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The influence of dietary n-3 fatty acids on brain phospholipid composition in Atlantic salmon (*Salmo salar*)

Jie Shen
Aquaculture

Contents

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Abbreviations

AA	Arachidonic acid (20:4n-6)
ALA	alpha-linolenic acid (18:3n-3)
BW	body weight
CC	Commercial control
DHA	Docosahexaenoic acid (22:6n-3)
DW	Dry weight
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid (20:5n-3)
FAME	Fatty acid methyl esters
GC	Gas chromatography
GWE	Gutted weight equivalent
LC-PUFA	Long-chain polyunsaturated fatty acids
MUFA	Monounsaturated fatty acids
n-3	Omega-3
n-6	Omega-6
NC	Negative control
PC	Phosphatidylcholine
PCA	Principal component analysis
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
PL	Phospholipids
PS	Phosphatidylserine
PUFA	Polyunsaturated fatty acids
S.E.M.	Standard error of mean
TLC	Thin-layer chromatography

Abstract

A feeding experiment was conducted to examine the percentages of different fatty acids in brain phospholipid subclasses under feeding dietary inclusion levels of EPA, DHA or a 1:1 mixture of EPA and DHA in farmed Atlantic salmon. The aim was to investigate how EPA alone, DHA alone or a mix of different doses influence the fatty acids in phospholipid subclasses, and how EPA and DHA deficiency influence the brain phospholipids. Fourteen dietary groups of Atlantic salmon, were fed diets with increasing inclusion levels of EPA, DHA or a 1:1 mixture of EPA and DHA, defined as 0, 0.5, 1, 1.5 or 2% of the feed dry weight, and a commercial diet. The 0 (negative control; NC), 2% and commercial dietary (commercial control; CC) groups were in triplicate, while the dietary groups containing 0.5, 1 and 1.5% were in duplicate tanks. This experiment started at the average fish body weight of 40g, and the brain was taken from 5 fish from each tank in each dietary group when the average fish body weight reached 200g (after 131 d) and 400g (after 186d). These samples were treated by lipid extraction, double TLC and GC to separate the different phospholipid subclasses and determine the fatty acid composition of each subclass.

In salmon brain, PC had higher 16:0 and 18:1n-9 and the lowest percentage of Σ n-3 fatty acids, PI was characterized by higher 18:0 and 20:4n-6, and PS and PE contained the highest percentages of 22:6n-3. Through PCA, only NC group is separated from the rest of the dietary groups, while there were no clear groupings of the other dietary groups. Furthermore, four phospholipid classes showed increased 20:5n-3 and 22:6n-3 incorporation and concomitantly decreased levels of Σ n-6 and Σ n-9 fatty acids, with increasing dietary levels of EPA, DHA or a mix of EPA and DHA. For the most of fatty acids, there was only a significant difference in the NC group. When the fish was fed a diet without EPA and DHA, the brain remained containing quite high amounts of EPA and DHA, and DHA was more retained than EPA.

In conclusion, phospholipids were characterized by a high degree of percentages of n-3 PUFA (20:5 and 22:6) and low percentages of n-6 PUFA, however, each phospholipid subclass had different characteristic fatty acids. The brain phospholipid fatty acid composition was conserved to a high degree and little affected by the diet.

Key words: Atlantic salmon brain, diet influence, EPA, DHA, PC, PS, PI, PE.

1. Introduction

The growing rate of global population is 1.4% per year, which means that by 2050 there will be approximately 9.1 billion people in the world (FAO, 2009). In other words, assuming consumption per capita stays constant, this implies a 40% increase in demand for animal protein. The United Nations, however, estimates actual demand to double (UN, 2012). Regarding that resources for increased land based protein production will be scarce, a key problem is how protein production in sea can be developed. Over the past few decades, aquaculture has been a major contributor to the increased requirements for animal protein production. The World Bank developed a scenario analysis in their report "Fish to 2030" predicting that aquaculture will continue to fill with the gap of supply demand for animal protein, and that by 2030, 62% of fish used for human consumption will be from this industry.

Fish is regarded as man's most important single source for high quality protein, providing 16% animal protein for human consumption, according to FAO of the United Nations (1997). Particularly, it is a vital protein source in regions where livestock is relatively scarce—fish supplies <10% of animal protein consumed in North America and Europe, but 17% in Africa, 26% in Asia and 22% in China (FAO, 2000). About one billion global population depend on fish as the primary animal protein source (FAO, 2000).

Although salmon can come from both wild and farmed sources, almost all commercially available Atlantic salmon is farmed. Compared to most other seafood categories, total global supply of salmonids is negligible (4.2%) (Marine Harvest, 2015). According to the statistics (Kontali Analyse), wild salmonids is varying between 0.7 and 1 million tonnes GWE (gutted weight equivalent), while farming salmonid are continuous growing. Since 1999, farmed salmonids dominated the total global supply. In 2014, the total supply of Atlantic salmon was 2.0 million tonnes GWE.

However, the continued growth of Atlantic salmon farming with a range of challenges should be focused. It includes environmental issues, economic sustainability and social sustainability. Salmon aquaculture carries with several aspects of potential direct threats to ecosystems at different levels. Three aspects mainly affect local ecosystems: 1) disease and parasites; 2) pollution issues (chemicals, antibiotics, nutrients released into the water through

feed and fish feces and 3) genetic and other threats to wild salmon and other local species populations. Related to social sustainability, the most basic question is whether the industry provide jobs. However, the question is not just how many jobs but also what kinds of jobs and the implications these have for the greater community. The welfare of the farmed fish should also be paid sustained attention (Bailey, 2014).

Salmon is a nutritious fish, which contains abundant amounts of vitamins, including vitamin D and the B vitamins B6, B12, niacin and riboflavin. Salmon is one of the richest sources of selenium, an element that helps detoxify mercury and has antioxidant properties (Alaska Seafood Marketing Institute, 2009). Best of all, salmon is rich in long chain omega-3 fatty acids, like EPA and DHA.

Most Atlantic salmon are anadromous, meaning that they undergo their greatest feeding and growing in the sea water; however, adults return to spawn in the native freshwater streams where the eggs hatch and juveniles grow through several distinct stages. Fig.1.1 shows the different stages in Atlantic salmon life cycle.

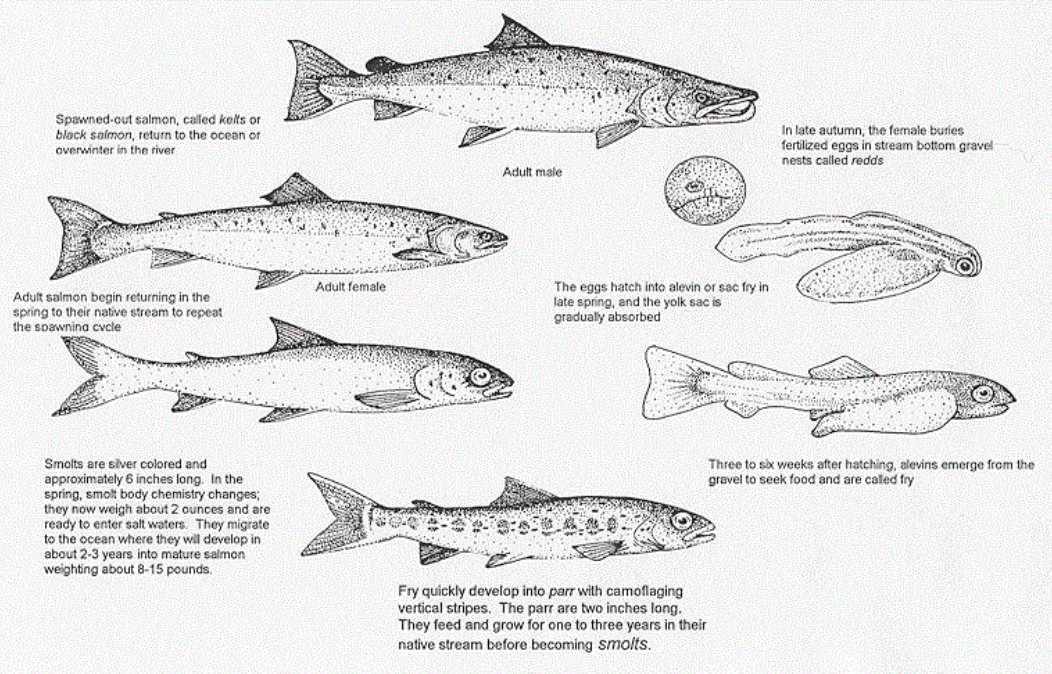

Figure 1.1 Atlantic salmon life cycle (Adapted from U.S. Fish & Wildlife Service)

2. Literature review

2.1 Salmon farming in Norway

Commercial aquaculture started around 1970s in Norway, since that time aquaculture played a major role in most coastal areas of Norway. Intensive farming of Atlantic salmon (*Salmo salar*) takes place in large nets in sheltered waters such as fjords or bays, which accounted for over 80% of the total Norwegian aquaculture production (FAO, 2016). Today, Norway has become the largest producer of farmed salmon in the world, and even aquaculture industry turns into fourth biggest export commodity (behind oil, gas and metals; Statistic Norway) (Liu et al., 2011). According to the statistical data (Marine Harvest, 2015) (Fig. 2.1), it revealed that wild catch of Atlantic salmon varied between 700 and 1000 thousand tonnes GWE, while the production of farmed salmon increased. In 2014, the total amount of farmed salmon is almost 2, 200 thousand tonnes GWE.

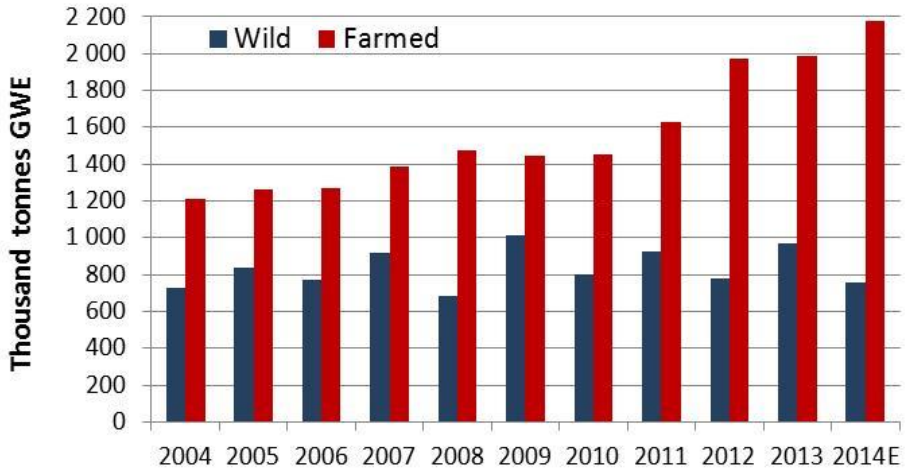

Figure 2.1 Production of wild and farmed Atlantic Salmon in Norway, 2004-2014 (Marine Harvest, 2015)

2.2 Salmon feed ingredients and alternatives

The two most important feed ingredients for farmed salmon have traditionally been fishmeal and fish oil, which contain high level of omega-3 essential fatty acids (Turchini et al., 2009). The function of fish oil involves animal health, including improved immunity against

disease, higher survival and growth, and reduced incidences of deformities (FAO, 1986). According to the record (SeaFish, 2011), 81% of the produced fish oil was utilized for the manufacture of aquafeeds (Shepherd, 2011) in 1 million tonnes of the globally production in 2009. And 317,000 tonnes of fish meal and 183,000 tonnes of fish oil, which amounted to 6% and 22%, respectively, went into feeds for Norwegian salmon industry in 2012 (Ytrestøyl et al., 2015). These numbers indicate that fishmeal and fish oil are the key resource for salmon aquaculture. In 1990, 90% of the ingredients in Norwegian salmon feed were of marine origin, whereas in 2013 only around 30% (Fig. 2.2). The origin of the marine ingredients in the salmon feed changes according to price and availability.

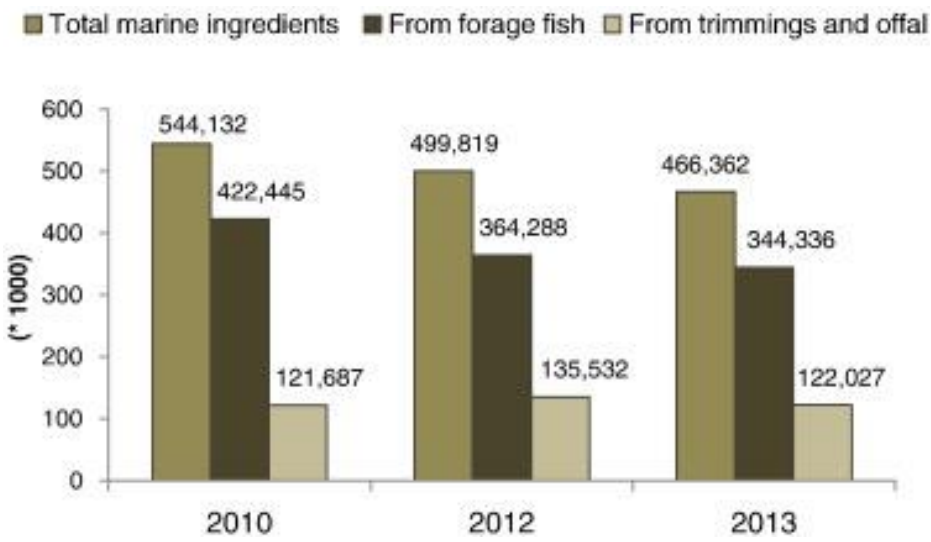

Figure 2.2 Use of marine ingredients in Norwegian salmon feed, 2010-2013 (in tonnes) (Ytrestøyl et al., 2015)

However, supplies of fishmeal and fish oil will be insufficient sooner or later (Hardy, 2010), due to a strong global demand that fishmeal and fish oil cannot keep pace with demand (Tacon et al., 2006). According to FAO (2012), global production of fish oil decreased 1.50 million tonnes to 1.07 million tonnes from 1994 to 2009. Moreover, environmental conditions mainly affected the fishmeal production throughout the years, such as El Niño years. It can be seen that finite fishmeal and fish oil cannot satisfy the rapidly growing aquaculture industry because of strong limits on availability of fishmeal and fish oil. Thus, it is significant to find alternative feed resources, which are cheap, sustainable and have all the essential nutrients and qualities of fishmeal and fish oil while reducing undesirable side effects such as decreased growth, worse

animal health and changes to the nutritional content of the end product (Schipp, 2008).

An effective solution is applied at the moment, based on substitution by less limited, alternative plant based resources. In other words, salmon are becoming vegetarian in a way. In case of fish oil, several vegetable of a low price and a high availability can be utilized as replacers, such as soybean, sunflower, palm, linseed and rapeseed oil (Naylor et al., 2009). Generally, reducing the marine ingredients in fish diets and increasing the plant ingredients leads to a lower level of long-chain n-3 PUFA (LC-PUFA) in the flesh of farmed fish and decreased nutritional value to human consumers (Tocher, 2003; Turchini et al., 2011). It is therefore a big challenge to replace fishmeal and fish oil completely. However, several experimental studies have showed that replacement of fish oil with vegetable oils for farmed salmon had good fish growth (Ruyter et al., 2000; Los & Murata, 1998; Torstensen et al., 2005). But, the diets which had less than 10% fishmeal gave reduced fish growth (Bendiksen et al., 2011). In addition, most studies said that using vegetable oils replacing fish oil does not affect feed intake significantly (Turchini et al., 2009). And some products (like SBM) are successful in replacing fishmeal in salmon diets, but to a certain extent (Refstie et al., 2000; Refstie et al., 2001). When high inclusion rates are applied, health problems can be encountered, related to the antinutritional factors present in some plant protein products (Francis et al., 2001). At present, the substitution of fishmeal and fish oil in Norwegian farmed salmon feed ingredients is feasible (Ytrestøyl et al., 2011). From the diagram of Norwegian Marine Harvest 2015 report (Fig. 2.3), vegetable meal accounted for almost half of the salmon feed diets. 15% and 21% of fishmeal and fish oil, respectively, was utilized in farmed salmon feed in 2014.

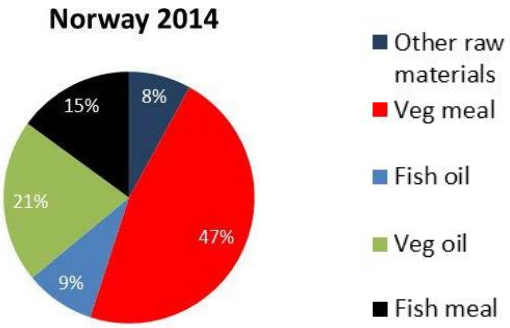

Figure 2.3 Percent of different salmon feed ingredients in Norway, 2014 (Marine Harvest, 2015)

2.3 Lipids, fatty acids and phospholipids

Lipids are the generic names assigned to a group of fat soluble compounds in the tissues of plants and animals. They are broadly classified as triacylglycerols, wax esters, phosphoglycerides, sphingolipids and sterols (Sargent et al., 2002). Fatty acids are an important component in most lipids. Fatty acids are designated on the basis of their chain lengths, their degree of unsaturation (number of double bonds), and the position of their double bonds (Sargent et al., 2002). The term "phospholipids" is usually taken to mean phosphoglycerides, which consists of two hydrophobic fatty acid "tails" and a hydrophilic "head", joined together by a glycerol molecule. These are the main constituent lipids of cellular membranes allowing the membrane surfaces to be hydrophobic or hydrophylic depending on the orientation of the lipid compounds into the intra or extracellular spaces (Halver, 1980). In salmon, the site having high level of lipid is the flesh (myosepta) and visceral adipose tissue, and the liver plays an important role in processing of triacylglycerols and phosphoglycerides, including modification of fatty acid chain lengths and the degree of unsaturation (Sargent et al., 1993).

Generally, fatty acids include saturated, monounsaturated and polyunsaturated fatty acids. Saturated fatty acids lack double bonds, monounsaturated fatty acids have only one double bonds, whereas PUFA contain two double bonds at least, having carbon chain lengths≥C18 and with three or more double bonds, are called long chain polyunsaturated fatty acids (LC-PUFA). PUFA, which is defined by the first double bond position relative to the methyl terminus of the fatty acids chain, can be mainly divided into n-3 fatty acids, n-6 fatty acids and n-9 fatty acids according to numbers, the position of first double bonds (Menon & Dhopeshwarkar, 1982).

The way in which a fatty acid is named is determined by the location of the first double bond, counted from the methyl end, that is, the omega (ω-) or the n- end. Eicosapentaenoic (EPA; 20:5 n-3) and docosahexaenoic (DHA; 22:6 n-3) acids are both important types of n-3 fatty acids and they feature prominently in fish lipid nutrition (Tur et al., 2012). In fish, EPA and DHA are key components of cell membranes. Especially, the retina and brain of fish have abundant DHA characteristically (Tocher & Harvie, 1988). Phospholipids are a class of lipids that are a major component of all cell membranes and have important roles to play in both fish and human nutrition (Sargent et al., 1999).

2.4 Dietary lipids, EFAs and phospholipids

As well as being major energy source, dietary lipids are also important in fish nutrition as carriers of lipid-soluble vitamins and minerals, and as the source of essential fatty acids (EFAs), which are required for the synthesis of new cellular lipid for growth, health, reproduction and body function (Sargent et al., 2002). Dietary EFAs are involved in maintaining cell membrane structure and function, and as precursors of eicosanoids (March, 1992). All vertebrate species have absolute dietary requirements for certain PUFA. However, their dietary requirements and precise nature of EFA vary with species. Besides, the quantitative requirement for EFA in dietary lipid level and the different stage of species' development maybe also change considerably (Sargent et al., 1995). For example, mammals generally need more n-6 than n-3 fatty acids for the requirement of PUFA (Bjerve et al., 1987; Yamada et al., 1981), whereas some fish species are reverse (Sargent et al., 1987). The requirement of n-3 LC-PUFA appeared to decrease in Gilthead sea bream (*Sparus Aurata*) from larval and early juvenile stage to older juvenile and pre-adult stage (Rodriguez et al., 1994; Ibeas et al., 1994). If a dietary deficiency occurs, the various deficiency symptoms, like stopping growing and reproducing, developing various pathologies and increasing mortality, will happen in fish (Glencross, 2009).

It is difficult to determine the accurate amount of EFA requirements for a given fish species (Bezard et al., 1994), because it involves the absolute requirements of each series of PUFA, the optimal balance between the two series (n-3 and n-6), the stage of fish development, and the *in vivo* fatty acid metabolism. In salmonids such as rainbow trout (*Oncorhyncus mykiss*), coho salmon (*Oncorhyncus keta*), chum salmon (*Oncorhyncus kisutch*), and Arctic charr (*Salvelinus alpinus*), the dietary requirements of EFAs have been examined by many researchers. It has been demonstrated that all these species require n-3 PUFA to satisfy normal growth and development, whereas n-6 PUFA has a big difference between these species (Table 2.1). The requirement for n-6 fatty acids appeared to be inaccurate in some salmonids, but all vertebrate species possible require a small amount of n-6 PUFA in order to form eicosanoids (Henderson & Tocher, 1987).

Table 2.1 EFA requirements of some freshwater and marine fish (Sargent et al., 1995)

Species	EFA	% of diet
Freshwater		
Rainbow trout (*Oncorhyncus mykiss*)	18:3n-3	0.7-1.0
	n-3 HUFA	0.4-0.5
Carp (*Cyprinus carpio*)	18:2n-6 & 18:3n-3	1.0 of each
Grass carp (*Ctenopharyngodon idella*)	18:2n-6 & 18:3n-3	1.0 & 0.5 respectively
Chum salmon (*Oncorhyncus keta*)	18:2n-6 & 18:3n-3	1.0 of each
Coho salmon (*Oncorhyncus kisutch*)	18:2n-6 & 18:3n-3	1.0 of each
Cherry salmon (*Oncorhyncus masou*)	18:3n-3 or n-3 HUFA	1.0
Tilapia (*T. zilli*)	18:2n-6	1.0
(*T. nilotica*)	18:2n-6	0.5
Eel (*Anguilla japonica*)	18:2n-6 & 18:3n-3	0.5 of each
Ayu (*Plecoglossus altivelis*)	18:3n-3 or 20:5n-3	1.0
Milkfish (*Chanos chanos*)	18:2n-6 & 18:3n-3	0.5 of each
Channel catfish (*Ictalurus punctatus*)	18:3n-3	1.0-2.0
	n-3 HUFA	0.5-0.75
Whitefish (*Coregonus laveratus*)	n-3 HUFA	1.0
Marine		
Turbot (*Scophthamus maximus*)	n-3 HUFA	0.8
Red sea bream (*Chrysoprys major*)	20:5n-3 or n-3 HUFA	0.5
	20:5n-3	1.0
	22:6n-3	0.5
Gilthead sea bream (*Sparus aurata*)	n-3 HUFA	0.9
Striped jack (*Pseudocaranx dentex*)	22:6n-3	1.7

For most marine fish, they have a very low activity of Δ5-desaturase, and therefore they need a dietary input of carbon-20 and carbon-22 PUFAs (Takeuchi et al., 1990). In contrast, freshwater fish species have the capacity to bio-convert alpha-linoleic (ALA; 18:3 n-3) into n-3 PUFA to satisfy the requirements of EFA (Sargent, 1997) (Fig. 2.4). Atlantic salmon is an anadromous fish, which begins life in freshwater and then spends most of their life in the sea and returns to freshwater to spawn. According to literature (Ruyter, 2000), Atlantic salmon need 18:3 n-3 (α-linolenic acid) and 18:2 n-6 (linoleic acid) as essential fatty acids and certain amounts of EPA and DHA.

