190
7.3.2 Growth experiment	192
7.3.3 Lipid extraction and isolation	193
7.3.4 Membrane lipid analysis – ESI-RP-LCMS	193
7.3.5 Storage lipid analysis - ¹³ C NMR spectroscopy	194
7.3.6 Principal components analysis (PCA)	195
7.3.7 Statistical analysis.....	195
7.4 Results.....	196
7.4.1 Growth experiment and fatty acid profiles of total lipid extracts	196
7.4.2 Fatty acid profiles of polar lipid extracts	196
7.4.3 Regiospecific analysis of the polar lipids extracts.....	198
7.4.4 Principal components analysis (PCA)	200
7.5.5 Regiospecificity of storage lipids	205
7.5 Discussion.....	207
7.5.1 Membrane Lipids	207
7.5.2 PCA.....	210
7.5.3 Storage lipids	212
7.6 Conclusions.....	214
7.7 Acknowledgements.....	214
7.8 References.....	215
CHAPTER 8	218
GENERAL DISCUSSION	218
8.1 General discussion	219
8.1.1 Capacity for the endogenous production of ω3 LC-PUFA.....	219
8.1.2 Advanced biochemistry and molecular biology	220
8.1.3 Replacement options for ω3 LC-PUFA oils and their commercial viability.....	221
8.1.4 Broader issues of sustainable intensive aquaculture.....	224
8.1.5 Future security of ω3 LC-PUFA oils and sustainable aquaculture.....	225
8.2 References.....	226

LIST OF TABLES

Table 1.1: Fatty acid profiles (g/100g) of possible plant/vegetable replacement oils for fish oil	11
Table 2.1: Ingredient and lipid composition (g/kg dry matter) of experimental diets.....	32
Table 2.2: Growth and efficiencies of Atlantic salmon fed experimental diets with canola oil (CO), 14% stearidonic acid oil (SO), 1:1 CO:SO (MX) and fish oil (FO) (mean \pm SE).	38
Table 2.3: Lipid content and FA and lipid class composition (g 100g ⁻¹) of red muscle samples of Atlantic salmon fed canola Oil (CO), 1:1 mix of canola oil:stearidonic oil (MX), stearidonic oil (SO) and fish oil (FO) diets	39
Table 2.4: Lipid content and FA and lipid class composition (mg g ⁻¹) of white muscle samples of Atlantic salmon fed canola Oil (CO), 1:1 mix of canola oil:stearidonic oil (MX), stearidonic oil (SO) and fish oil (FO) diets	41
Table 2.5: Mass balance of absolute amount T _f (g) and total eaten T _e (g) plus total initial T _i (g) of ω 3 LC-PUFA in Atlantic salmon fed experimental feeds with canola oil (CO), 14% stearidonic acid oil (SO), 1:1 CO:SO (MX) and fish oil (FO).	42
Table 3.1: Ingredient and lipid composition (g/kg dry matter) of experimental diets.....	64
Table 3.2: Characteristics of the real-time PCR primers	70
Table 3.3: Growth and efficiencies of Atlantic salmon fed experimental diets with canola oil (CO), 14% stearidonic acid oil (SO) and fish oil (FO) (mean \pm SEM).	73
Table 3.4: Fatty acid content and lipid class composition of the whole carcass of Atlantic salmon smolt fed canola oil (CO), stearidonic acid oil (SO) diets and fish oil (FO) (mg g ⁻¹ total fatty acids)	74
Table 3.5: Fatty acid content and lipid class composition of the white muscle samples of Atlantic salmon smolt fed canola oil (CO), stearidonic acid oil (SO) diets and fish oil (FO) (mg g ⁻¹ total fatty acids)	76
Table 3.6: Fatty acid content and lipid class composition of the liver of Atlantic salmon smolt fed canola oil (CO), stearidonic acid oil (SO) diets and fish oil (FO) (mg g ⁻¹ total fatty acids)	77
Table 3.7: Apparent digestibility coefficient (ADC) for the crude protein (N), energy (kJ), and fatty acids for diets containing different oils	78
Table 4.1: Ingredient and lipid composition (g/kg dry matter) of experimental diets....	100
Table 4.2: Growth and efficiencies of Atlantic salmon fed experimental diets with palm oil (PO), thraustochyrid oil (TO), 4:1 PO:TO (MX) and fish oil (FO) (mean \pm SE).	106
Table 4.3: Lipid content and FA and lipid class composition (as percent of total) of red muscle samples of Atlantic salmon fed palm oil (PO), 4:1 mix of palm oil: thraustochyrid oil (MX), thraustochyrid oil (TO) and fish oil (FO) diets	108
Table 4.4: Lipid content and FA and lipid class composition (as percent of total) of white muscle samples of Atlantic salmon fed palm oil (PO), 4:1 mix of palm oil: thraustochyrid oil (MX), thraustochyrid oil (TO) and fish oil (FO) diets	110
Table 4.5: Apparent digestibility coefficients (ADC) for different fatty acid fractions for diets containing different oil sources	111
Table 5.1: Ingredient, lipid and sterol composition (g/kg dry matter) of experimental diets.....	133
Table 5.2: Sterol content of white muscle and livers of Atlantic salmon smolt fed canola oil (CO), Echium oil (SO) and fish oil (FO).	137