Dietary phospholipids play an important role in the absorption of lipid in fish, as shown by increased the efficiency of transport of dietary lipids and fatty acids from the gut to the rest of the body probably through enhanced lipoprotein synthesis (Tocher et al., 2008). In addition, dietary phospholipids can improve culture performance of majority of fish species (Coutteau et al., 1997). For instance, some evidences (Kanazawa et al., 1981; Kanazawa et al., 1983) have been markedly increased growth performance in both larvae and juvenile, improved survival rates and stress resistance, as well as reduced the incidence of spinal malformation in case of

dietary intact phospholipids in freshwater and marine species, including Atlantic salmon.

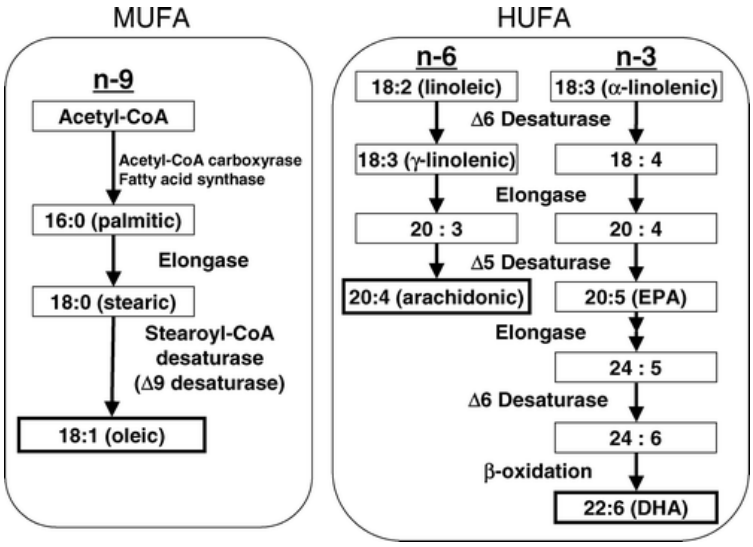

Figure 2.4 A simplified scheme showing the biosynthesis of MUFA and PUFA, indicating pathways of elongation and desaturation (Nakamura & Nara, 2004)

2.5 Structure and function of the fish brain

Compared to the familiar shape and form of a mammal brain, fish brains are rather odd looking and may vary greatly between species. It is elongated, and has distinct sections. In most fishes, the brain is much smaller than a mammal's brain in relation to its body and in some cases may occupy only about 6% of the brain cavity in an elasmobranch (Kruska, 1988). The remaining space is filled by the adipose tissue (Kotrschal et al., 1988). Fish brain can be divided into many typical parts: a stem, a cerebellum, midbrain (diencephalon), hindbrain, a pair optic lobe, and the telencephalon (forebrain). Somatosensory information reaches the brain primarily via specialized cranial nerves, especially trigeminus (V), facialis (VII), vagus (X) and three lateral line nerves, rather than through ascending fiber systems of the spinal cord.

The cerebellum originates from its rostral roof and a pair of optic lobes (tectum opticum) cap the mesencephalon, and have a major function in movements. The telencephalon is composed of paired cerebral hemispheres with olfactory bulbs attached to the rostral hemispheres in most fish, and its function is mainly in processing and integration of sensory and motor information. A research has also indicated that the forebrain may play a part in learning and retaining automatic responses to things that are unpleasant, and even in spatial awareness (Rodriguez et al., 2002). The midbrain sits directly behind, and is connected to the

forebrain. It is main purpose is to process and understand the signals sent from the eyes to the brain. Other functions of the midbrain include learning and controlling muscular reactions. The hindbrain is mostly responsible for controlling swimming, in other words, deciding the direction of fish swimming (Kotrschal et al., 1988). Overall, the fish brain is responsible for coordinating and regulating of body functions according to the stimuli from external or internal.

2.6 Lipids in the fish brain

In the fish brain, there is a high level of fatty acids, including n-3 PUFA and n-6 PUFA, but it has more n-3 series than n-6 series. Especially, the retina and brain of fish have abundant DHA characteristically (Tocher & Harvie, 1988), and thus, DHA- deficient diets result in impaired visual performance (Bell et al., 1995). Glucolipids are present in fish brains, but at a much lower proportion in contrast with the mammalian brain and the concentration of glucolipids varies with the environmental temperature (Lenas et al., 2010). Compared to the mammalian brain, the lipid composition of the fish brain appears to be affected by nutrition (Pagliarani et al., 1986; Tocher et al., 1988). For example, in brain of Atlantic salmon, the relative levels of 18:2 n-6 and 20:4 n-6 increased in all classes of lipid in fish fed the vegetable oil based diets (Amlund, 2012).

Table 2.2 Total lipid content of brain from different fish species (percent of wet weight, mean ± S.D., n=3) (Stoknes et al., 2004)

Fish species	Lipid content (%)	Yield (%)[a]
Cod	4.8±0.1	0.4±0.0
Saithe	6.2±0.1	0.8±0.0
Salmon	7.1/5.3[b]±0.0/0.2[b]	0.3/0.2[b]±0.0
Trout	7.0±0.1	0.2±0.0
Redfish	52.5±1.4	1.6±0.2
Portuguese dogfish	3.8±0.1	0.4±0.1
Black dogfish	3.2±0.0	0.7±0.1
Leafscale gulper shark	4.1±0.1	0.5±0.0

[a] Of total wet head weight.
[b] Lipid content and yield of brain tissue versus brain fluid.

According to the results (Stoknes et al., 2004) (Table 2.2), the brain of Atlantic salmon contained about 7% fat, however, it is maybe too high due to the lipid layer on the top of the brain in redfish. Brain of salmon had a higher percentage of the fatty acids 14:0, 22:1, 18:2 n-

6, 18:3 n-3, and 22:5 n-3 than other lean species. All species in the experiment contained the same amount of EPA (20:5 n-3), except for salmon, which had more EPA than others. In addition, redfish was examined to show a different fatty acid composition in the brain than other species. Particularly, it contained high amounts of the fatty acids 14:0, 16:1, 22:1, and 18:4 n-3, and the lowest amount of DHA (22:6 n-3). For salmon, the fluid surrounded the brain separated the brain tissue, and both the fluid and the brain tissue determined the composition of fatty acids. The results also showed that the brain fluid contained mainly monounsaturated fatty acids, and the fatty acid 18:1 n-9 was dominant, whereas the brain tissue was composed mainly of PUFAs, especially highly amounts of EPA and DHA.

2.7 Dietary fatty acids influence the phospholipid fatty acid composition

It has been assumed that the function of n-3 PUFAs is mainly in modulating the structure and function of biological membranes, including membrane organization, elasticity and ion permeability. Specifically, EPA has been suggested to have a function in neuroprotection, like anti-oxidative activity (Bos et al., 2016). A study (Betancor et al., 2014) investigated interactions between different levels of DHA and lipid and fatty acid compositions in post-smolt Atlantic salmon. Briefly, it showed that total lipid content and fatty acid composition of the liver, brain, head kidney and gill were greatly unaffected by changes in the dietary LC-PUFA, whereas phospholipid fatty acid composition was affected to some extent. The brain slightly reflected diet, with increased dietary LC-PUFA leading to minor changes in PL fatty acids. Although dietary LC-PUFA influenced the PL fatty acids slightly, it seems reasonable to assume that n-3 LC-PUFAs may also play an important role in PL fatty acid composition of brain and brain function in fish.

Although the fatty acids are abundantly present in the fish brain, the information about its influence on the fish brain development and function is lacking. Many articles about mammalian brain are existing. For instance, EPA and DHA are necessary for the development and function of brain and maintenance of normal electrical nervous systems, and even for improving the visual activity (Holub, 1990; Lauritzen et al., 2001). Dietary DHA improves the ability of learning and development of the mammalian brain, and also has a positive effect in a

variety of diseases, such as hypertension, atherosclerosis and certain cancers (Horrocks & Yeo, 1999). DHA has a specific localization in the retina and brain of humans and other mammals, and the long-chain n-3 fatty acids are rapidly incorporated into cell membrane phospholipids where it is regarded they influence the metabolism/ metabolic events within the cells (Reviewed by Sinclair et al., 2000). Both the human and fish brain are structured similarly, and they share a few more structure with similar function as well. Therefore, some similar connection between fatty acids and brain function may be present.

The present study therefore aimed to examine the composition and percentages of the different fatty acids in Atlantic salmon brain lipid, in addition to learn how EPA and DHA deficiency influence the brain phospholipids and how EPA alone, DHA alone or a mix different doses influence the fatty acids of brain by comparative analysis. Furthermore, the study was to determine how different dietary levels of n-3 fatty acids influence the phospholipid fractions of the brain.

3. Materials and methods

3.1 Materials

Table 3.1 Chemicals and equipment

Chemicals and equipment	Producer
Chloroform	VWR International, PA, USA
Methanol	VWR International, PA, USA
Ethanol	VWR International, PA, USA
Benzene	VWR International, PA, USA
Hexane	VWR International, PA, USA
Petroleum ether	Sigma Chemical Co., St Louis. MO, USA
Diethyl ether	Sigma Chemical Co., St Louis. MO, USA
2,2-dimethoxypropane	Sigma Chemical Co., St Louis. MO, USA
Acetic acid	Merck, Darmstadt, Germany
2',7'-dichlorofluorescein	Merck, Darmstadt, Germany
Methanolic HCl	Supelco Inc., Bellefonte, PA, USA
Butylated hydroxytoluen (2,6-Di-t-butyl-p-cresol, BHT)	Sigma Chemical Co., St Louis. MO, USA
TLC silica gel 60g 25 Glass plates	Merck, Darmstadt, Germany
Hewlett packard 6890 gas chromatograph	Avondale, PA, USA

3.2 Methods

3.2.1 Experimental fish and diets

The experiment was carried out at NOFIMA's research station at Sunndalsøra, Norway, with Atlantic salmon reared on a commercial diet to an average body weight (BW) of 40 grams. The fish were distributed into 33 tanks, from the time that the feeding trial was started with 40g salmon in seawater. The water temperature was measured daily and changed between 6.3 and 13.8 °C. The oxygen saturation level in the water was kept at 85% or above.

The experimental fish were raised for a period of 186 days to reach an average BW of 400

grams. All fish tanks were divided into 14 dietary groups, which were fed one of the 14 experimental diets or a control diet with a composition close to a commercial diet. The experimental diets were formulated with increasing inclusion levels of EPA, DHA, or a 1:1 mixture of EPA + DHA, defined as 0, 0.5, 1, 1.5 or 2% of the feed dry weight (DW) (Table 3.2). The 0%, 2% and control dietary groups were in triplicate tanks, while the dietary groups containing 0.5, 1 and 1.5% were in duplicate tanks. As noted, one diet, was completely deficient of EPA and DHA, and is hereafter named negative control (NC). The diet with a composition close to a commercial diet with an EPA and DHA content of 2.2% EPA and DHA is hereafter named positive control (i.e. commercial control, CC).

Total lipid content was kept constant basically, on a level of 23.9 ± 0.65% (mean ± s.d.) of the feed DW. The same basal formulation was used in all the experimental diets and only the lipid coating varied between them. A different formulation was used in the commercial control diet. All diets were produced by Nofima feed technology center (Norway).

Table 3.2 FA composition (g per 100 g feed DW) and total lipid content (%) of the experimental diets

	CC[1]	NC[2]	0.5% EPA	1% EPA	1.5% EPA	2% EPA	0.5% DHA	1% DHA	1.5% DHA	2% DHA	0.5% EPA+DHA	1% EPA+DHA	1.5% EPA+DHA	2% EPA+DHA
14:00	0.7	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
16:00	3.0	3.5	3.4	3.2	3.1	3.0	3.4	3.3	3.2	3.1	3.3	3.2	3.3	3.1
18:00	0.8	0.9	0.9	0.8	0.8	0.8	0.9	0.9	0.9	0.9	0.9	0.8	0.9	0.8
20:00	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
22:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24:00:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.1	0.1
Σ Saturated[3]	4.6	4.7	4.6	4.4	4.2	4.1	4.7	4.5	4.5	4.4	4.5	4.4	4.5	4.3
16:1n–7	0.8	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.6	0.6	0.6	0.6
18:1(n–7)+(n–9)+(n–11)	7.6	9.4	9.1	8.7	8.4	7.9	9.2	8.7	8.4	8.1	8.9	8.7	8.7	8.3
20:1(n–7)+(n–9)+(n–11)	0.7	0.2	0.2	0.3	0.3	0.4	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3
22:1(n–7)+(n–9)+(n–11)	0.5	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.1
24:1n–9	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Σ Monounsaturated[4]	9.7	10.3	10.1	9.7	9.4	8.9	10.1	9.7	9.3	9.0	9.8	9.6	9.8	9.4
18:2n–6	4.6	4.8	4.7	4.5	4.3	4.2	4.7	4.5	4.4	4.2	4.6	4.5	4.5	4.3
18:3n–6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:2n–6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:3n–6	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Σ n–6[5]	4.9	4.9	4.8	4.6	4.5	4.3	4.8	4.6	4.5	4.3	4.7	4.6	4.7	4.4
18:3n–3	1.1	1.0	1.0	0.9	0.9	0.9	1.0	0.9	0.9	0.9	1.0	0.9	0.9	0.9
18:4n–3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	n.d.	n.d.	n.d.	n.d.	n.d.
20:4n–3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:5n–3	1.1	0.0	0.5	1.0	1.5	2.0	0.1	0.2	0.2	0.3	0.3	0.6	0.9	1.2
22:5n–3	0.2	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.2	0.0	0.1	0.1	0.1
22:6n–3	1.1	0.0	0.1	0.3	0.4	0.5	0.5	0.9	1.4	1.9	0.3	0.5	0.9	1.2
Σ n–3[6]	3.6	1.1	1.7	2.4	3.0	3.6	1.7	2.2	2.7	3.5	1.7	2.2	3.0	3.6
EPA + DHA	2.2	0.0	0.6	1.2	1.8	2.5	0.6	1.1	1.6	2.3	0.6	1.1	1.8	2.4
Σ Polyunsaturated[7]	8.7	6.0	6.5	7.0	7.5	8.0	6.5	6.8	7.2	7.8	6.4	6.9	7.6	8.0
Total lipid	25.8	23.4	23.7	23.8	23.7	23.9	23.8	23.6	23.6	23.9	23.3	23.5	24.7	24.4

[1] CC = commercial control; [2] NC = negative control; [3] Includes 15:0, 17:0; [4] Includes 14:1n–5, 17:1n–7; [5] Includes 16:2n–6, 20:4n–6, 22:2n–6; [6] Includes 20:3n–3; [7] Includes 16:2n–6, 16:3n–4, 20:3n–3, 20:4n–6, 22:2n–6.

3.2.2 Sampling

The brain was taken out from 5 fish per tank in each dietary group when the average BW of fish reached 200 grams (after 131 d) and 400 grams (after 186 d), and these samples were immediately frozen in liquid nitrogen and kept frozen at -70 °C until analyzed. Of course, the fish were anaesthetized before being sacrificed.

3.2.3 Lipid extraction

Folch extraction (Folch et al., 1957) is one method for lipid extraction, which is based on lipids' solubility in organics solvent using 1-step solvent extraction with mixture of 0.9% NaCl and chloroform/methanol (2:1) followed by a wash with 0.9% NaCl. Using the method of Folch extraction, total lipids were extracted from brain tissue samples. Each sample consisted on a pool of 5 fish brains from the same tank. These brains were homogenized with dry ice using a blender. The resulting brain homogenate was collected in plastic bags and stored at -40 °C with the bags open to allow the dry ice to evaporate. Once the dry ice was evaporated, the bags were closed and kept at -40 °C until analysis. Approximately 50 mg from each sample was homogenized in 1.8 ml 0.9% NaCl and 15 ml chloroform:methanol (2:1) with anti-oxidant butylated hydroxytoluen (BHT) 0.7mg/l for 60 seconds with a knife homogenizator. The homogenized sample was added 1.8 ml 0.9% NaCl and continue to be homogenized for 5 seconds. The extract was shaken and kept at room temperature for an hour, when the mixture partitions into two layers. The lower phase is composed of chloroform- methanol- water in the proportions 86:14:1 (by volume) and contains virtually all of the lipids, while the upper phase consists of the same solvents in the proportions of 3:48:47 (by volume), respectively, and contains much of the polar water phase. The polar water-soluble methanol phase and protein was carefully removed, and the nonpolar chloroform phase containing the lipid was stored at -40 °C until further separation by TLC.

3.2.4 Separation of lipid groups with TLC

Thin-layer chromatography consists of a stationary phase immobilized on a glass or plastic plate and a solvent. The sample, either liquid or dissolved in a volatile solvent, is deposited as a spot on the stationary phase. One edge of the plate is then placed in a solvent reservoir and the solvent moves up the plate by capillary action. When the solvent front reached the other

edge of the stationary phase, the plate is removed from the solvent reservoir (Fig. 3.1). The separated spots are visualized with ultraviolet light or by placing the plate in iodine vapor. The different components in the mixture move up the plate at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

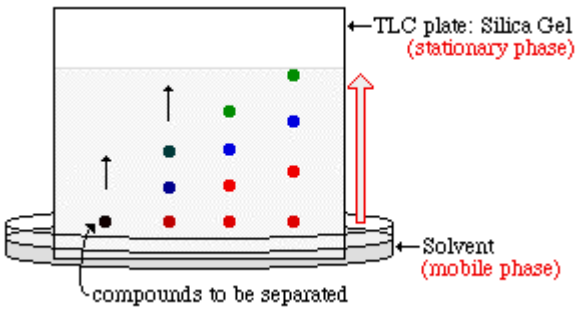

Figure 3.1 Scheme of a Thin Layer Chromatography

The chloroform phase was kept in the hood until they reached room temperature and then evaporated at 60 °C with nitrogen overflow to avoid samples being oxidized. In advance, the top side of the TLC plates was marked, washed with pure methanol, and then dried in the incubator at 120 °C for 20 min and stored in an exsiccator. Next with a pencil, a thin mark is made at the bottom of the plate to apply the sample spots. Then samples solutions which were added 3-5 droplets of pure chloroform, are applied on the spots marked on the line in equal distance. The mobile phase, composed of petroleum ether, diethyl ether and acetic acid in the ratios of 113:20:1 by volume, respectively, was poured into a TLC chamber to a leveled few centimeters above the chamber bottom. A moistened filter paper in the mobile phase was placed on the inner wall of the chamber to maintain equal humidity (and thereby avoiding edge effect as well). Now, the plate prepared with sample spotting was placed in the TLC chamber so that the side of the plate with the sample line was facing the mobile phase. Then the chamber was closed with a lid and the plate was immersed, such that the sample spots were well above the level of mobile phase for development. Allow sufficient time for the migration of the samples. Then remove the plates and allow them to dry. After spraying the plates with a solution of 2% 2',7'-dichlorofluorescein (a fluorogenic dye that measures hydroxyl, peroxyl and other reactive oxygen species activity and a highly fluorescent compound which can be detected by

fluorescence spectroscopy with maximum excitation and emission spectra of 495 nm and 529 nm respectively) in 96% methanol and detection in UV light (366 nm), spots corresponding to phospholipids (due to different affinity to stationary phase) were scraped off. Because the distance traveled by a substance relative to the distance traveled by the solvent front depends upon the molecular structure of that substance, TLC can be used to separate and identify substances. The distance relationship is expressed as an R_f value given in Eq. 3.1.

$$R_f = \frac{\text{distance traveled by substance}}{\text{distance traveled by solvent front}} \qquad \text{(Equation 3.1)}$$

Then, the phospholipid fraction of each sample was dissolved in Arvidson's solution (Arvidson, 1968). Arvidson's solvent is composed of chloroform-methanol-acetic acid-water 50:39:1:10 (by volume). Arvidson is a relatively polar solvent, which makes it possible to solubilize all the phospholipids classes. In addition, add the acetic acid, mainly to suppress some acids or alkaline substances arising spots tailing. The tubes were capped and stored at -40 °C freezer to the next day. The phospholipids samples were centrifuged at 2000 rpm for few seconds firstly, added 0.5 ml 0.9% NaCl and then centrifuged again. Two clear phases were obtained. The upper phase containing dichlorofluorescein but no lipid was sucked off and discarded and the chloroform phase was pipetted and stored at -40 °C until phospholipids analysis.

3.2.5 Separation of phospholipids groups with TLC

Similar to the previous separation, the total phospholipid fraction, of the samples were allowed to reach the room temperature and then evaporated at 60 °C with nitrogen overflow to avoid samples being oxidized. Sample was added 3-5 droplets of pure chloroform and then the whole samples were applied to the TLC plate to form a small concentrated line at a marked position around 2 cm from the bottom of the plate. Use a mixture of chloroform, methanol, acetic acid and water in the ratios of 100:75:6:2 by volume as the mobile phase. A moistened filter paper was placed in TLC chamber in order to maintain equilibrium before the TLC plate with the samples was put into the chamber. Allow sufficient time for the migration of the samples and then remove the plates and allow them to dry. After drying, the lipids were visualized, by spraying the plates and the spots revealed were identified under UV-light by comparison with known standards (Sigma Chemical Co., St Louis. MO, USA). The spots

corresponding to SM, PC, PE, PI and PS were scraped off into glass tubes, added 10 ul chloroform C23:0 (0.6176g/50 ml) as an internal standard and used for further quantification of the fatty acids, then trans-methylated overnight with 2 ml benzene, 2 ml methanolic HCl and 200 ul 2,2-dimethoxypropane at room temperature (Mason & Waller, 1964). After 24 hours, these samples were added 2 ml hexane and 3 ml 6% $NaHCO_3$ and after that two phases were obtained. Carefully transfer the upper phase into a glass tube, which contains the fatty acid methyl esters (FAMEs).

3.2.6 Gas chromatography (GC)

Gas Chromatography is a very sensitive method for the separation and quantification of chemicals, and it is perfect for the analysis of fatty acid components. Like in any other chromatographic technique, separation of compounds depends on their partition between a stationary and a mobile phase. In gas chromatography, the mobile phase is a gas that is moved through the column, while the stationary phase is a liquid film that coats the column filling (in packed columns) or the column wall (in capillary columns). Hence, the correct name for gas chromatography is “Gas Liquid Chromatography”, abbreviated GLC. Compounds are injected into the column and carried through it by the mobile phase; depending on their partition into the stationary phase, they move slower or faster. A sensitive detector is required at the end of the column to detect and quantify the compounds as they leave the column.

Intact triacylglycerols and free fatty acids are not very volatile and therefore difficult to analyze using GC (which require that the lipids be capable of being volatized in the instrument). For this reason, lipids are usually derivatized prior to analysis to increase their volatility. Triacylglycerols are first saponified which breaks them down to glycerol and free fatty acids, and are then methylated.

Firstly, the samples were evaporated at 60 °C with nitrogen overflow to avoid FAMEs being oxidized. The samples were dissolved in hexane with nitrogen as antioxidant for GC running. The volume of hexane is dependent on sample volume/weight. FAMEs were separated and quantified by GLC using a 60 m*0.25 mm ID capillary column (SGE, Pty Ltd. Victoria, Australia). Hydrogen was used as carrier gas and temperature programming was from 50 °C to 170 °C at 4°C/min, next to 200 °C at 0.5 °C/min and then to 300 °C at 10 °C/ min. Individual

methyl esters were identified by comparison with known standards and by reference to published data (Ackman, 1980; Tocher & Harvie, 1988).

3.2.7 Statistical analysis

The statistical analysis was performed by using a multivariate statistical approach (Principal Component Analysis, PCA) through the Unscrambler software (CAMO, Corvallis, OR, USA), Version 10.3. This has enabled to get an overall overview of the differences in the fatty acid composition among the different dietary groups. Then significant differences for fatty acids in each dietary group were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's new multiple range test. Successively, the data were statistically analyzed and calculated of the mean, standard error mean by using Microsoft Excel, Version 2016. In the graphs of this paper, data were presented as mean values ± standard error mean (SEM) (n=2 or 3, whereby 'n' represents the number of tanks).

4. Results

4.1 PCA of the main phospholipids in brain

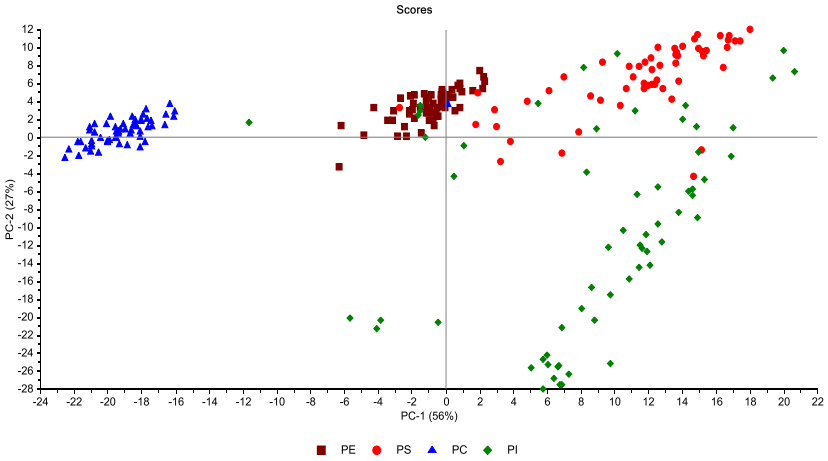

Figure 4.1 PCA of the fatty acid composition of the main phospholipids (including PC, PS, PI and PE) in the brain of Atlantic salmon. PCA: Principal Component Analysis; PC: Phosphatidylcholine; PS: Phosphatidylserine; PI: Phosphatidylinositol; PE: Phosphatidylethanolamine. The blue points represent the component level of PC in the different dietary groups when fish body weight (BW) reached 200g and 400g. Correspondingly, red points are PS, green points are PI and brown points are PE. Each phospholipid subclass contains 66 points, whereby every point represents a pooled sample of 5 fish. PC-1 represents a vector along the longest axis in the multidimensional 'cloud' of data points and usually explains the dominant part of the actual variance. The second component (PC-2) runs perpendicular to PC-1.

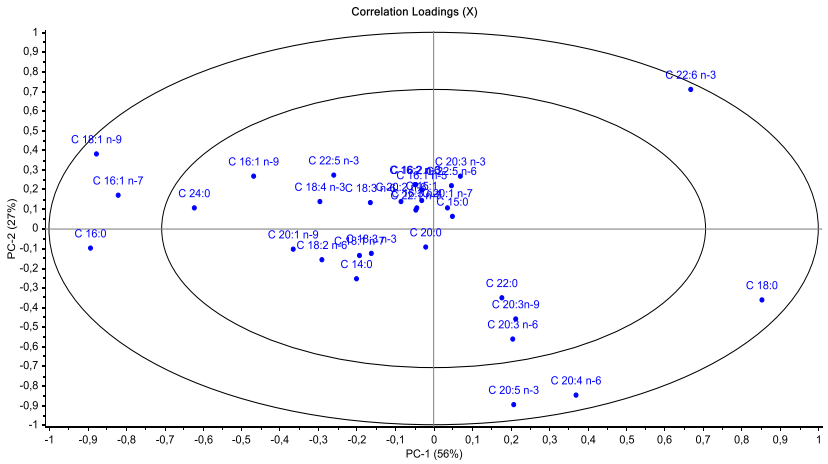

Figure 4.2 Correlation loading plot of the fatty acid composition of the main phospholipids (including PC, PS, PI and PE) in the brain of Atlantic salmon. PC: Phosphatidylcholine; PS: Phosphatidylserine; PI: Phosphatidylinositol; PE: Phosphatidylethanolamine. Those fatty acids in the inner oval have common features and are not significantly different between the different phospholipid classes.

The principal component analysis (PCA) in Unscrambler was done in order to obtain an overview of how the fatty acid compositions of phospholipid subclasses (including PC, PS, PI and PE) in the brain of Atlantic salmon were influenced by different dietary levels of EPA, DHA or a mix of EPA and DHA. All dietary groups were included in the analysis of the brain of fish at body weight (BW) 200g and 400g (Fig. 4.1). Two significant principal components were found through PCA. PC-1 represents a vector along the longest axis in the multidimensional 'cloud' of data points and usually explains the dominant part of the actual variance. The second component (PC-2) runs perpendicular to PC-1. Two main components (PC-1 and PC-2) taken from all the data can reflect 83% information, which was reliable to some extent. Also we can see that the PC, PS, PI, PE were divided into four completely clear groupings in the PCA analyses, meaning that the fatty acid composition of each phospholipid was unique to each group. However, compared to PC, PS and PE in brain, there were larger variation in the fatty acid composition of PI, making the grouping of this phospholipid subclass less clear, probably due to the low lipid level in this fraction used in GC so that it affected the accuracy of data. The PI fraction may also, to a larger extent, be influenced by the fatty acid composition of the diet.

By plotting the scores according to the principal components, it can be found that some fatty acids in the dataset were specifically characteristic and significantly different between each phospholipid subclass (Fig. 4.2).

Fig. 4.3 show the percentage of some key fatty acids in the different phospholipid subclasses. Since the pattern is the same for all dietary groups, we have chosen only to present the extreme groups (NC and CC). The major fatty acids in the PC fraction in the brain was 16:0, 18:1n-9 and 22:6n-3. Correspondingly, 18:0 and 22:6n-3 were the major fatty acids in the PS fraction, and 18:1n-9 and 22:6n-3 were the major fatty acids in the PE fraction. The fatty acid pattern of the PI fraction was less defined. In the PI fraction, the 18:0 was the dominating fatty acid, then followed by more equal percentages of the fatty acids 18:1n-9, 20:4n-6, 20:5n-3 and 22:6n-3.

4.2 PCA of phospholipid subclasses in brain

In the Fig. 4.4, the graphs (**a**-PC and **c**-PS) indicated that only the NC group (lower left square) was separated from the rest of the dietary groups. Those fatty acids located in the outer oval in the figures **b** and **d**, showed the significant difference. The NC group was characterized by different levels of 18:0, 18:1n-9 and 20:3n-9 compared to the other dietary groups. There were no clear groupings of the other dietary groups, but the PCA plot indicated that the EPA and DHA groups were characterized by 22:6n-3 (PC), and for PS the NC group was characterized by 18:2n-6, 20:3n-9 and 22:0 compared to the majority of the other dietary groups in the upper right square that seemed to be characterized by 18:0 and 22:6n-3.

Although it was not so clear in Fig. 4.5, the graphs (**a**-PI and **c**-PE) revealed that the NC group was separated from the majority of the other dietary groups. In the figures **b** and **d**, their correlation loadings showed the significant fatty acids located in the outer oval. The NC group (lower left square) was characterized by different levels of 16:0, 18:1n-9, 18:3n-3 and 18:2n-6 compared to the other dietary groups. There were also no clear groupings of the other dietary groups, but the PCA plot showed that the EPA and DHA groups seemed to be characterized by 18:0, 20:5n-3 and 22:6n-3 (PI), and for PE the NC group (upper left square) was characterized by 20:2n-6, 20:3n-6 and 20:4n-6 compared to the majority of the other dietary groups in the left low square that was characterized by 16:0 and 18:1n-9.

4.3 Fatty acid composition of phospholipid subclasses in brain

4.3.1 Fatty acid composition of PC

The different dietary levels of EPA, DHA or a mix of EPA and DHA affected the percentages of fatty acids in PC (Fig. 4.6, Fig. 4.7 and Fig. 4.8). In general, the dietary treatments had no significant effects on the percentages of 16:0 and 18:0, and little effects on Σ saturated fatty acids. 16:0 had higher proportions in the PC compared to the other fractions (PS, PI and PE). Furthermore, the percentages of Σ n-9 fatty acids (18:1n-9 and 20:3n-9) and Σ n-6 fatty acids (18:2n-6, 20:3n-6 and 20:4n-6) increased with decreasing dietary levels of EPA, DHA or a mix of EPA and DHA. Among these fatty acids, they had low proportions of total fatty acids except 18:1n-9. Also the percentages of 18:1n-9 showed more markedly

increases in PC compared to the other fractions (PS, PI and PE). The percentages of 20:3n-9 were significantly increased at BW 200g, and no significant differences were detected at BW 400g. Significant increases in Σ n-6 fatty acids (18:2n-6, 20:3n-6 and 20:4n-6) were seen in NC group. On the contrary, the percentages of Σ n-3 fatty acids (20:5n-3, 22:5n-3 and 22:6n-3) reduced significantly with decreasing dietary levels of EPA, DHA or a mix of EPA and DHA. The variations of 22:5n-3 were more significant than 20:5n-3. For the sum of PUFA, it occupied a large proportion in all groups. More details about the fatty acid compositions of PC at BW 200g and 400g, see attachments (Fig. 5.1 and Fig. 5.2).

4.3.2 Fatty acid composition of PS

The percentages of fatty acids in PS were influenced by different dietary levels of EPA, DHA or a mix of EPA and DHA (Fig. 4.9, Fig. 4.10 and Fig. 4.11). As can be seen from the figures, there were no significant effects on Σ saturated fatty acids (16:0 and 18:0) by the dietary treatments. The only exception was in NC group at BW 400g, which showed that the percentages of 16:0 and 18:0 exchanged mutually and expressed significantly increased or decreased. Besides, the percentages of Σ n-9 fatty acids (18:1n-9 and 20:3n-9) and Σ n-6 fatty acids (18:2n-6, 20:3n-6 and 20:4n-6) increased with decreasing dietary levels of EPA, DHA or a mix of EPA and DHA, whereby no significant increases were seen in 18:1n-9, and significant in 20:3n-9 seemed to increase at BW 200g. And the significant increases in the percentages of Σ n-6 fatty acids (18:2n-6, 20:3n-6 and 20:4n-6) were seen in NC group, especially in 20:4n-6. In contrast, the percentages of Σ n-3 fatty acids (20:5n-3 and 22:6n-3) reduced with decreasing dietary levels of EPA, DHA or a mix of EPA and DHA, whereby the percentages of 20:5n-3 and 22:6n-3 varied significantly in most of dietary groups. Compared to the other fractions (PC, PI and PE), PS was rich in Σ n-3 fatty acids. More details about the fatty acid compositions of PS at BW 200g and 400g, see attachments (Fig. 5.3 and Fig. 5.4).

4.3.3 Fatty acid composition of PI

The percentages of fatty acids in PI were affected by different dietary levels of EPA, DHA or a mix of EPA and DHA (Fig. 4.12, Fig. 4.13 and Fig. 4.14). Although each fatty acid had larger variations in PI compared to the other fractions (PC, PS and PE), the trends for fatty acids were seen in brain. The dietary treatments had no significant effects on the percentages

of Σ saturated fatty acids (16:0 and 18:0). Furthermore, the percentages of Σ n-9 fatty acids (18:1n-9 and 20:3n-9) and Σ n-6 fatty acids (18:2n-6, 20:3n-6 and 20:4n-6) increased with decreasing dietary levels of EPA, DHA or a mix of EPA and DHA. The percentages of 20:1n-9 did not show significant increase, while the percentages of 20:3n-9 were significantly increased. And the most significantly increases in 20:3n-6 were seen in PI compared to the other fractions (PC, PS and PE). In contrast, the percentages of Σ n-3 fatty acids (20:5n-3 and 22:6n-3) reduced with decreasing dietary levels of EPA, DHA or a mix of EPA and DHA, whereby the significantly decreases in the percentages of 20:5n-3 were observed at BW 200g. More details about the fatty acid compositions of PI at BW 200g and 400g, see attachments (Fig. 5.5 and Fig. 5.6).

4.3.4 Fatty acid composition of PE

The percentages of fatty acids in PE showed some changes by different dietary levels of EPA, DHA or a mix of EPA and DHA (Fig. 4.15, Fig. 4.16 and Fig. 4.17). It can be seen from the figures, there was little effects on Σ saturated (16:0 and 18:0) by the dietary treatments, and the variations of 18:0 were more pronounced than those of 16:0. Besides, the percentages of Σ n-9 (18:1n-9 and 20:3n-9) and Σ n-6 (18:2n-6, 20:3n-6 and 20:4n-6) increased with decreasing dietary levels of EPA, DHA or a mix of EPA and DHA. Among these fatty acids, the significantly increases in the percentages of 20:3n-9 were seen at BW 200g, and the percentages of 20:3n-6 increased significantly at BW 400g. And the most significantly increases in 20:4n-6 were observed in PE compared to the other fractions (PC, PS and PI). On the contrary, the percentages of Σ n-3 (20:5n-3 and 22:6n-3) significantly reduced with decreasing dietary levels of EPA, DHA or a mix of EPA and DHA. The variations of 20:5n-3 were more markedly than 22:6n-3. More details about the fatty acid compositions of PE at BW 200g and 400g, see attachments (Fig. 5.7 and Fig. 5.8).

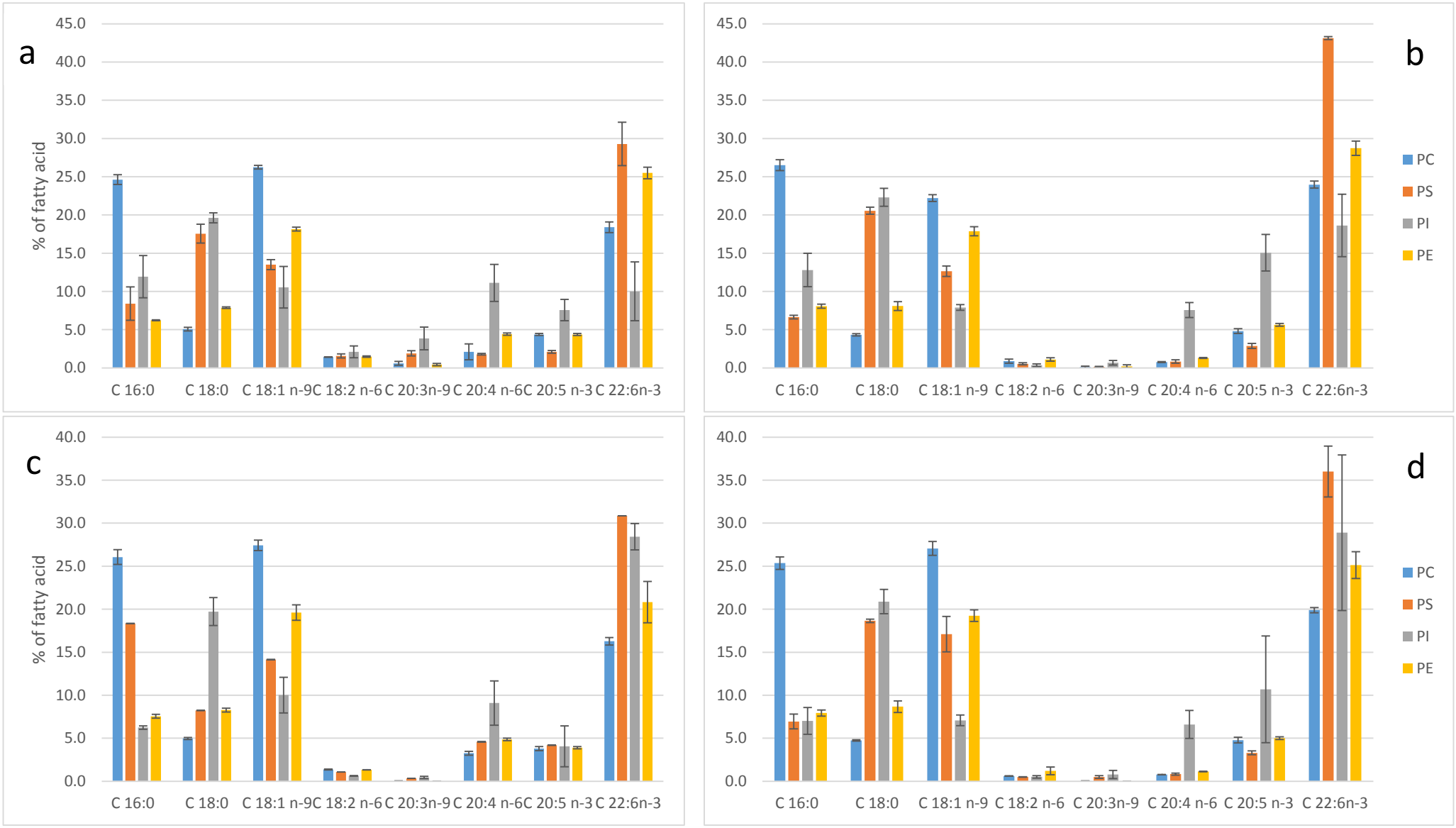

Figure 4.3 Percentage of fatty acids in different phospholipid subclasses at fish body weight (BW) 200g and 400g. PC: Phosphatidylcholine (blue); PS: Phosphatidylserine (orange); PI: Phosphatidylinositol (gray); PE: Phosphatidylethanolamine (yellow). Data are expressed as mean ± standard error mean (SEM) (n=3). Each group includes 3 tanks, whereby every tank represents a pooled sample of 5 fish. The figure **a** is 0% EPA+DHA (NC) group, and **b** is 2.2% EPA+DHA (CC) group at BW 200g. The figure **c** is 0% EPA+DHA (NC) group, and **d** is 2.2% EPA+DHA (CC) group at BW 400g. NC: negative control; CC: commercial control.

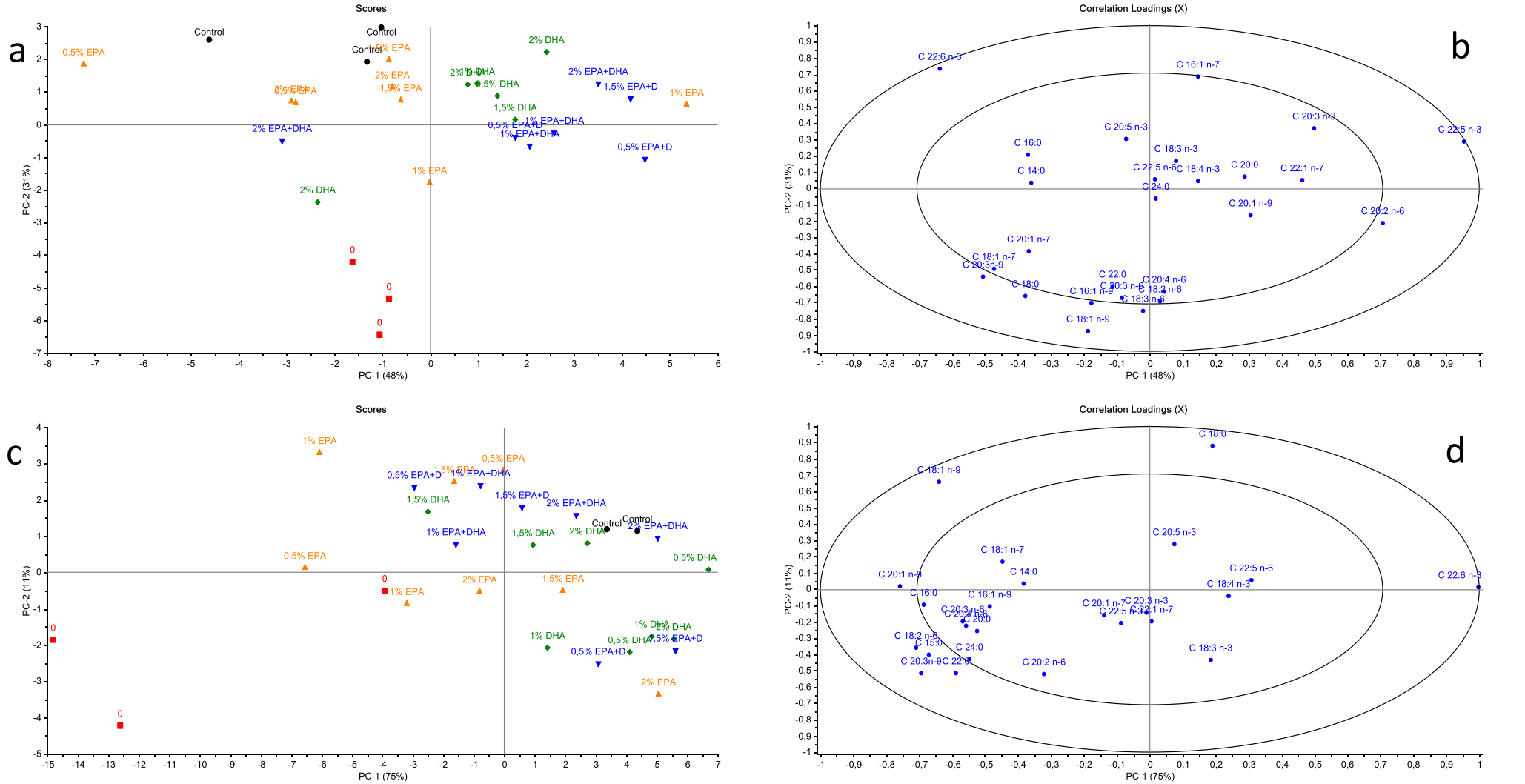

Figure 4.4 PCA of different dietary groups in PC and PS groups at fish body weight (BW) 200g. PCA: Principal Component Analysis; PC: Phosphatidylcholine; PS: Phosphatidylserine. In the **a** and **c** graphs, the red "0" means negative control (NC), and the black "control" represents commercial control (CC). The orange codes represent different EPA groups (0.5%, 1%, 1.5%, 2%), and the green and blue codes represent DHA groups and EPA+DHA groups, respectively. Each dietary group contains 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish. In the **b** and **d** graphs, those fatty acids in the inner oval have common features and are not significantly different between the different dietary groups. Graphs **a** and **b** belong to PC, and graphs **c** and **d** are PS. PC-1 represents a vector along the longest axis in the multidimensional 'cloud' of data points and usually explains the dominant part of the actual variance. The second component (PC-2) runs perpendicular to PC-1.