Table 5.3: Sterol composition (mg/100g) of white muscle of Atlantic salmon smolt fed canola oil (CO), Echium oil (SO) and fish oil (FO) diets.....	138
Table 5.4: Sterol composition (mg/100g) of liver of Atlantic salmon smolt fed canola oil (CO), Echium oil (SO) and fish oil (FO) diets	139
Table 5.5: Apparent digestibility coefficients (ADC) (%) for the different sterols in canola oil (CO), Echium oil (SO) and fish oil (FO) diets fed to Atlantic salmon ..	140
Table 6.1: FA and lipid class composition and content of the total lipid fraction of a commercial formulated diet and white and red muscle and gill tissue of Atlantic salmon <i>Salmo salar</i> fed that diet when held at 19oC	161
Table 6.2: FA and lipid class composition and content of the total lipid fraction of white and red muscle and gill tissue of Atlantic salmon <i>Salmo salar</i> fed commercial formulated diet when held at 15°C	163
Table 6.3: FA and lipid class percentage composition comparison and content of the polar lipid fraction of white and red muscle and gill tissue of Atlantic salmon <i>Salmo salar</i> fed commercial formulated diet when held at 19°C	165
Table 6.4a: Composition of the polar lipid fraction of white and red muscle and gill tissue of Atlantic salmon <i>Salmo salar</i> fed commercial formulated diet when held at 19°C	167
Table 6.4b: Composition of the polar lipid fraction of white and red muscle and gill tissue of Atlantic salmon <i>Salmo salar</i> fed commercial formulated diet when held at 19°C	168
Table 6.5: Percentage of fatty acid in the <i>sn</i> -2 position in the triacylglycerol fraction from Atlantic salmon <i>Salmo salar</i> tissues when held at 19°C and fed a commercial formulated diet determined by ¹³ C NMR spectroscopy.....	169
Table 7.1: Ingredient and lipid composition (g/kg dry matter) of experimental diets....	191
Table 7.2: FA content and lipid class composition of the polar lipid extract of white muscle samples of Atlantic salmon fed: palm oil (PO), 1:4 mix of thraustochyrid oil: palm oil (MX), thraustochyrid oil (TO) and fish oil (FO) diets. Initial fish (Int) were fed a FO diet.....	197
Table 7.3: Percentage composition of molecular species of phospholipids as determined by ESI RP LC-MS of the white muscle of Atlantic salmon	199
Table 7.4: Frequencies and statistical significance for one way ANOVA for principal component scores.....	205
Table 7.5: FA percentage composition and phosphatidylinositol molecular species of the polar lipid extract of gill samples of Atlantic salmon fed: palm oil (PO) 1:4 mix of thraustochyrid oil: palm oil (MX), thraustochyrid oil (TO) and fish oil (FO) diets. Initial fish (Int) were fed a FO diet.	208

TABLE OF FIGURES

Figure 1.1: Representation of the ω 3 and ω 6 LC-PUFA biosynthetic pathway from their C_{18} FA precursors in Atlantic salmon.	9
Figure 3.1: Differential gene expression in livers of Atlantic salmon fed diets rich in stearidonic rich oil (SO); canola oil (CO) and fish oil (FO).	80
Figure 3.2: Differential gene expression in white muscle of Atlantic salmon fed diets rich in stearidonic rich oil (SO); canola oil (CO) and fish oil (FO).	81
Figure 4.1: Blood osmolarity (mOsm) of Atlantic salmon on last day of trial (day 63) and 14 days later (day 77) after the salinity was raised from 0 to 32 ppm over four diets.	118
Figure 6.1: Abundances of SFA, MUFA, total ω 3 PUFA and total PUFA from the total lipid extract (TLE) for the diet, red muscle, white muscle and gills from Atlantic salmon <i>Salmo salar</i> grown at 15 and 19°C.	171
Figure 7.1: Principal component analysis of the fatty acid composition of the total solvent extract of Atlantic salmon: a) red muscle b) white muscle c) liver and d) gill - fed 4 experimental diets; palm oil (PO), 4:1 mix of palm oil: thraustochyrid oil (MX), thraustochyrid oil (TO) and fish oil (FO).	201
Figure 7.2: Principal component analysis of the fatty acid composition of the polar lipid extract of Atlantic salmon: a) red muscle b) white muscle c) liver and d) gill - fed 4 experimental diets; palm oil (PO), 4:1 mix of palm oil: thraustochyrid oil (MX), thraustochyrid oil (TO) and fish oil (FO).	202
Figure 7.3: Principal component analysis of the molecular species from liquid chromatography-mass spectrometry of the polar lipid extract of Atlantic salmon: a) red muscle b) white muscle c) liver and d) gill - fed 4 experimental diets; palm oil (PO), 4:1 mix of palm oil: thraustochyrid oil (MX), thraustochyrid oil (TO) and fish oil (FO).	204
Figure 7.4: Percentage of fatty acids in the <i>sn</i> -2 position in the triacylglycerol fraction from Atlantic salmon <i>Salmo salar</i> red muscle fed: palm oil (PO) 1:4 mix of thraustochyrid oil: palm oil (MX), thraustochyrid oil (TO) diets and fish oil (FO).	206