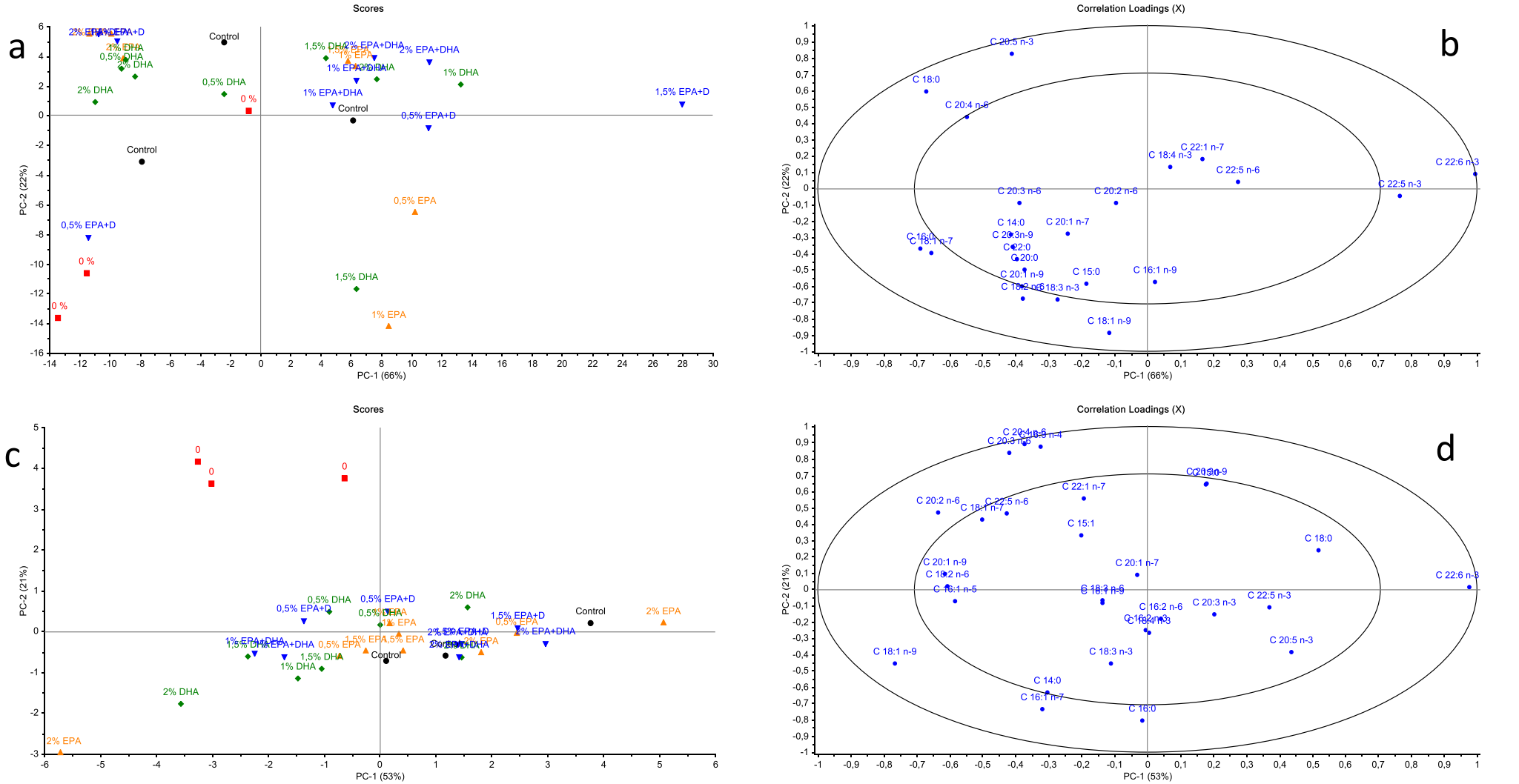

Figure 4.5 PCA of different dietary groups in PI and PE groups at fish body weight (BW) 200g. PCA: Principal Component Analysis; PI: Phosphatidylinositol; PE: Phosphatidylethanolamine. In the **a** and **c** graphs, the red "0" means negative control (NC), and the black "control" represents commercial control (CC). The orange codes represent different EPA groups (0.5%, 1%, 1.5%, 2%), and the green and blue codes represent DHA groups and EPA+DHA groups, respectively. Each dietary group contains 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish. In the **b** and **d** graphs, those fatty acids in the inner oval have common features and are not significantly different between the different dietary groups. Graphs **a** and **b** belong to PI, and graphs **c** and **d** are PE. PC-1 represents a vector along the longest axis in the multidimensional 'cloud' of data points and usually explains the dominant part of the actual variance. The second component (PC-2) runs perpendicular to PC-1.

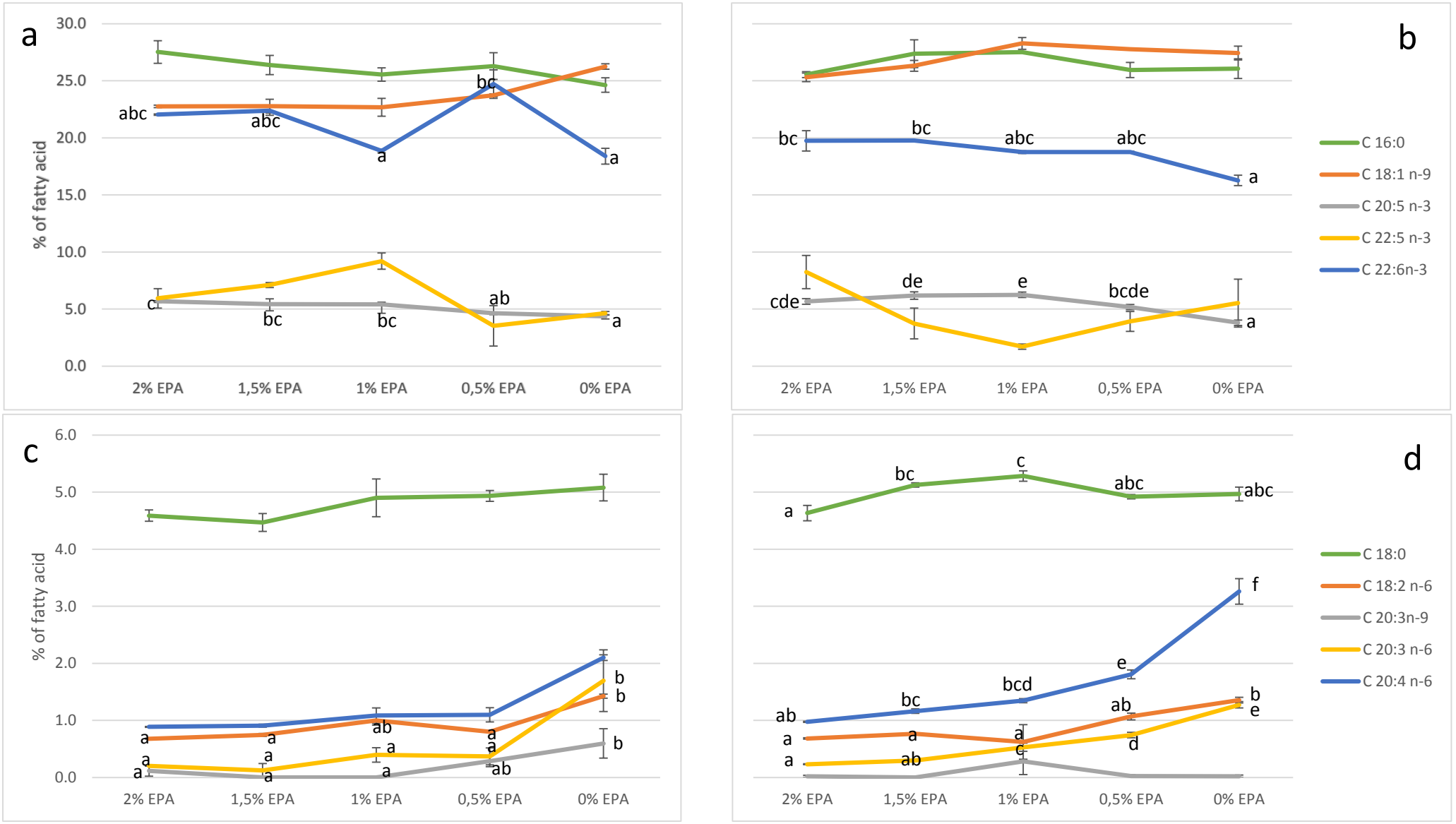

Figure 4.6 Percentage of fatty acids in the PC of brain lipids from fish fed different dietary levels of EPA at fish body weight (BW) 200g and 400g in Atlantic salmon. PC: Phosphatidylcholine; EPA: Eicosapentaenoic acid (20:5 n-3). Data are expressed as mean ± standard error mean (SEM) (n=2 or 3). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish. Different lower-case letters indicate significant differences within the dietary group ($P < 0.05$, ANOVA followed by Duncan's new multiple range test). The figures **a** and **c** are at BW 200g, and **b** and **d** are at BW 400g.

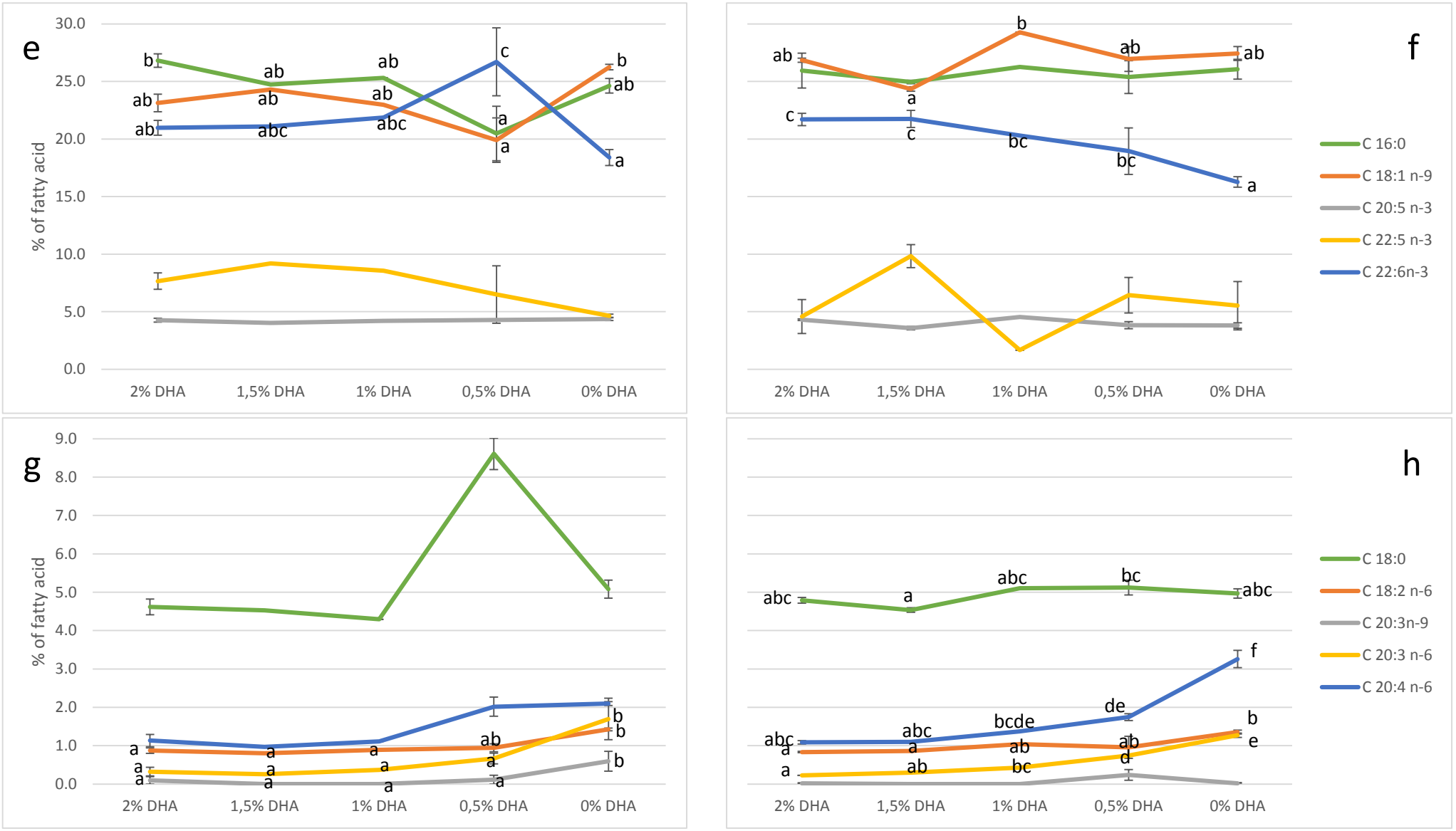

Figure 4.7 Percentage of fatty acids in the PC of brain lipids from fish fed different dietary levels of DHA at fish body weight (BW) 200g and 400g in Atlantic salmon. PC: Phosphatidylcholine; DHA: Docosahexaenoic acid (22:5 n-3). Data are expressed as mean ± standard error mean (SEM) (n=2 or 3). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish. Different lower-case letters indicate significant differences within the dietary group ($P < 0.05$, ANOVA followed by Duncan's new multiple range test). The figures **e** and **g** are at BW 200g, and **f** and **h** are at BW 400g.

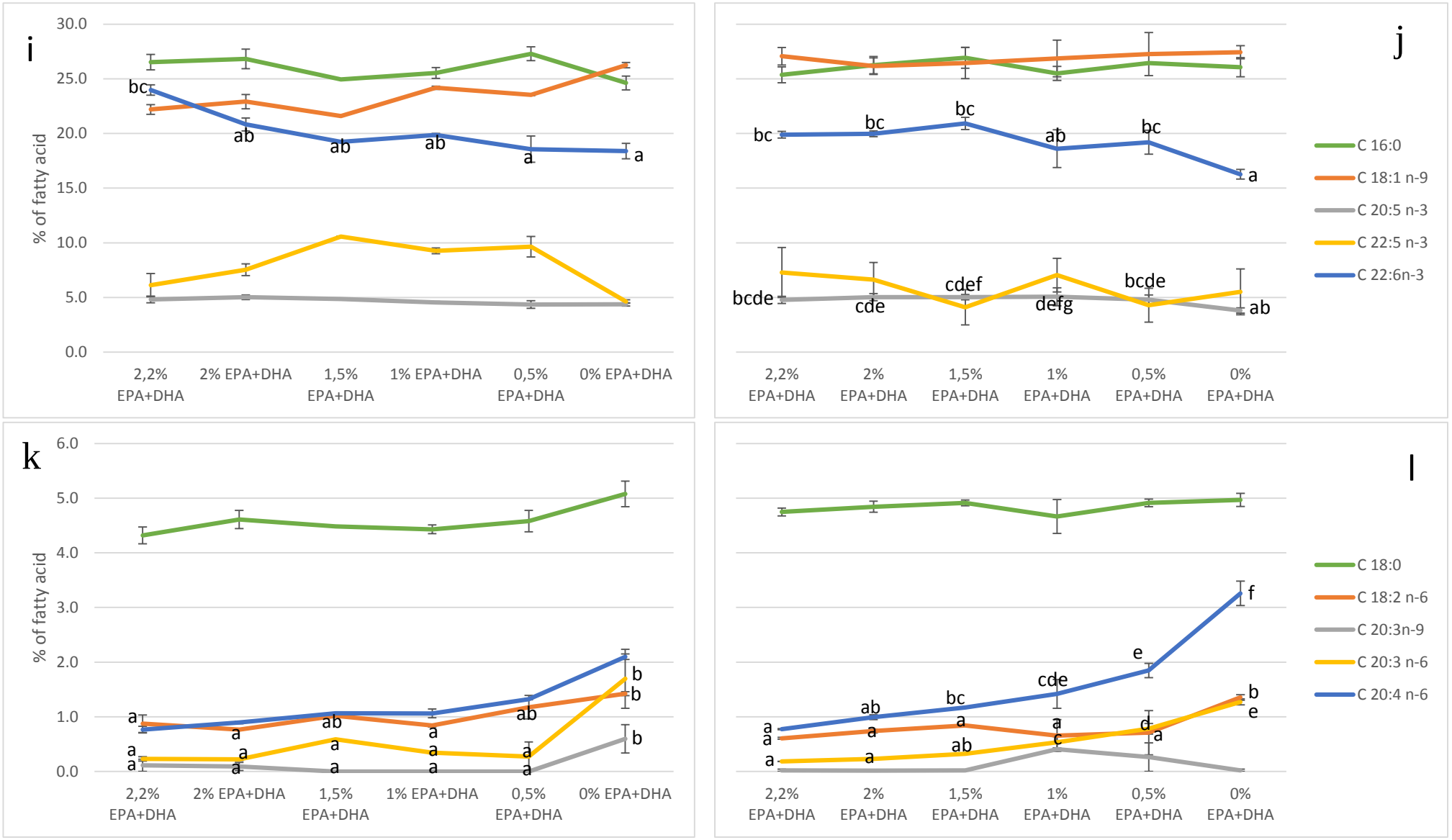

Figure 4.8 Percentage of fatty acids in the PC of brain lipids from fish fed different dietary levels of EPA+DHA at fish body weight (BW) 200g and 400g in Atlantic salmon. PC: Phosphatidylcholine; EPA: Eicosapentaenoic acid (20:5 n-3); DHA: Docosahexaenoic acid (22:5 n-3). Data are expressed as mean ± standard error mean (SEM) (n=2 or 3). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish. Different lower-case letters indicate significant differences within the dietary group ($P < 0.05$, ANOVA followed by Duncan's new multiple range test). The figures **i** and **k** are at BW 200g, and **j** and **l** are at BW 400g.

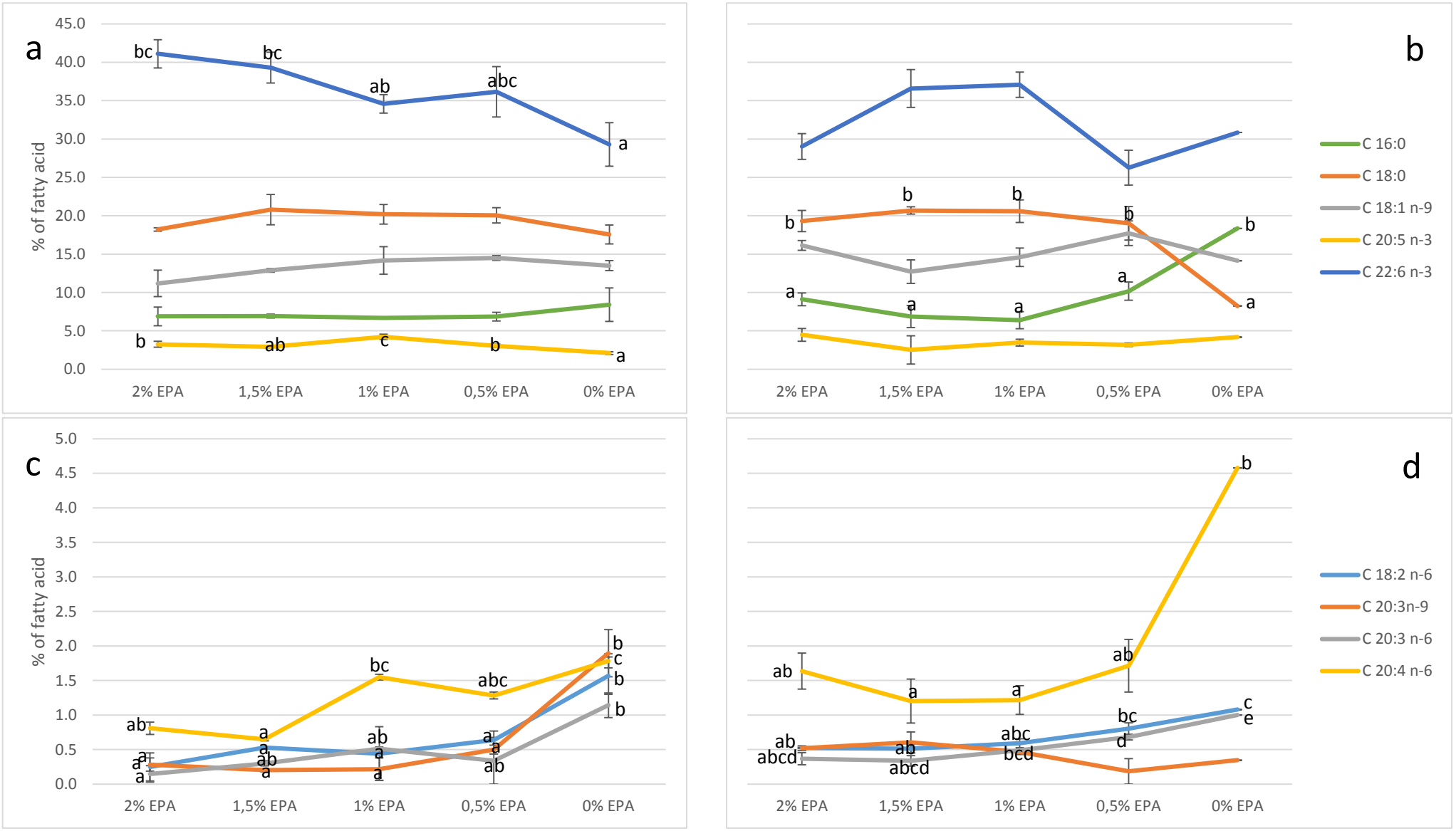

Figure 4.9 Percentage of fatty acids in the PS of brain lipids from fish fed different dietary levels of EPA at fish body weight (BW) 200g and 400g in Atlantic salmon. PS: Phosphatidylserine; EPA: Eicosapentaenoic acid (20:5 n-3). Data are expressed as mean ± standard error mean (SEM) (n=2 or 3). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish. Different lower-case letters indicate significant differences within the dietary group ($P < 0.05$, ANOVA followed by Duncan's new multiple range test). The figures **a** and **c** are at BW 200g, and **b** and **d** are at BW 400g.

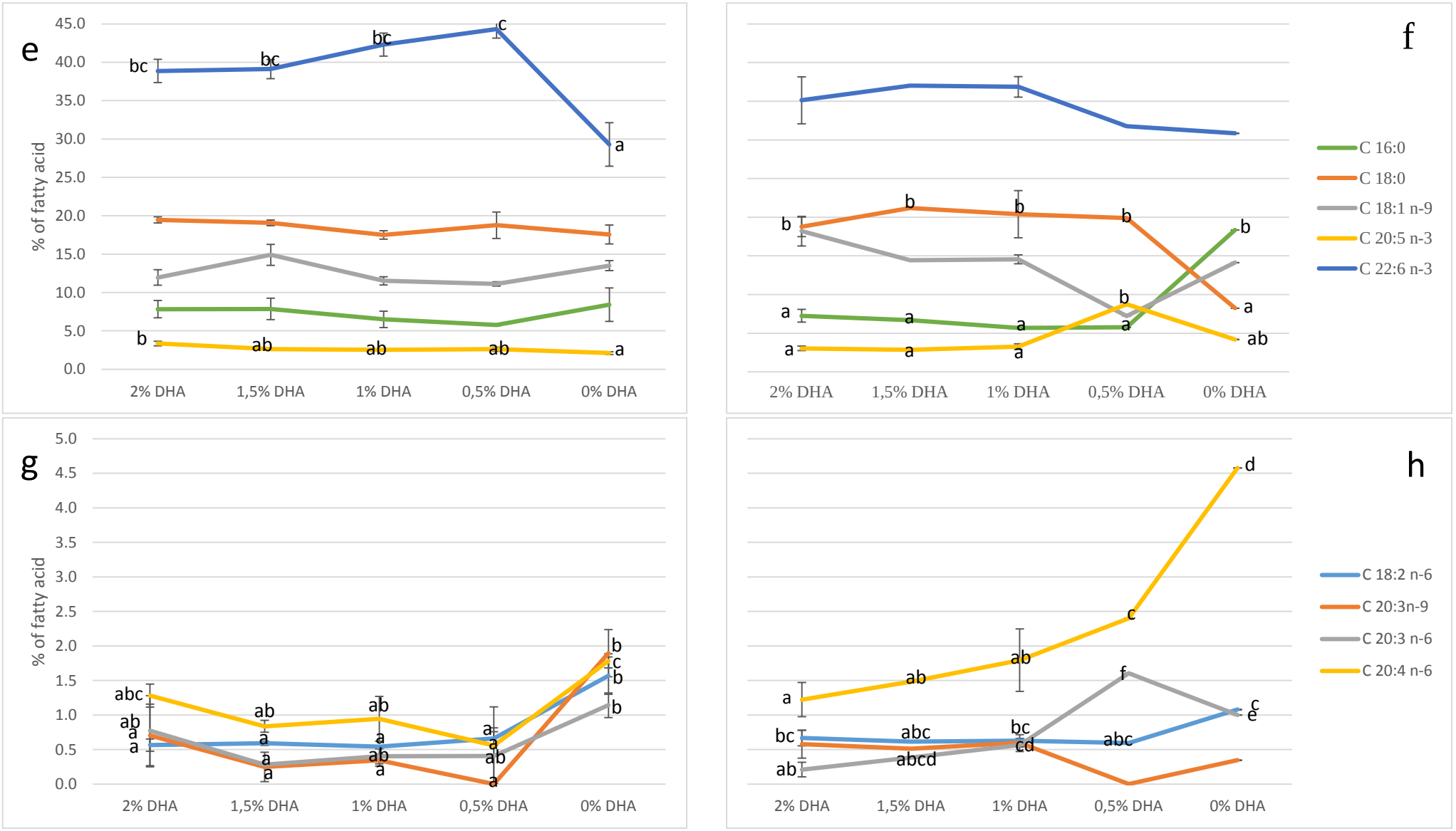

Figure 4.10 Percentage of fatty acids in the PS of brain lipids from fish fed different dietary levels of DHA at fish body weight (BW) 200g and 400g in Atlantic salmon. PS: Phosphatidylserine; DHA: Docosahexaenoic acid (22:5 n-3). Data are expressed as mean ± standard error mean (SEM) (n=2 or 3). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish. Different lower-case letters indicate significant differences within the dietary group ($P < 0.05$, ANOVA followed by Duncan's new multiple range test). The figures **e** and **g** are at BW 200g, and **f** and **h** are at BW 400g.

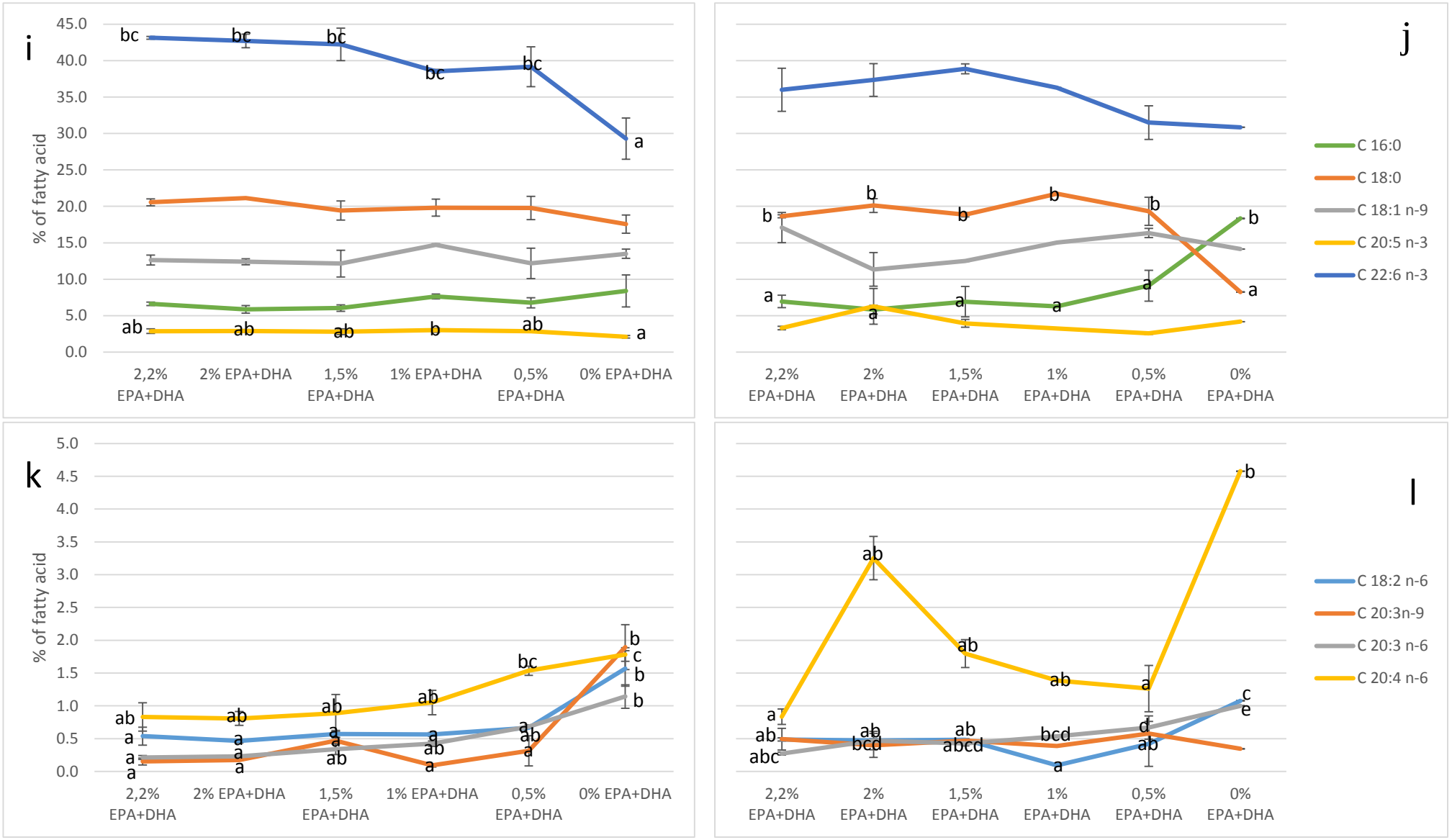

Figure 4.11 Percentage of fatty acids in the PS of brain lipids from fish fed different dietary levels of EPA+DHA at fish body weight (BW) 200g and 400g in Atlantic salmon. PS: Phosphatidylserine; EPA: Eicosapentaenoic acid (20:5 n-3); DHA: Docosahexaenoic acid (22:5 n-3). Data are expressed as mean ± standard error mean (SEM) (n=2 or 3). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish. Different lower-case letters indicate significant differences within the dietary group ($P < 0.05$, ANOVA followed by Duncan's new multiple range test). The figures **i** and **k** are at BW 200g, and **j** and **l** are at BW 400g.

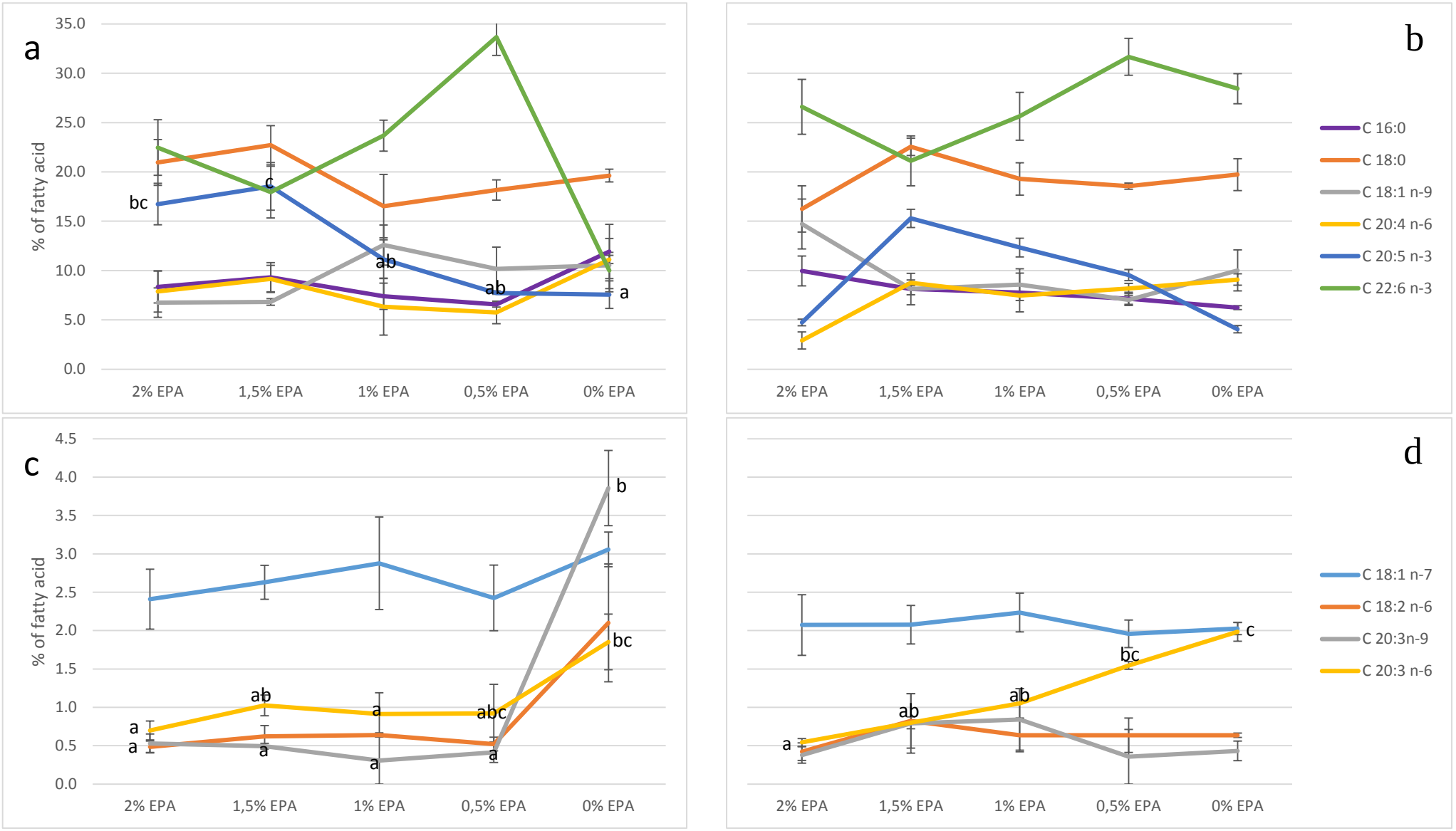

Figure 4.12 Percentage of fatty acids in the PI of brain lipids from fish fed different dietary levels of EPA at fish body weight (BW) 200g and 400g in Atlantic salmon. PI: Phosphatidylinositol; EPA: Eicosapentaenoic acid (20:5 n-3). Data are expressed as mean ± standard error mean (SEM) (n=2 or 3). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish. Different lower-case letters indicate significant differences within the dietary group ($P < 0.05$, ANOVA followed by Duncan's new multiple range test). The figures **a** and **c** are at BW 200g, and **b** and **d** are at BW 400g.

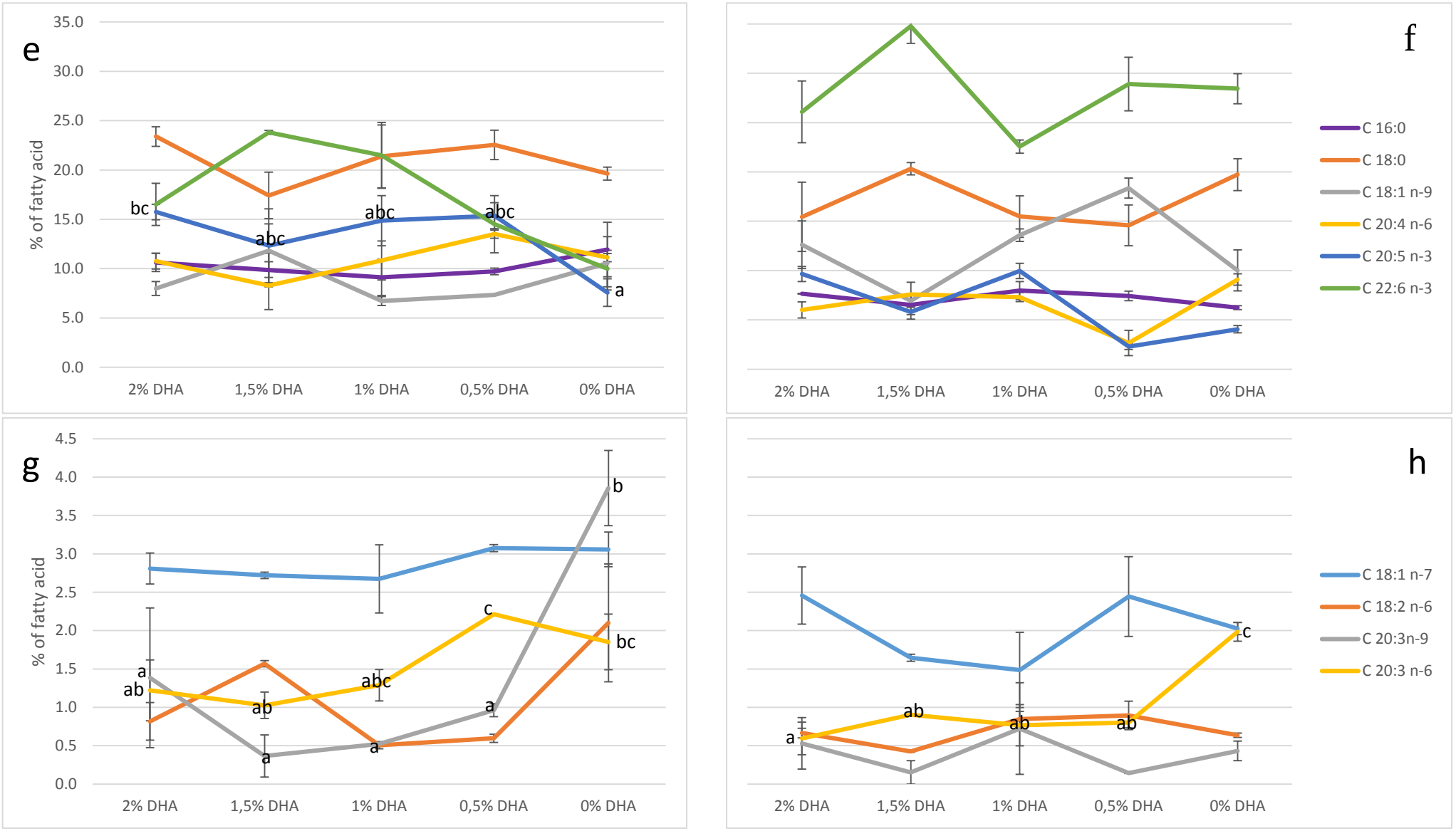

Figure 4.13 Percentage of fatty acids in the PI of brain lipids from fish fed different dietary levels of DHA at fish body weight (BW) 200g and 400g in Atlantic salmon. PI: Phosphatidylinositol; DHA: Docosahexaenoic acid (22:5 n-3). Data are expressed as mean ± standard error mean (SEM) (n=2 or 3). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish. Different lower-case letters indicate significant differences within the dietary group ($P < 0.05$, ANOVA followed by Duncan's new multiple range test). The figures **e** and **g** are at BW 200g, and **f** and **h** are at BW 400g.

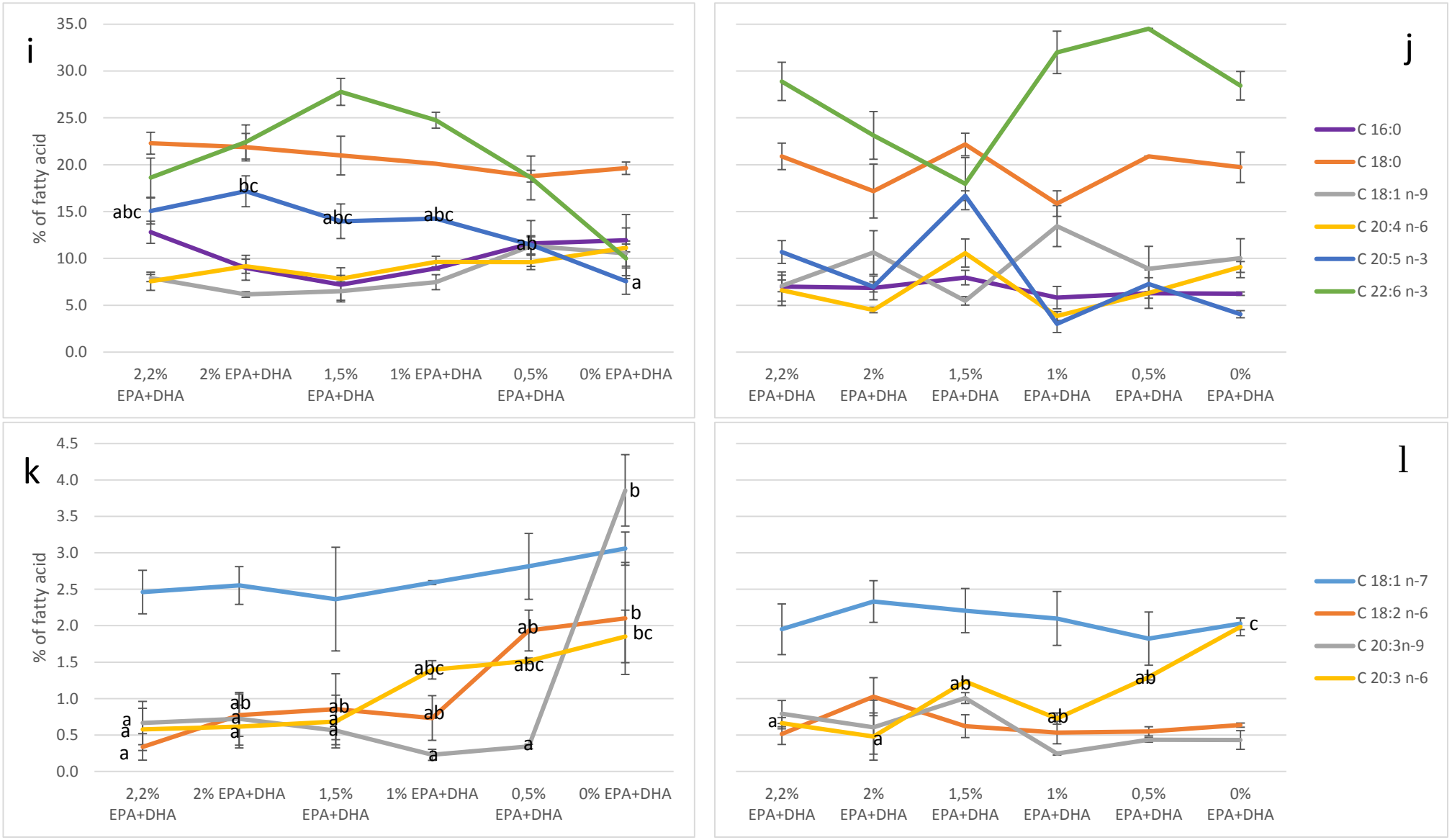

Figure 4.14 Percentage of fatty acids in the PI of brain lipids from fish fed different dietary levels of EPA+DHA at fish body weight (BW) 200g and 400g in Atlantic salmon. PI: Phosphatidylinositol; EPA: Eicosapentaenoic acid (20:5 n-3); DHA: Docosahexaenoic acid (22:5 n-3). Data are expressed as mean ± standard error mean (SEM) (n=2 or 3). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish. Different lower-case letters indicate significant differences within the dietary group ($P < 0.05$, ANOVA followed by Duncan's new multiple range test). The figures **i** and **k** are at BW 200g, and **j** and **l** are at BW 400g.

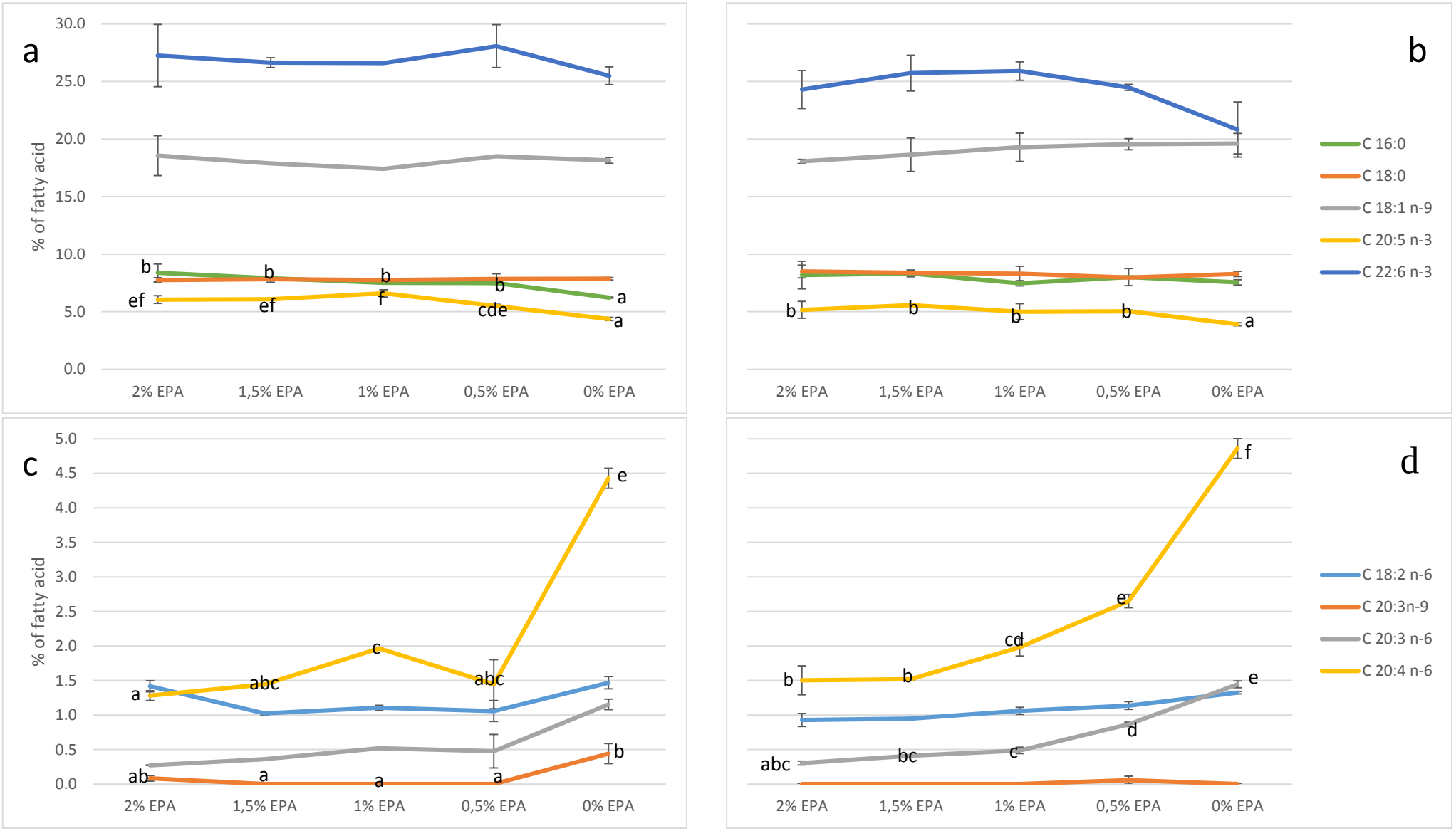

Figure 4.15 Percentage of fatty acids in the PE of brain lipids from fish fed different dietary levels of EPA at fish body weight (BW) 200g and 400g in Atlantic salmon. PE: Phosphatidylethanolamine; EPA: Eicosapentaenoic acid (20:5 n-3). Data are expressed as mean ± standard error mean (SEM) (n=2 or 3). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish. Different lower-case letters indicate significant differences within the dietary group ($P < 0.05$, ANOVA followed by Duncan's new multiple range test). The figures **a** and **c** are at BW 200g, and **b** and **d** are at BW 400g.