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- Nichols, P.D. and Carter, C.G assisted with the general supervision of all aspects of this thesis. These included experimental design, interpretation of data and proof reading manuscripts (10% of chapters)
- Bridle, A. R. assisted in the gene expression sample preparation and data collection and contributed to the proofing of chapter 3. (10% of chapter)
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Barnes, J. (PhD candidate) with Carter, C.G. designed and performed the elevated temperature Atlantic salmon trial in chapter 6. Other than fish weight data no other data from this trial is used in this thesis.

We the undersigned agree with the above stated “proportion of work undertaken” for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis.

Supervisor:

Professor Chris Carter

Assoc supervisor:

Dr Peter Nichols

Deputy head of school:

Dr. John Purser

LIST OF ABBREVIATIONS

The following abbreviations are used in this thesis:

^{13}C NMR, ^{13}C nuclear magnetic resonance
ARA, arachidonic acid
ADC, apparent digestibility coefficients
ALA, α -linolenic acid
ANOVA, 1-way analysis of variance
BSFTA, N,O-bis(trimethylsilyl)-trifluoroacetamide
 CDCl_3 , deuterated chloroform
CHD, coronary heart disease
CMC, carboxymethyl cellulose
DHA, docosahexaenoic acid
DM, dry matter
DPA, docosapentaenoic acid
EPA, eicosapentaenoic acid
ESI-RP-LCMS, electrospray ionization reversed-phase liquid chromatography-mass spectrometry
ETA, eicosatetraenoic acid
FA, fatty acid(s)
FAD5, fatty acid Δ^5 desaturase
FAD6, fatty acid Δ^6 desaturase
FAE, fatty acid elongase
FAME, fatty acid(s) methyl ester
FC, total feed consumption
FER, feed efficiency ratio
FFA, free fatty acids
GLA, γ -linolenic acid
GC, gas chromatography
GC-MS, gas chromatography mass spectroscopy
HPLC, high pressure liquid chromatography
HIS, hepatosomatic index
HNF, hepatic nuclear factors
LA, linolenic acid
LC, long chain ($\geq\text{C}_{20}$)
LDL, low density lipoprotein
LXR, liver X receptor
mRNA, messenger ribonucleic acid
MUFA, monounsaturated fatty acid(s)
NOE, nuclear overhauser effect
NRQ, normalised relative quantities
OA, oleic acid
PCB, polychlorinated biphenyls
PC, phosphatidylcholine
PCA, principal components analysis
PE, phosphatidylethanolamine
PG, phosphatidylglycerol
PI, phosphatidylinositol

PKS, polyketide synthases
PL, polar lipid
PLFA, polar lipid fatty acid
PPAR, peroxisome proliferators-activated receptors
PS, phosphatidylserine
PUFA, polyunsaturated fatty acid(s)
RT-PCR, real-time quantitative polymerase chain reaction
RXR, retinoid X receptor
SCO, single cell oils
SDA, stearidonic acid
S.E. standard error
SFA, saturated fatty acid(s)
SGP, salmon genome project
SGR, specific growth rate
SREP-1c, sterol regulatory element protein-1c
ST, sterol(s)
TAG, triacylglycerol
TLC-FID, thin layer chromatography-flame ionisation detection
TLE, total lipid extract
tr, trace amounts
UPL undetermined polar lipid
UFA, unsaturated fatty acid(s)
 ω 3, omega 3
 ω 3 LC-PUFA, omega 3 long chain ($\geq C_{20}$)-polyunsaturated fatty acid(s)
 ω 6, omega 6
WW, wet weight