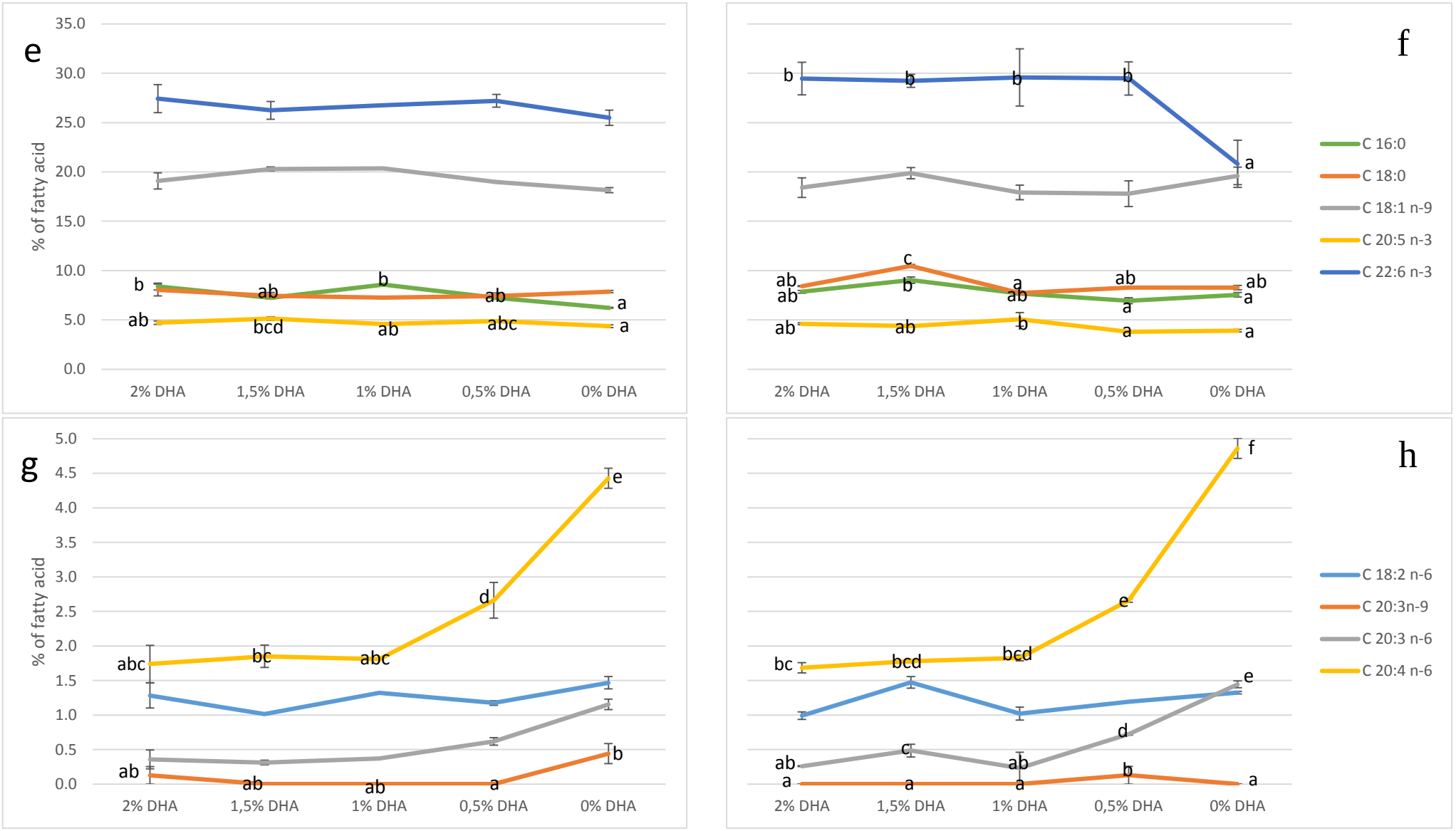

Figure 4.16 Percentage of fatty acids in the PE of brain lipids from fish fed different dietary levels of DHA at fish body weight (BW) 200g and 400g in Atlantic salmon. PE: Phosphatidylethanolamine; DHA: Docosahexaenoic acid (22:5 n-3). Data are expressed as mean ± standard error mean (SEM) (n=2 or 3). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish. Different lower-case letters indicate significant differences within the dietary group ($P < 0.05$, ANOVA followed by Duncan's new multiple range test). The figures **e** and **g** are at BW 200g, and **f** and **h** are at BW 400g.

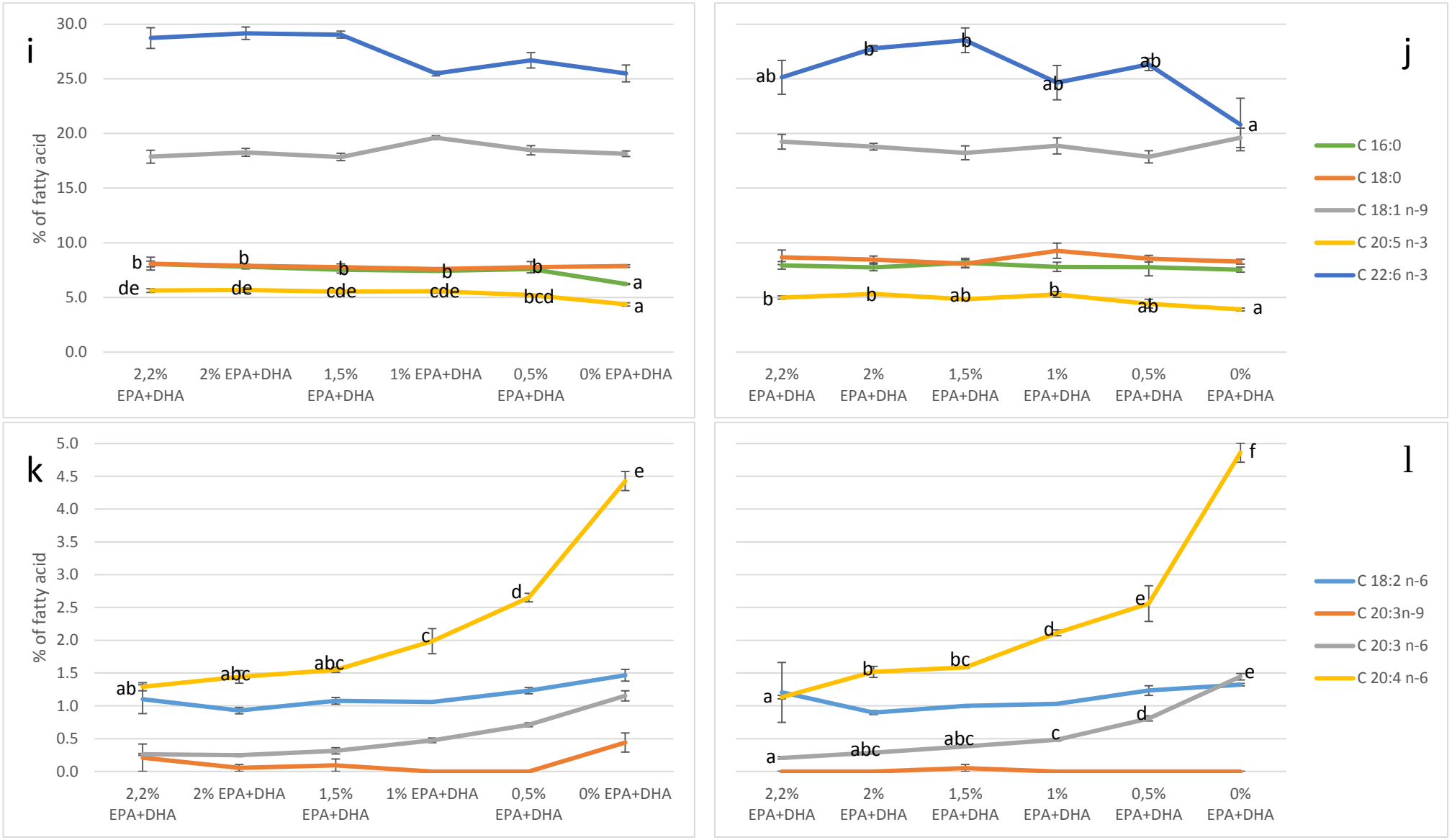

Figure 4.17 Percentage of fatty acids in the PE of brain lipids from fish fed different dietary levels of EPA+DHA at fish body weight (BW) 200g and 400g in Atlantic salmon. PE: Phosphatidylethanolamine; EPA: Eicosapentaenoic acid (20:5 n-3); DHA: Docosahexaenoic acid (22:5 n-3). Data are expressed as mean ± standard error mean (SEM) (n=2 or 3). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish. Different lower-case letters indicate significant differences within the dietary group ($P < 0.05$, ANOVA followed by Duncan's new multiple range test). The figures **i** and **k** are at BW 200g, and **j** and **l** are at BW 400g.

5. Discussion

5.1 PCA of the main phospholipid subclasses (PC, PS, PI and PE) in brain

The score plot (PC-1 vs. PC2) of the fatty acid data of brain showed an overview to compare the fatty acid composition/pattern of the different phospholipid subclasses. Apparently, the PC, PS, PI and PE were divided into four completely clear groupings, and each phospholipid subclass PC, PS, PI and PE have a distinct fatty acid profile. Although they all belong to the phosphoglycerides, they have different structures (variation in the head group). It is found by Tocher & Harvie (1988) that the distribution of fatty acids and specific PUFA between different phosphoglycerides was essentially similar in rat and fish brain tissue. Söderberg et al. (1991) established that PC and PE are the major subclasses of phospholipid in human brain and that PS and PI make up the balance.

For each phospholipid subclass by PCA analysis, it revealed that only the NC group was clearly separated from the rest of the dietary EPA and DHA groups, indicating that the brain conserved the fatty acids in the membrane phospholipids to a large extent. It was only when the feed was totally devoid of EPA and DHA, that some significant changes appeared in the membrane lipids. This is in contrast to what is found in other tissues by others, where for instance, the fatty acid composition of total phospholipids in the liver of Atlantic salmon was clearly affected by the diet level of n-3 fatty acids (Thomassen et al., 2016; Betancor et al., 2014). On the contrary, the fatty acid composition of lipid classes in brain and liver of sea bass (*Dicentrarchus labrax*) were equally affected by the dietary fatty acid input (Pagliarani et al. 1986). Therefore, our data shows that the fatty acid composition of brain phospholipids of Atlantic salmon are well conserved and little affected by the fatty acid compositions of the diets, consistent with other studies in Atlantic salmon (Bell et al. 1989; Brodtkorb et al., 1997), gilthead sea bream (*Sparus aurata*) (Benedito-Palos et al., 2010) or turbot (*Scophthalmus maximus*) (Bell et al., 1999), and also with mammal brain studies (Carrié et al., 2000; Abbott et al., 2012). This maybe implied a slower turnover of lipids and fatty acids in the brain of Atlantic salmon, or a preference of transporting fatty acids to the brain from other tissue.

5.2 The influence of dietary n-3 fatty acids on fatty acid composition of individual phospholipid subclasses in brain

5.2.1 The influence of dietary n-3 fatty acids in PC

In salmon brain, PC contained relative high percentages of the fatty acids 16:0 and 18:1n-9 and lower percentages of Σ n-3 fatty acids, compared to the other fractions (PS, PI and PE). The fatty acids 16:0 and 18:1n-9, together with n-3 PUFA, were the primary fatty acids selectively incorporated into membrane phospholipids in juvenile gilthead seabream (*Sparus aurata*) (Ibeas et al. 1996). There was a small increase in the percentage of 18:1n-9 in NC group. Ruyter et al. (2000) detected that in salmon fed a semi-purified diet with no n-3 or n-6 PUFA, both 18:1n-9 and 20:3n-9 significantly increased in blood phospholipids in the 3rd and 4th month of the study. Furthermore, although the primary fatty acids in PC are 16:0, 18:1n-9 and 22:6n-3, the percentages of Σ n-3 fatty acids had a lower level, especially in 22:6n-3 compared to the other fractions (PS, PI and PE). Similar results were reported for PC fraction in brain in Atlantic salmon (Brodtkorb et al. 1997), in rainbow trout and cod (Tocher & Harvie, 1988). Bell & Dick (1991) reported that a lower proportion of 22:6n-3 containing species resulted in that PC was the most saturated and contained the shortest average chain length in cod brain. In the present study, brain PC showed increased 20:5n-3 and 22:6n-3 incorporation and concomitantly decreased levels of Σ n-6 and Σ n-9 fatty acids, with increasing dietary levels of EPA, DHA or a mix of EPA and DHA. Similar consequence was observed in mice brain PC (Petursdottir et al., 2008). PC is the most abundant phospholipid component in all cells, and also plays a role in membrane-mediated cell signalling and phosphatidylcholine transfer protein (PCTP) activation of other enzymes (Kanno et al., 2007).

5.2.2 The influence of dietary n-3 fatty acids in PS

PS was characterized by the high percentage of fatty acid 18:0 and the enrichment of 22:6n-3 in salmon brain. These two fatty acids were also the main fatty acids in PS. The dietary treatments had little effect on the percentages of 18:0. Besides, PS had the highest Σ n-3 fatty acids (20:5n-3 and 22:6n-3), especially 22:6n-3. These are many investigations to prove that DHA is necessary for development of the nervous system in brain of fish. It was shown that DHA is specifically accumulated in brain lipid (Mourente et al., 1991; Mourente & Tocher,

1993; Nieminen et al., 2014). Also they found that DHA was incorporated in brain in preference to other fatty acids by turbot. This characteristic is in accordance with results of Tocher & Harvie (1988), who reported that PS and PE contained the highest percentages of 22:6n-3 in all species. Bell & Dick (1991) observed that Di22:6 (22:6/22:6) was abundant in both PS and PE subclasses, especially PS (24.0%), while 16:0/22:6, 18:1/22:6 and 18:0/22:6 were the other major species in PE, and 18:0/22:6 and 18:1/22:6 in PS. In the present study, although brain phospholipid fatty acid composition had little effects by dietary fatty acid input, brain PS revealed that reduced 20:5n-3 and 22:6n-3 incorporation and increased levels of Σ n-6 and Σ n-9 fatty acids were followed by decreasing dietary levels of EPA, DHA or a mix of EPA and DHA. This is similar with other studies in brain (Brodtkorb et al., 1997), liver and muscle (Thomassen et al., 2016) of Atlantic salmon. In mammals, brain PS is rich in DHA, and DHA supplementation is known to improve hippocampal function (Kim, 2007). A decrease of the DHA content in PS has been reported in cognitive impairment (Petursdottir et al., 2007; Cunnane et al., 2012).

5.2.3 The influence of dietary n-3 fatty acids in PI

PI differed from the other phospholipid subclasses in having high levels of 18:0 and 20:4n-6, which were also the primary fatty acids. The percentages of 18:0 had no significant changes, while the increases in 20:4n-6 were seen in PI. Compared to the other fractions (PC, PS and PE), PI had the highest proportion of 20:4n-6. Although other phospholipid subclasses may provide fatty acids for eicosanoid synthesis, in fish tissues 20:4n-6 is overwhelmingly concentrated in PI (Bell, 1989; Bell et al., 1983; Tocher & Sargent, 1984). The importance of 20:4n-6 in fish nutrition has been pointed to and discussed by Bell & Sargent (2003). The very different contents of the 18:0-20:4n-6 and 18:0-20:5n-3 species in brain and retinal PI was predictable from earlier fatty acid composition data (Tocher & Harvie, 1988). The major species in PI was 18:0-20:4n-6, which is the normal situation in fish and mammals. However, sometimes brain PI is dominated by 18:0-20:5n-3 species, such as in rainbow trout (Bell & Tocher, 1989) and cod (Bell & Dick, 1990). In the present study, the percentage of 20:5n-3 did not exceed the 22:6n-3 percentage in salmon brain PI, which is not in accordance with the results of Brodtkorb et al. (1997), who detected the content of 20:5n-3 was lower than the

22:6n-3 content, and reported that this may be of considerable relevance to eicosanoid metabolism in the brain. Furthermore, there was very little lipid in the PI fraction in our study, making it more difficult with accurate measurements. For instance, each fatty acid had larger variation, compared to the other fractions (PC, PS and PE). Brain PI is unusual in containing a greater concentration of EPA when compared to the other fractions (PC, PS and PE). This is in accordance with the findings of Tocher & Harvie (1988) and Bell & Dick (1991). In the present study, brain PI showed increased 20:5n-3 and 22:6n-3 incorporation and concomitantly decreased levels of Σ n-6 and Σ n-9 fatty acids, with increasing dietary levels of EPA, DHA or a mix of EPA and DHA. This is in accordance with the results of Brodtkorb et al. (1997). Bell et al. (1995) found that with increasing dietary AA (20:4n-6), the level of AA increased, and EPA reduced in juvenile turbot (*Scophthalmus maximus*) brain PI.

5.2.4 The influence of dietary n-3 fatty acids in PE

In PE from brain, it was rich in the percentage of 22:6n-3, which was also the dominating fatty acid. Similar results can be seen (Tocher & Harvie, 1988; Bell & Dick, 1991). The high concentration of PE in brain and neural tissues in comparison with its content in other tissues in all vertebrates including fish has long been known and may play a special role for PE in these tissues (McColl & Rossiter, 1952). PE may serve a function solely as the location and source of 22:6n-3, but also be especially important for regulation of localized membrane structure and fluidity in neural tissues. The fatty acid compositions of PE from the fish retinas, especially cod, implies the existence of di-PUFA and di-22:6n-3 molecular species in the fish (Tocher & Harvie, 1988). However, any special role for PE in fish brain function is purely speculative.

The percentage of 20:3n-9 was nearly zero in four phospholipid subclasses in brain studied upon feeding different dietary levels of EPA, DHA or a mix of EPA and DHA. And the percentage of 20:3n-9 increased in NC group, which was also observed in Atlantic salmon liver and muscle (Ruyter et al., 2000; Thomassen et al., 2016). EFA deficiency is known to induce Δ9-desaturase activity in rainbow trout, which also results in increased levels of 18:1n-9 and 20:3n-9 in lipids (Tocher et al., 1996). Consequently, 20:3n-9 may be used as an indicator of

essential fatty acid deficiency.

In the present experiment, the 18:2n-6 and 20:3n-6 content in brain was low and similar in four phospholipid subclasses, contributing only 0.5-1.5% of the total fatty acid content. Also salmon cannot synthesize 18:2n-6, and must intake from the diet. With decreasing dietary levels of EPA, DHA or a mix of EPA and DHA, the increased percentages of Σ n-6 fatty acids (18:2n-6, 20:3n-6 and 20:4n-6) was found, especially in NC group. Because salmon try to compensate for reduction of n-3 fatty acids by making higher percentage of n-6 PUFAs. It was also found in Atlantic salmon by Thomassen et al. (2016) and in rainbow trout by Leray et al. (1985). Ruyter & Thomassen (1999) showed that 18:2n-6 and 18:3n-3 can be elongated and desaturated in Atlantic salmon liver, and that this conversion and the activity of reverse metabolism (retroconversion) back to very long-chain PUFA is significantly enhanced by EFA deficiency. The level of 20:3n-6 increase due to a diet deficient in n-3 fatty acids in several fish species: rainbow trout (Castledine & Buckley, 1982), common and grass carp (Takeuchi, 1996) and tilapia (Kanazawa et al., 1980).

It was obvious that the percentages of 20:5n-3 and 22:6n-3 significantly increased with increasing dietary levels of EPA, DHA or a mix of EPA and DHA. Besides, the level of 20:5n-3 was much lower compared to the level of 22:6n-3 in brain phospholipids. This indicates the different degree of incorporation of 20:5n-3 and 22:6n-3 into the brain lipids when the dietary input of these fatty acids are increased. Similar result was reported in Atlantic salmon (Brodtkorb et al., 1997), and rainbow trout and cod (Tocher & Harvie, 1988).

The diet without EPA and DHA did not render the brain devoid of EPA and DHA. Instead, throughout the growth period of fish fed without EPA and DHA, the brain remained containing relatively large amounts of EPA and DHA. Similar finding in European sea bass (*Dicentrarchus labrax*) brain existed (Skalli et al., 2006). The brain was probably prioritized over other tissue producing large amounts of EPA and DHA from 18:3n-3 (ALA). When the supply of dietary fatty acids is scarce, they seem to be used preferentially to maintain the fatty acid composition of the membrane phospholipids. Furthermore, it was observed that the percentages of EPA were consistently lower than DHA in phospholipid subclasses of salmon brain. This consequence suggested a selective deposition and retention of DHA in the brain. This effect has been previously observed in salmon brain (Brodtkorb et al. 1997). Mourente & Tocher (1992) found

that DHA was incorporated in brain in preference to other fatty acids by turbot. The effects of the increasing percentages of 22:6n-3 in the dietary EPA groups indicated that 20:5n-3 was desaturated and elongated further to 22:6n-3.

As can be seen from the figures, the percentage of DHA increased markedly with increasing EPA level. However, the percentage of EPA did not have a clear increase but rather fluctuated with increasing dietary DHA level. These findings demonstrated that it has a certain degree of bioconversion of EPA to DHA through elongation and desaturation in brain or in the other tissues, which then transported to the brain, when salmon were only supplied with EPA. In contrast, there was no data supporting DHA to EPA retroconversion of any significance. Bioconversion of EPA to DHA and a lack of proof for DHA to EPA retroconversion matches with the previously described observation that DHA was more retained in the brain than EPA. Overall, it seems that when the supply of n-3 PUFA is limited, there is a greater effort to produce large amounts of EPA and DHA, and DHA was more retained than EPA.

6. Conclusion

The study focused on how different dietary levels of EPA, DHA or a mix of EPA and DHA influence the percentages of fatty acids in phospholipid subclasses, and how EPA and DHA deficiency influence the brain phospholipids in Atlantic salmon. In the course of this analysis, it was demonstrated that decreased levels of dietary EPA, DHA or a mix of EPA and DHA resulted in a reduction of EPA and DHA content, and an increase of Σ n-6 fatty acids (18:2n-6, 20:3n-6 and 20:4n-6) and Σ n-9 fatty acids (18:1n-9 and 20:3n-9). However, the fatty acid composition of brain phospholipid was relatively conserved and little affected by the diet. PC had higher 16:0 and 18:1n-9 and the lowest percentages of Σ n-3 fatty acids, PI was characterized by higher 18:0 and 20:4n-6, and PS and PE contained the highest percentages of 22:6n-3 in salmon brain. Furthermore, when the fish was fed a diet without EPA and DHA, the brain remained containing quite high amounts of EPA and DHA, and DHA was more retained than EPA.

In summary, EPA and DHA are essential for salmon brain, and play different important roles in brain function. On the whole, phospholipids were characterized by high percentages of n-3 PUFA (20:5 and 22:6) and low percentages of n-6 PUFA, however, each phospholipid subclass had different characteristic fatty acids. By PCA analysis, it revealed that only the NC group was clearly separated from the rest of the dietary EPA and DHA groups, showing that the fatty acid composition of brain phospholipid was relatively conserved and little affected by the diet.

Additional research should focus upon how n-3 fatty acids deficiency influence brain function, even function of each phospholipid subclass.

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Appendix

PC 200g	NC	0.5% EPA	1% EPA	1.5% EPA	2% EPA
C 14:0	0.3 ± 0.12	0.3 ± 0.02	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.04
C 16:0	24.6 ± 0.63	26.3 ± 1.16	25.5 ± 0.58	26.4 ± 0.83	27.5 ± 0.99
C 18:0	5.1 ± 0.23	4.9 ± 0.10	4.9 ± 0.33	4.5 ± 0.16	4.6 ± 0.10
C 20:0	0.1 ± 0.01	0.0 ± 0.04	0.0 ± 0.00	0.2 ± 0.05	0.1 ± 0.00
C 22:0	0.4 ± 0.10	0.0 ± 0.01	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Sum N-0	31.1 ± 0.67	31.8 ± 1.47	30.8 ± 0.83	31.9 ± 0.88	32.9 ± 1.06
C 16:1 n-9	1.7 ± 0.22	1.2 ± 0.11	1.3 ± 0.06	1.3 ± 0.05	1.4 ± 0.05
C 18:1 n-9	26.3 ± 0.25	23.7 ± 0.13	22.7 ± 0.78	22.8 ± 0.59	22.8 ± 0.10
C 20:1 n-9	2.2 ± 0.05	1.5 ± 0.23	1.2 ± 0.38	1.9 ± 0.32	1.4 ± 0.04
C 20:3 n-9	0.6 ± 0.26	0.3 ± 0.10	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.09
C 18:2 n-6	1.4 ± 0.04	0.8 ± 0.01	1.0 ± 0.08	0.7 ± 0.03	0.7 ± 0.01
C 18:3 n-6	0.2 ± 0.03	0.0 ± 0.00	0.1 ± 0.09	0.0 ± 0.00	0.0 ± 0.00
C 20:2 n-6	0.4 ± 0.02	0.1 ± 0.07	0.3 ± 0.05	0.3 ± 0.02	0.1 ± 0.10
C 20:3 n-6	1.7 ± 0.54	0.4 ± 0.15	0.4 ± 0.13	0.1 ± 0.12	0.2 ± 0.00
C 20:4 n-6	2.1 ± 0.05	1.1 ± 0.12	1.1 ± 0.13	0.9 ± 0.03	0.9 ± 0.01
C 22:5 n-6	0.1 ± 0.14	0.0 ± 0.05	0.1 ± 0.05	0.2 ± 0.18	0.1 ± 0.07
Sum N-6	6.0 ± 0.57	2.4 ± 0.30	3.0 ± 0.19	2.3 ± 0.12	2.0 ± 0.17
C 18:3 n-3	0.1 ± 0.07	0.1 ± 0.01	0.3 ± 0.01	0.2 ± 0.01	0.1 ± 0.01
C 18:4 n-3	0.3 ± 0.01	0.2 ± 0.01	0.3 ± 0.09	0.3 ± 0.09	0.2 ± 0.00
C 20:3 n-3	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.04	0.1 ± 0.04
C 20:5 n-3	4.4 ± 0.14	4.6 ± 0.07	5.4 ± 0.17	5.4 ± 0.46	5.7 ± 0.05
C 22:5 n-3	4.6 ± 0.16	3.5 ± 1.78	9.2 ± 0.71	7.1 ± 0.22	5.9 ± 0.84
C 22:6 n-3	18.4 ± 0.70	24.7 ± 1.25	18.9 ± 0.16	22.4 ± 0.41	22.0 ± 0.06
Sum N-3	27.8 ± 0.87	33.2 ± 0.44	34.0 ± 0.80	35.4 ± 0.71	34.1 ± 0.91

PC 200g	NC	0.5% DHA	1% DHA	1.5% DHA	2% DHA
C 14:0	0.3 ± 0.12	1.0 ± 0.65	0.3 ±	0.3 ±	0.4 ± 0.04
C 16:0	24.6 ± 0.63	20.5 ± 2.36	25.3 ±	24.7 ±	26.8 ± 0.59
C 18:0	5.1 ± 0.23	8.6 ± 0.41	4.3 ±	4.5 ±	4.6 ± 0.20
C 20:0	0.1 ± 0.01	0.1 ± 0.02	0.2 ±	0.1 ±	0.1 ± 0.02
C 22:0	0.4 ± 0.10	0.0 ± 0.02	0.0 ±	0.0 ±	0.1 ± 0.08
Sum N-0	31.1 ± 0.67	30.2 ± 0.71	30.6 ±	30.1 ±	32.5 ± 0.75
C 16:1 n-9	1.7 ± 0.22	1.0 ± 0.17	1.2 ±	1.2 ±	1.3 ± 0.10
C 18:1 n-9	26.3 ± 0.25	19.9 ± 1.93	23.0 ±	24.3 ±	23.1 ± 0.76
C 20:1 n-9	2.2 ± 0.05	1.6 ± 0.02	2.3 ±	1.8 ±	1.8 ± 0.18
C 20:3 n-9	0.6 ± 0.26	0.1 ± 0.11	0.0 ±	0.0 ±	0.1 ± 0.10
C 18:2 n-6	1.4 ± 0.04	0.9 ± 0.10	0.9 ±	0.8 ±	0.9 ± 0.07
C 18:3 n-6	0.2 ± 0.03	0.0 ± 0.04	0.0 ±	0.0 ±	0.0 ± 0.00
C 20:2 n-6	0.4 ± 0.02	0.4 ± 0.03	0.4 ±	0.3 ±	0.2 ± 0.13
C 20:3 n-6	1.7 ± 0.54	0.7 ± 0.14	0.4 ±	0.3 ±	0.3 ± 0.11
C 20:4 n-6	2.1 ± 0.05	2.0 ± 0.25	1.1 ±	1.0 ±	1.1 ± 0.16
C 22:5 n-6	0.1 ± 0.14	0.1 ± 0.11	0.2 ±	0.0 ±	0.2 ± 0.04
Sum N-6	6.0 ± 0.57	4.2 ± 0.83	3.0 ±	2.4 ±	2.8 ± 0.25
C 18:3 n-3	0.1 ± 0.07	0.1 ± 0.01	0.2 ±	0.2 ±	0.2 ± 0.01
C 18:4 n-3	0.3 ± 0.01	0.2 ± 0.01	0.3 ±	0.3 ±	0.2 ± 0.02
C 20:3 n-3	0.0 ± 0.00	0.0 ± 0.01	0.1 ±	0.0 ±	0.1 ± 0.04
C 20:5 n-3	4.4 ± 0.14	4.3 ± 0.05	4.2 ±	4.0 ±	4.3 ± 0.17
C 22:5 n-3	4.6 ± 0.16	6.5 ± 2.49	8.6 ±	9.2 ±	7.7 ± 0.72
C 22:6 n-3	18.4 ± 0.70	26.7 ± 2.95	21.9 ±	21.1 ±	21.0 ± 0.65
Sum N-3	27.8 ± 0.87	37.8 ± 2.49	35.2 ±	34.7 ±	33.4 ± 2.32

PC 200g	NC	0.5% EPA+DHA	1% EPA+DHA	1.5% EPA+DHA	2% EPA+DHA	CC
C 14:0	0.3 ± 0.12	0.3 ± 0.04	0.3 ± 0.00	0.3 ±	0.4 ± 0.06	0.4 ± 0.04
C 16:0	24.6 ± 0.63	27.3 ± 0.63	25.5 ± 0.51	25.0 ±	26.8 ± 0.90	26.5 ± 0.71
C 18:0	5.1 ± 0.23	4.6 ± 0.20	4.4 ± 0.08	4.5 ±	4.6 ± 0.17	4.3 ± 0.15
C 20:0	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.5 ±	0.2 ± 0.01	0.1 ± 0.05
C 22:0	0.4 ± 0.10	0.0 ± 0.00	0.0 ± 0.00	0.0 ±	0.1 ± 0.05	0.0 ± 0.04
Sum N-0	31.1 ± 0.67	32.6 ± 0.74	30.9 ± 0.52	31.1 ±	32.2 ± 1.22	31.8 ± 0.73
C 16:1 n-9	1.7 ± 0.22	1.3 ± 0.04	1.3 ± 0.04	1.2 ±	1.3 ± 0.07	1.2 ± 0.05
C 18:1 n-9	26.3 ± 0.25	23.5 ± 0.07	24.2 ± 0.14	21.6 ±	22.9 ± 0.66	22.2 ± 0.45
C 20:1 n-9	2.2 ± 0.05	2.1 ± 0.35	1.7 ± 0.04	2.6 ±	1.9 ± 0.11	1.8 ± 0.23
C 20:3 n-9	0.6 ± 0.26	0.0 ± 0.00	0.0 ± 0.00	0.0 ±	0.1 ± 0.07	0.1 ± 0.11
C 18:2 n-6	1.4 ± 0.04	1.2 ± 0.22	0.8 ± 0.03	1.0 ±	0.8 ± 0.01	0.9 ± 0.16
C 18:3 n-6	0.2 ± 0.03	0.0 ± 0.05	0.0 ± 0.00	0.0 ±	0.0 ± 0.00	0.0 ± 0.00
C 20:2 n-6	0.4 ± 0.02	0.3 ± 0.09	0.3 ± 0.03	0.6 ±	0.2 ± 0.15	0.2 ± 0.10
C 20:3 n-6	1.7 ± 0.54	0.3 ± 0.27	0.3 ± 0.02	0.6 ±	0.2 ± 0.02	0.2 ± 0.04
C 20:4 n-6	2.1 ± 0.05	1.3 ± 0.01	1.1 ± 0.08	1.1 ±	0.9 ± 0.02	0.8 ± 0.06
C 22:5 n-6	0.1 ± 0.14	0.0 ± 0.00	0.0 ± 0.00	0.0 ±	0.1 ± 0.10	0.1 ± 0.07
Sum N-6	6.0 ± 0.57	3.1 ± 0.11	2.6 ± 0.15	3.3 ±	2.2 ± 0.28	2.1 ± 0.26
C 18:3 n-3	0.1 ± 0.07	0.2 ± 0.03	0.1 ± 0.00	0.4 ±	0.2 ± 0.01	0.3 ± 0.11
C 18:4 n-3	0.3 ± 0.01	0.1 ± 0.12	0.2 ± 0.00	0.5 ±	0.3 ± 0.01	0.3 ± 0.02
C 20:3 n-3	0.0 ± 0.00	0.1 ± 0.00	0.1 ± 0.01	0.3 ±	0.1 ± 0.05	0.1 ± 0.04
C 20:5 n-3	4.4 ± 0.14	4.4 ± 0.35	4.5 ± 0.09	4.9 ±	5.0 ± 0.21	4.8 ± 0.30
C 22:5 n-3	4.6 ± 0.16	9.6 ± 0.94	9.3 ± 0.26	10.6 ±	7.5 ± 0.54	6.1 ± 1.07
C 22:6 n-3	18.4 ± 0.70	18.6 ± 1.20	19.9 ± 0.14	19.2 ±	20.8 ± 0.59	24.0 ± 0.47
Sum N-3	27.8 ± 0.87	32.9 ± 0.00	34.1 ± 0.49	35.9 ±	33.9 ± 2.21	35.5 ± 0.82

Figure 5.1 Fatty acid compositions (% of total fatty acids) of PC at fish body weight (BW) 200g. PC: Phosphatidylcholine; NC: negative control (0% EPA+DHA); CC: commercial control (2.2% EPA+DHA); EPA: Eicosapentaenoic acid (20:5 n-3); DHA: Docosahexaenoic acid (22:5 n-3). Data are expressed as mean (n=1, 2 or 3) ± standard error of mean (SEM). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish.

PC 400g	NC	0.5% EPA	1% EPA	1.5% EPA	2% EPA
C 14:0	0.3 ± 0.00	0.3 ± 0.00	0.3 ± 0.03	0.3 ± 0.00	0.3 ± 0.02
C 16:0	26.1 ± 0.87	26.0 ± 0.66	27.5 ± 0.02	27.4 ± 1.22	25.5 ± 0.27
C 18:0	5.0 ± 0.12	4.9 ± 0.04	5.3 ± 0.09	5.1 ± 0.04	4.6 ± 0.13
C 20:0	0.1 ± 0.01	0.1 ± 0.00	0.1 ± 0.01	0.1 ± 0.05	0.1 ± 0.01
C 22:0	0.1 ± 0.04	0.1 ± 0.00	0.1 ± 0.03	0.1 ± 0.00	0.1 ± 0.01
Sum N-0	32.1 ± 0.14	31.9 ± 0.09	33.5 ± 0.16	33.5 ± 0.07	31.2 ± 0.21
C 16:1 n-9	1.5 ± 0.03	1.4 ± 0.02	1.4 ± 0.07	2.2 ± 0.74	1.4 ± 0.06
C 18:1 n-9	27.4 ± 0.60	27.8 ± 0.04	28.3 ± 0.51	26.3 ± 0.48	25.3 ± 0.37
C 20:1 n-9	1.8 ± 0.02	1.7 ± 0.01	1.6 ± 0.04	1.5 ± 0.04	1.5 ± 0.03
C 20:3 n-9	0.0 ± 0.02	0.0 ± 0.02	0.3 ± 0.23	0.0 ± 0.00	0.0 ± 0.02
C 18:2 n-6	1.4 ± 0.05	1.1 ± 0.06	0.6 ± 0.30	0.8 ± 0.01	0.7 ± 0.01
C 18:3 n-6	0.0 ± 0.04	0.0 ± 0.03	0.0 ± 0.02	0.0 ± 0.01	0.0 ± 0.00
C 20:2 n-6	0.4 ± 0.03	0.3 ± 0.00	0.2 ± 0.15	0.3 ± 0.01	0.3 ± 0.00
C 20:3 n-6	1.3 ± 0.06	0.7 ± 0.05	0.5 ± 0.07	0.3 ± 0.01	0.2 ± 0.01
C 20:4 n-6	3.3 ± 0.22	1.8 ± 0.08	1.3 ± 0.04	1.2 ± 0.04	1.0 ± 0.01
C 22:5 n-6	0.1 ± 0.13	0.1 ± 0.09	0.1 ± 0.08	0.1 ± 0.00	0.1 ± 0.01
Sum N-6	6.5 ± 0.39	4.1 ± 0.12	2.8 ± 0.53	2.7 ± 0.02	2.3 ± 0.02
C 18:3 n-3	0.1 ± 0.00	0.1 ± 0.01	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00
C 18:4 n-3	0.3 ± 0.01	0.1 ± 0.11	0.3 ± 0.08	0.2 ± 0.00	0.2 ± 0.00
C 20:3 n-3	0.1 ± 0.00	0.1 ± 0.00	0.0 ± 0.04	0.1 ± 0.00	0.1 ± 0.01
C 20:5 n-3	3.8 ± 0.24	5.2 ± 0.23	6.2 ± 0.24	6.2 ± 0.33	5.7 ± 0.25
C 22:5 n-3	5.5 ± 2.10	3.9 ± 0.87	1.7 ± 0.23	3.7 ± 1.35	8.2 ± 1.46
C 22:6 n-3	16.3 ± 0.45	18.8 ± 0.08	18.8 ± 0.12	19.8 ± 0.02	19.7 ± 0.89
Sum N-3	26.0 ± 1.91	28.1 ± 0.66	27.2 ± 0.15	30.1 ± 2.01	34.0 ± 0.97

PC 400g	NC	0.5% DHA	1% DHA	1.5% DHA	2% DHA
C 14:0	0.3 ± 0.00	0.3 ± 0.06	0.2 ±	0.2 ± 0.01	0.2 ± 0.01
C 16:0	26.1 ± 0.87	25.4 ± 1.43	26.3 ±	25.0 ± 0.15	25.9 ± 1.52
C 18:0	5.0 ± 0.12	5.1 ± 0.19	5.1 ±	4.5 ± 0.06	4.8 ± 0.08
C 20:0	0.1 ± 0.01	0.0 ± 0.04	0.1 ±	0.0 ± 0.05	0.1 ± 0.00
C 22:0	0.1 ± 0.04	0.0 ± 0.01	0.1 ±	0.1 ± 0.01	0.1 ± 0.00
Sum N-0	32.1 ± 0.14	31.4 ± 0.13	32.3 ±	30.3 ± 0.02	31.7 ± 0.07
C 16:1 n-9	1.5 ± 0.03	1.2 ± 0.04	1.3 ±	1.3 ± 0.09	1.3 ± 0.00
C 18:1 n-9	27.4 ± 0.60	26.9 ± 1.08	29.3 ±	24.3 ± 0.19	26.8 ± 0.18
C 20:1 n-9	1.8 ± 0.02	1.8 ± 0.20	1.8 ±	1.5 ± 0.07	1.7 ± 0.08
C 20:3 n-9	0.0 ± 0.02	0.2 ± 0.14	0.0 ±	0.0 ± 0.00	0.0 ± 0.01
C 18:2 n-6	1.4 ± 0.05	1.0 ± 0.29	1.0 ±	0.9 ± 0.01	0.8 ± 0.02
C 18:3 n-6	0.0 ± 0.04	0.0 ± 0.00	0.0 ±	0.0 ± 0.00	0.0 ± 0.00
C 20:2 n-6	0.4 ± 0.03	0.3 ± 0.29	0.4 ±	0.3 ± 0.01	0.3 ± 0.00
C 20:3 n-6	1.3 ± 0.06	0.7 ± 0.02	0.4 ±	0.3 ± 0.01	0.2 ± 0.00
C 20:4 n-6	3.3 ± 0.22	1.7 ± 0.09	1.4 ±	1.1 ± 0.03	1.1 ± 0.04
C 22:5 n-6	0.1 ± 0.13	0.1 ± 0.10	0.0 ±	0.1 ± 0.01	0.2 ± 0.09
Sum N-6	6.5 ± 0.39	3.8 ± 0.38	3.2 ±	2.7 ± 0.02	2.7 ± 0.02
C 18:3 n-3	0.1 ± 0.00	0.2 ± 0.06	0.1 ±	0.1 ± 0.01	0.1 ± 0.01
C 18:4 n-3	0.3 ± 0.01	0.3 ± 0.07	0.2 ±	0.2 ± 0.01	0.2 ± 0.00
C 20:3 n-3	0.1 ± 0.00	0.0 ± 0.00	0.1 ±	0.0 ± 0.04	0.1 ± 0.00
C 20:5 n-3	3.8 ± 0.24	3.8 ± 0.30	4.5 ±	3.6 ± 0.15	4.3 ± 0.07
C 22:5 n-3	5.5 ± 2.10	6.4 ± 1.55	1.7 ±	9.8 ± 1.00	4.6 ± 1.48
C 22:6 n-3	16.3 ± 0.45	18.9 ± 2.02	20.3 ±	21.8 ± 0.74	21.7 ± 0.54
Sum N-3	26.0 ± 1.91	29.7 ± 2.36	26.9 ±	35.5 ± 0.43	31.0 ± 1.86

PC 400g	NC	0.5% EPA+DHA	1% EPA+DHA	1.5% EPA+DHA	2% EPA+DHA	CC
C 14:0	0.3 ± 0.00	0.3 ± 0.03	0.2 ± 0.07	0.3 ± 0.02	0.3 ± 0.01	0.3 ± 0.01
C 16:0	26.1 ± 0.87	26.4 ± 0.04	25.5 ± 0.64	26.9 ± 0.96	26.3 ± 0.78	25.4 ± 0.73
C 18:0	5.0 ± 0.12	4.9 ± 0.07	4.7 ± 0.31	4.9 ± 0.05	4.8 ± 0.10	4.7 ± 0.07
C 20:0	0.1 ± 0.01	0.0 ± 0.04	0.1 ± 0.03	0.0 ± 0.05	0.1 ± 0.03	0.1 ± 0.00
C 22:0	0.1 ± 0.04	0.1 ± 0.06	0.0 ± 0.00	0.1 ± 0.01	0.1 ± 0.05	0.1 ± 0.05
Sum N-0	32.1 ± 0.14	32.3 ± 0.02	30.7 ± 0.62	32.8 ± 0.13	32.1 ± 0.09	31.1 ± 0.09
C 16:1 n-9	1.5 ± 0.03	1.4 ± 0.12	1.3 ± 0.23	2.1 ± 0.71	1.9 ± 0.60	1.3 ± 0.08
C 18:1 n-9	27.4 ± 0.60	27.3 ± 1.97	26.9 ± 1.67	26.4 ± 1.42	26.2 ± 0.76	27.1 ± 0.80
C 20:1 n-9	1.8 ± 0.02	1.3 ± 0.18	1.4 ± 0.27	1.5 ± 0.12	1.5 ± 0.06	1.7 ± 0.01
C 20:3 n-9	0.0 ± 0.02	0.3 ± 0.26	0.4 ± 0.01	0.0 ± 0.02	0.0 ± 0.02	0.0 ± 0.02
C 18:2 n-6	1.4 ± 0.05	0.7 ± 0.40	0.7 ± 0.29	0.8 ± 0.00	0.7 ± 0.03	0.6 ± 0.02
C 18:3 n-6	0.0 ± 0.04	0.0 ± 0.00	0.1 ± 0.09	0.0 ± 0.01	0.0 ± 0.00	0.0 ± 0.00
C 20:2 n-6	0.4 ± 0.03	0.2 ± 0.19	0.3 ± 0.03	0.3 ± 0.01	0.3 ± 0.01	0.2 ± 0.01
C 20:3 n-6	1.3 ± 0.06	0.8 ± 0.10	0.5 ± 0.10	0.3 ± 0.00	0.2 ± 0.01	0.2 ± 0.00
C 20:4 n-6	3.3 ± 0.22	1.8 ± 0.13	1.4 ± 0.26	1.2 ± 0.00	1.0 ± 0.04	0.8 ± 0.01
C 22:5 n-6	0.1 ± 0.13	0.2 ± 0.05	0.1 ± 0.09	0.1 ± 0.01	0.1 ± 0.06	0.1 ± 0.00
Sum N-6	6.5 ± 0.39	3.8 ± 0.41	3.0 ± 0.05	2.8 ± 0.01	2.4 ± 0.05	1.9 ± 0.03
C 18:3 n-3	0.1 ± 0.00	0.2 ± 0.05	0.3 ± 0.07	0.1 ± 0.00	0.1 ± 0.04	0.1 ± 0.01
C 18:4 n-3	0.3 ± 0.01	0.3 ± 0.08	0.2 ± 0.23	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.00
C 20:3 n-3	0.1 ± 0.00	0.0 ± 0.04	0.1 ± 0.08	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00
C 20:5 n-3	3.8 ± 0.24	4.8 ± 0.42	5.1 ± 0.82	5.0 ± 0.24	5.0 ± 0.33	4.8 ± 0.33
C 22:5 n-3	5.5 ± 2.10	4.3 ± 1.54	7.0 ± 1.54	4.1 ± 1.61	6.6 ± 1.56	7.3 ± 2.29
C 22:6 n-3	16.3 ± 0.45	19.2 ± 1.10	18.6 ± 1.72	20.9 ± 0.55	20.0 ± 0.26	19.9 ± 0.30
Sum N-3	26.0 ± 1.91	28.8 ± 2.13	31.3 ± 4.22	30.5 ± 2.90	32.0 ± 1.97	32.4 ± 1.84

Figure 5.2 Fatty acid compositions (% of total fatty acids) of PC at fish body weight (BW) 400g. PC: Phosphatidylcholine; NC: negative control (0% EPA+DHA); CC: commercial control (2.2% EPA+DHA); EPA: Eicosapentaenoic acid (20:5 n-3); DHA: Docosahexaenoic acid (22:5 n-3). Data are expressed as mean (n=1, 2 or 3) ± standard error of mean (SEM). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish.

PS 200g	NC	0.5% EPA	1% EPA	1.5% EPA	2% EPA
C 14:0	0.4 ± 0.18	0.4 ± 0.06	0.2 ± 0.10	0.2 ± 0.05	0.1 ± 0.05
C 16:0	8.4 ± 2.19	6.9 ± 0.57	6.7 ± 0.04	6.9 ± 0.26	6.9 ± 1.22
C 18:0	17.6 ± 1.24	20.1 ± 1.00	20.2 ± 1.28	20.8 ± 1.97	18.2 ± 0.21
C 20:0	0.3 ± 0.18	0.0 ± 0.00	0.1 ± 0.06	0.1 ± 0.02	0.1 ± 0.04
C 22:0	1.1 ± 0.15	0.0 ± 0.00	0.0 ± 0.04	0.1 ± 0.08	0.4 ± 0.22
Sum N-0	30.1 ± 2.35	27.5 ± 0.31	27.2 ± 1.36	28.1 ± 1.91	25.7 ± 1.39
C 16:1 n-9	0.6 ± 0.15	0.7 ± 0.09	0.6 ± 0.01	0.4 ± 0.03	0.4 ± 0.03
C 18:1 n-9	13.5 ± 0.64	14.5 ± 0.31	14.2 ± 1.80	12.9 ± 0.24	11.2 ± 1.71
C 20:1 n-9	2.4 ± 0.19	1.6 ± 0.10	1.4 ± 0.08	1.2 ± 0.08	0.6 ± 0.47
C 20:3 n-9	1.9 ± 0.34	0.5 ± 0.06	0.2 ± 0.16	0.2 ± 0.02	0.3 ± 0.10
C 18:2 n-6	1.6 ± 0.27	0.6 ± 0.13	0.4 ± 0.39	0.5 ± 0.01	0.2 ± 0.20
C 20:2 n-6	0.5 ± 0.09	0.2 ± 0.19	0.3 ± 0.06	0.2 ± 0.01	0.6 ± 0.34
C 20:3 n-6	1.1 ± 0.18	0.3 ± 0.34	0.5 ± 0.02	0.3 ± 0.04	0.1 ± 0.12
C 20:4 n-6	1.8 ± 0.10	1.3 ± 0.05	1.5 ± 0.04	0.6 ± 0.02	0.8 ± 0.09
C 22:4 n-6	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 22:5 n-6	0.0 ± 0.00	0.0 ± 0.00	0.3 ± 0.04	0.3 ± 0.04	0.2 ± 0.01
Sum N-6	5.0 ± 0.38	2.4 ± 0.71	3.1 ± 0.42	1.9 ± 0.01	2.0 ± 0.08
C 18:3 n-3	0.1 ± 0.06	0.0 ± 0.00	0.0 ± 0.04	0.1 ± 0.01	0.2 ± 0.07
C 18:4 n-3	0.0 ± 0.00	0.2 ± 0.17	0.1 ± 0.06	0.2 ± 0.02	0.1 ± 0.06
C 20:3 n-3	0.2 ± 0.11	0.0 ± 0.00	0.2 ± 0.03	0.2 ± 0.02	0.1 ± 0.06
C 20:5 n-3	2.1 ± 0.18	3.0 ± 0.24	4.2 ± 0.34	2.9 ± 0.10	3.3 ± 0.39
C 22:5 n-3	5.2 ± 0.66	4.6 ± 1.66	6.5 ± 0.30	6.6 ± 0.09	6.9 ± 0.50
C 22:6 n-3	29.3 ± 2.83	36.2 ± 3.28	34.6 ± 1.21	39.3 ± 2.01	41.1 ± 1.84
Sum N-3	36.9 ± 3.53	44.0 ± 1.55	45.6 ± 1.11	49.3 ± 2.03	51.5 ± 1.90

PS 200g	NC	0.5% DHA	1% DHA	1.5% DHA	2% DHA
C 14:0	0.4 ± 0.18	0.0 ± 0.00	0.1 ± 0.08	0.3 ± 0.12	0.2 ± 0.04
C 16:0	8.4 ± 2.19	5.8 ± 0.13	6.5 ± 1.07	7.9 ± 1.40	7.8 ± 1.14
C 18:0	17.6 ± 1.24	18.8 ± 1.72	17.5 ± 0.55	19.1 ± 0.37	19.5 ± 0.40
C 20:0	0.3 ± 0.18	0.0 ± 0.00	0.1 ± 0.09	0.1 ± 0.04	0.1 ± 0.05
C 22:0	1.1 ± 0.15	0.0 ± 0.00	0.1 ± 0.12	0.2 ± 0.08	0.4 ± 0.32
Sum N-0	30.1 ± 2.35	24.7 ± 1.73	24.6 ± 0.11	27.6 ± 0.87	28.3 ± 1.55
C 16:1 n-9	0.6 ± 0.15	0.6 ± 0.15	0.5 ± 0.07	0.4 ± 0.06	0.5 ± 0.04
C 18:1 n-9	13.5 ± 0.64	11.1 ± 0.29	11.5 ± 0.53	14.9 ± 1.37	11.9 ± 1.01
C 20:1 n-9	2.4 ± 0.19	0.7 ± 0.67	1.4 ± 0.09	1.5 ± 0.15	1.2 ± 0.08
C 20:3 n-9	1.9 ± 0.34	0.0 ± 0.00	0.3 ± 0.09	0.2 ± 0.21	0.7 ± 0.45
C 18:2 n-6	1.6 ± 0.27	0.7 ± 0.10	0.5 ± 0.01	0.6 ± 0.03	0.6 ± 0.09
C 20:2 n-6	0.5 ± 0.09	0.0 ± 0.00	0.4 ± 0.13	0.3 ± 0.05	0.2 ± 0.08
C 20:3 n-6	1.1 ± 0.18	0.4 ± 0.41	0.4 ± 0.11	0.3 ± 0.02	0.8 ± 0.51
C 20:4 n-6	1.8 ± 0.10	0.6 ± 0.56	0.9 ± 0.32	0.8 ± 0.09	1.3 ± 0.17
C 22:4 n-6	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 22:5 n-6	0.0 ± 0.00	0.0 ± 0.00	0.2 ± 0.23	0.3 ± 0.07	0.2 ± 0.11
Sum N-6	5.0 ± 0.38	1.6 ± 0.25	2.6 ± 0.52	2.2 ± 0.25	3.0 ± 0.42
C 18:3 n-3	0.1 ± 0.06	0.0 ± 0.00	0.1 ± 0.05	0.0 ± 0.03	0.0 ± 0.02
C 18:4 n-3	0.0 ± 0.00	0.0 ± 0.00	0.5 ± 0.30	0.2 ± 0.01	0.3 ± 0.21
C 20:3 n-3	0.2 ± 0.11	0.0 ± 0.00	0.1 ± 0.12	0.2 ± 0.04	0.9 ± 0.74
C 20:5 n-3	2.1 ± 0.18	2.6 ± 0.27	2.5 ± 0.09	2.6 ± 0.04	3.4 ± 0.29
C 22:5 n-3	5.2 ± 0.66	4.7 ± 0.12	4.1 ± 0.15	3.4 ± 0.15	3.7 ± 0.21
C 22:6 n-3	29.3 ± 2.83	44.3 ± 1.15	42.3 ± 1.50	39.1 ± 1.24	38.9 ± 1.51
Sum N-3	36.9 ± 3.53	51.6 ± 1.53	49.7 ± 1.61	45.5 ± 1.45	47.2 ± 2.40

PS 200g	NC	0.5% EPA+DHA	1% EPA+DHA	1.5% EPA+DHA	2% EPA+DHA	CC
C 14:0	0.4 ± 0.18	0.2 ± 0.00	0.2 ± 0.03	0.1 ± 0.04	0.2 ± 0.02	0.2 ± 0.01
C 16:0	8.4 ± 2.19	6.8 ± 0.69	7.6 ± 0.33	6.0 ± 0.44	5.9 ± 0.52	6.6 ± 0.24
C 18:0	17.6 ± 1.24	19.8 ± 1.60	19.8 ± 1.16	19.4 ± 1.31	21.1 ± 0.04	20.6 ± 0.47
C 20:0	0.3 ± 0.18	0.2 ± 0.04	0.2 ± 0.01	0.2 ± 0.06	0.1 ± 0.03	0.1 ± 0.06
C 22:0	1.1 ± 0.15	0.1 ± 0.13	0.0 ± 0.00	0.2 ± 0.01	0.1 ± 0.06	0.0 ± 0.00
Sum N-0	30.1 ± 2.35	27.2 ± 1.99	27.9 ± 0.80	26.2 ± 1.46	27.6 ± 0.42	27.4 ± 0.30
C 16:1 n-9	0.6 ± 0.15	0.6 ± 0.03	0.5 ± 0.03	0.6 ± 0.26	0.4 ± 0.01	0.4 ± 0.04
C 18:1 n-9	13.5 ± 0.64	12.2 ± 2.08	14.7 ± 0.12	12.2 ± 1.83	12.4 ± 0.41	12.6 ± 0.67
C 20:1 n-9	2.4 ± 0.19	1.3 ± 0.02	1.3 ± 0.00	1.1 ± 0.38	1.1 ± 0.01	1.0 ± 0.02
C 20:3 n-9	1.9 ± 0.34	0.3 ± 0.23	0.1 ± 0.02	0.5 ± 0.05	0.2 ± 0.00	0.2 ± 0.05
C 18:2 n-6	1.6 ± 0.27	0.7 ± 0.01	0.6 ± 0.03	0.6 ± 0.05	0.5 ± 0.03	0.5 ± 0.14
C 20:2 n-6	0.5 ± 0.09	0.3 ± 0.04	0.2 ± 0.01	0.3 ± 0.01	0.1 ± 0.07	0.1 ± 0.06
C 20:3 n-6	1.1 ± 0.18	0.7 ± 0.03	0.4 ± 0.04	0.3 ± 0.08	0.2 ± 0.01	0.2 ± 0.03
C 20:4 n-6	1.8 ± 0.10	1.5 ± 0.07	1.1 ± 0.19	0.9 ± 0.29	0.8 ± 0.11	0.8 ± 0.22
C 22:4 n-6	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 22:5 n-6	0.0 ± 0.00	0.3 ± 0.02	0.3 ± 0.04	0.1 ± 0.13	0.3 ± 0.02	0.1 ± 0.07
Sum N-6	5.0 ± 0.38	3.5 ± 0.04	2.5 ± 0.24	2.2 ± 0.44	1.9 ± 0.04	1.7 ± 0.24
C 18:3 n-3	0.1 ± 0.06	0.2 ± 0.07	0.0 ± 0.00	0.1 ± 0.08	0.1 ± 0.01	0.1 ± 0.01
C 18:4 n-3	0.0 ± 0.00	0.2 ± 0.09	0.2 ± 0.02	0.2 ± 0.08	0.1 ± 0.01	0.1 ± 0.06
C 20:3 n-3	0.2 ± 0.11	0.5 ± 0.35	0.2 ± 0.00	0.2 ± 0.01	0.1 ± 0.07	0.1 ± 0.07
C 20:5 n-3	2.1 ± 0.18	2.9 ± 0.15	3.0 ± 0.29	2.8 ± 0.39	2.9 ± 0.19	2.9 ± 0.32
C 22:5 n-3	5.2 ± 0.66	5.0 ± 0.59	4.3 ± 0.03	4.9 ± 0.16	5.4 ± 0.18	4.3 ± 0.00
C 22:6 n-3	29.3 ± 2.83	39.2 ± 2.73	38.5 ± 0.26	42.2 ± 2.23	42.7 ± 0.93	43.1 ± 0.19
Sum N-3	36.9 ± 3.53	47.9 ± 3.67	46.2 ± 0.01	50.4 ± 2.93	51.2 ± 1.23	50.6 ± 0.62

Figure 5.3 Fatty acid compositions (% of total fatty acids) of PS at fish body weight (BW) 200g. PS: Phosphatidylserine; NC: negative control (0% EPA+DHA); CC: commercial control (2.2% EPA+DHA); EPA: Eicosapentaenoic acid (20:5 n-3); DHA: Docosahexaenoic acid (22:5 n-3). Data are expressed as mean (n=1, 2 or 3) ± standard error of mean (SEM). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish.

PS 400g	NC	0.5% EPA	1% EPA	1.5% EPA	2% EPA
C 14:0	0.1 ±	0.8 ± 0.66	0.4 ± 0.12	0.3 ± 0.27	0.1 ± 0.05
C 16:0	18.4 ±	10.2 ± 1.18	6.4 ± 1.11	6.9 ± 1.44	9.1 ± 0.83
C 18:0	8.2 ±	19.0 ± 2.19	20.6 ± 1.46	20.7 ± 0.47	19.3 ± 1.38
C 20:0	0.1 ±	0.2 ± 0.07	0.1 ± 0.02	0.2 ± 0.03	0.1 ± 0.01
C 22:0	0.2 ±	0.2 ± 0.01	0.1 ± 0.05	0.3 ± 0.02	0.3 ± 0.01
Sum N-0	27.2 ±	30.6 ± 4.10	27.7 ± 0.27	28.5 ± 2.27	29.2 ± 0.46
C 16:1 n-9	0.8 ±	1.1 ± 0.22	0.6 ± 0.02	0.7 ± 0.27	0.7 ± 0.03
C 18:1 n-9	14.1 ±	17.7 ± 1.61	14.6 ± 1.21	12.7 ± 1.53	16.1 ± 0.64
C 20:1 n-9	1.3 ±	1.6 ± 0.19	1.3 ± 0.15	1.5 ± 0.37	0.9 ± 0.30
C 20:3 n-9	0.3 ±	0.2 ± 0.18	0.5 ± 0.04	0.6 ± 0.15	0.5 ± 0.04
C 18:2 n-6	1.1 ±	0.8 ± 0.08	0.6 ± 0.06	0.5 ± 0.06	0.5 ± 0.01
C 20:2 n-6	0.2 ±	0.2 ± 0.03	0.2 ± 0.10	0.3 ± 0.01	0.1 ± 0.11
C 20:3 n-6	1.0 ±	0.7 ± 0.04	0.5 ± 0.01	0.3 ± 0.08	0.4 ± 0.09
C 20:4 n-6	4.6 ±	1.7 ± 0.38	1.2 ± 0.21	1.2 ± 0.32	1.6 ± 0.26
C 22:4 n-6	0.0 ±	0.2 ± 0.21	0.1 ± 0.15	0.2 ± 0.16	0.1 ± 0.10
C 22:5 n-6	0.0 ±	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.14	0.0 ± 0.00
Sum N-6	5.9 ±	3.7 ± 0.19	2.6 ± 0.31	2.6 ± 0.55	2.8 ± 0.85
C 18:3 n-3	0.1 ±	0.2 ± 0.11	0.1 ± 0.07	0.1 ± 0.03	0.0 ± 0.04
C 18:4 n-3	0.0 ±	0.3 ± 0.03	0.1 ± 0.02	0.2 ± 0.00	0.3 ± 0.14
C 20:3 n-3	0.1 ±	0.2 ± 0.03	0.1 ± 0.05	0.1 ± 0.00	0.1 ± 0.00
C 20:5 n-3	4.2 ±	3.2 ± 0.24	3.5 ± 0.44	2.5 ± 1.85	4.5 ± 0.83
C 22:5 n-3	4.2 ±	4.7 ± 0.97	6.5 ± 0.18	6.9 ± 0.85	6.2 ± 0.39
C 22:6 n-3	30.8 ±	26.3 ± 2.27	37.1 ± 1.65	36.6 ± 2.47	29.0 ± 1.67
Sum N-3	39.5 ±	34.9 ± 3.31	47.3 ± 1.91	46.4 ± 5.13	40.2 ± 1.14

PS 400g	NC	0.5% DHA	1% DHA	1.5% DHA	2% DHA
C 14:0	0.1 ±	0.2 ±	0.3 ± 0.28	0.2 ±	0.4 ± 0.30
C 16:0	18.4 ±	5.8 ±	5.7 ± 0.01	6.7 ±	7.3 ± 0.83
C 18:0	8.2 ±	19.9 ±	20.4 ± 3.06	21.2 ±	18.8 ± 1.27
C 20:0	0.1 ±	0.0 ±	0.1 ± 0.08	0.2 ±	0.1 ± 0.06
C 22:0	0.2 ±	1.2 ±	0.0 ± 0.00	0.3 ±	0.1 ± 0.06
Sum N-0	27.2 ±	27.3 ±	26.9 ± 2.33	29.1 ±	26.9 ± 0.73
C 16:1 n-9	0.8 ±	0.2 ±	0.7 ± 0.13	0.7 ±	1.0 ± 0.29
C 18:1 n-9	14.1 ±	7.2 ±	14.5 ± 0.57	14.4 ±	18.2 ± 1.91
C 20:1 n-9	1.3 ±	1.0 ±	1.5 ± 0.10	1.2 ±	1.5 ± 0.20
C 20:3 n-9	0.3 ±	0.0 ±	0.6 ± 0.06	0.5 ±	0.6 ± 0.20
C 18:2 n-6	1.1 ±	0.6 ±	0.6 ± 0.09	0.6 ±	0.7 ± 0.11
C 20:2 n-6	0.2 ±	0.3 ±	0.3 ± 0.03	0.3 ±	0.3 ± 0.02
C 20:3 n-6	1.0 ±	1.6 ±	0.6 ± 0.09	0.4 ±	0.2 ± 0.11
C 20:4 n-6	4.6 ±	2.4 ±	1.8 ± 0.45	1.5 ±	1.2 ± 0.25
C 22:4 n-6	0.0 ±	0.4 ±	0.0 ± 0.00	0.0 ±	0.1 ± 0.10
C 22:5 n-6	0.0 ±	0.0 ±	0.0 ± 0.00	0.0 ±	0.0 ± 0.00
Sum N-6	5.9 ±	12.2 ±	3.3 ± 0.49	2.8 ±	2.5 ± 0.28
C 18:3 n-3	0.1 ±	0.1 ±	0.0 ± 0.00	0.1 ±	0.0 ± 0.02
C 18:4 n-3	0.0 ±	0.2 ±	0.1 ± 0.07	0.1 ±	0.0 ± 0.05
C 20:3 n-3	0.1 ±	0.0 ±	0.0 ± 0.00	0.2 ±	0.1 ± 0.05
C 20:5 n-3	4.2 ±	8.7 ±	3.3 ± 0.37	2.9 ±	3.0 ± 0.29
C 22:5 n-3	4.2 ±	3.3 ±	4.3 ± 0.89	3.7 ±	3.5 ± 0.17
C 22:6 n-3	30.8 ±	31.8 ±	36.9 ± 1.33	37.0 ±	35.1 ± 3.03
Sum N-3	39.5 ±	44.1 ±	44.5 ± 1.78	43.9 ±	41.8 ± 3.57

PS 400g	NC	0.5% EPA+DHA	1% EPA+DHA	1.5% EPA+DHA	2% EPA+DHA	NC
C 14:0	0.1 ±	0.6 ± 0.29	0.1 ±	0.3 ± 0.27	0.0 ± 0.03	0.1 ±
C 16:0	18.4 ±	9.1 ± 2.12	6.3 ±	6.9 ± 2.08	5.8 ± 0.59	18.4 ±
C 18:0	8.2 ±	19.3 ± 1.93	21.7 ±	18.9 ± 0.29	20.1 ± 0.95	8.2 ±
C 20:0	0.1 ±	0.1 ± 0.06	0.1 ±	0.1 ± 0.00	0.1 ± 0.02	0.1 ±
C 22:0	0.2 ±	0.1 ± 0.12	0.1 ±	0.3 ± 0.01	0.3 ± 0.10	0.2 ±
Sum N-0	27.2 ±	29.7 ± 1.85	28.3 ±	26.9 ± 2.65	26.6 ± 1.64	27.2 ±
C 16:1 n-9	0.8 ±	0.8 ± 0.20	0.4 ±	0.9 ± 0.43	0.4 ± 0.02	0.8 ±
C 18:1 n-9	14.1 ±	16.3 ± 0.63	15.0 ±	12.5 ± 0.09	11.4 ± 2.30	14.1 ±
C 20:1 n-9	1.3 ±	1.6 ± 0.10	1.4 ±	1.1 ± 0.04	1.1 ± 0.13	1.3 ±
C 20:3 n-9	0.3 ±	0.6 ± 0.01	0.4 ±	0.5 ± 0.02	0.4 ± 0.18	0.3 ±
C 18:2 n-6	1.1 ±	0.4 ± 0.34	0.1 ±	0.5 ± 0.02	0.5 ± 0.03	1.1 ±
C 20:2 n-6	0.2 ±	0.3 ± 0.02	0.3 ±	0.3 ± 0.01	0.4 ± 0.12	0.2 ±
C 20:3 n-6	1.0 ±	0.7 ± 0.18	0.5 ±	0.4 ± 0.04	0.5 ± 0.14	1.0 ±
C 20:4 n-6	4.6 ±	1.3 ± 0.35	1.4 ±	1.8 ± 0.21	3.3 ± 0.33	4.6 ±
C 22:4 n-6	0.0 ±	0.0 ± 0.00	0.0 ±	0.2 ± 0.16	0.3 ± 0.05	0.0 ±
C 22:5 n-6	0.0 ±	0.1 ± 0.12	0.0 ±	0.0 ± 0.00	0.0 ± 0.00	0.0 ±
Sum N-6	5.9 ±	2.8 ± 0.09	2.3 ±	3.1 ± 0.40	4.9 ± 2.02	5.9 ±
C 18:3 n-3	0.1 ±	0.0 ± 0.04	0.1 ±	0.1 ± 0.01	0.1 ± 0.03	0.1 ±
C 18:4 n-3	0.0 ±	0.1 ± 0.10	0.1 ±	0.2 ± 0.03	0.3 ± 0.21	0.0 ±
C 20:3 n-3	0.1 ±	0.1 ± 0.08	0.2 ±	0.1 ± 0.01	0.1 ± 0.01	0.1 ±
C 20:5 n-3	4.2 ±	2.6 ± 0.16	3.2 ±	4.0 ± 0.53	6.3 ± 2.46	4.2 ±
C 22:5 n-3	4.2 ±	4.2 ± 0.37	5.1 ±	5.1 ± 0.36	5.1 ± 0.92	4.2 ±
C 22:6 n-3	30.8 ±	31.5 ± 2.31	36.3 ±	38.9 ± 0.69	37.3 ± 2.24	30.8 ±
Sum N-3	39.5 ±	38.5 ± 3.05	45.0 ±	48.4 ± 1.61	49.3 ± 0.52	39.5 ±

Figure 5.4 Fatty acid compositions (% of total fatty acids) of PS at fish body weight (BW) 400g. PS: Phosphatidylserine; NC: negative control (0% EPA+DHA); CC: commercial control (2.2% EPA+DHA); EPA: Eicosapentaenoic acid (20:5 n-3); DHA: Docosahexaenoic acid (22:5 n-3). Data are expressed as mean (n=1, 2 or 3) ± standard error of mean (SEM). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish.

PI 200g	NC	0.5% EPA	1% EPA	1.5% EPA	2% EPA
C 14:0	0.5 ± 0.27	0.2 ± 0.04	0.5 ± 0.06	0.2 ± 0.02	0.2 ± 0.12
C 16:0	11.9 ± 2.76	6.6 ± 0.26	7.4 ± 1.33	9.3 ± 1.48	8.3 ± 1.63
C 18:0	19.6 ± 0.66	18.2 ± 1.03	16.5 ± 3.20	22.7 ± 1.98	21.0 ± 2.32
C 20:0	0.7 ± 0.09	0.1 ± 0.06	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.03
C 22:0	1.8 ± 0.56	0.2 ± 0.01	0.2 ± 0.10	0.3 ± 0.03	0.3 ± 0.01
Sum N-0	36.6 ± 3.74	35.4 ± 0.90	35.9 ± 0.26	30.3 ± 2.19	28.7 ± 3.80
C 16:1 n-9	0.5 ± 0.13	0.6 ± 0.37	0.6 ± 0.40	0.3 ± 0.05	0.3 ± 0.10
C 18:1 n-9	10.5 ± 2.71	10.2 ± 2.22	12.6 ± 2.03	6.8 ± 0.34	6.7 ± 1.48
C 20:1 n-9	2.8 ± 0.60	1.4 ± 0.04	1.5 ± 0.25	1.3 ± 0.07	1.2 ± 0.26
C 20:3 n-9	3.9 ± 0.49	0.4 ± 0.13	0.3 ± 0.31	0.5 ± 0.04	0.5 ± 0.12
C 18:2 n-6	2.1 ± 0.77	0.5 ± 0.09	0.6 ± 0.03	0.6 ± 0.14	0.5 ± 0.07
C 20:2 n-6	0.3 ± 0.13	0.1 ± 0.09	0.2 ± 0.07	0.2 ± 0.08	0.1 ± 0.06
C 20:3 n-6	1.9 ± 0.36	0.9 ± 0.38	0.9 ± 0.28	1.0 ± 0.13	0.7 ± 0.12
C 20:4 n-6	11.1 ± 0.42	5.8 ± 1.15	6.3 ± 2.89	9.2 ± 1.38	7.9 ± 2.09
C 22:4 n-6	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 22:5 n-6	0.0 ± 0.00	0.1 ± 0.13	0.0 ± 0.00	0.1 ± 0.12	0.1 ± 0.08
Sum N-6	15.8 ± 2.02	15.2 ± 0.56	13.4 ± 0.12	10.4 ± 1.27	9.5 ± 2.21
C 18:3 n-3	0.5 ± 0.16	0.0 ± 0.03	0.1 ± 0.03	0.1 ± 0.07	0.1 ± 0.04
C 18:4 n-3	0.1 ± 0.09	0.1 ± 0.00	0.1 ± 0.09	0.1 ± 0.10	0.1 ± 0.06
C 20:3 n-3	0.9 ± 0.94	0.1 ± 0.05	0.2 ± 0.18	0.0 ± 0.00	0.0 ± 0.00
C 20:5 n-3	7.6 ± 1.40	7.7 ± 0.13	11.1 ± 1.97	18.5 ± 2.41	16.7 ± 2.10
C 22:5 n-3	1.7 ± 0.53	3.7 ± 0.30	4.0 ± 0.05	3.3 ± 1.09	3.9 ± 1.62
C 22:6 n-3	10.0 ± 1.84	33.7 ± 1.88	23.7 ± 1.58	18.0 ± 2.62	22.5 ± 2.82
Sum N-3	20.9 ± 5.36	32.3 ± 1.37	32.5 ± 2.00	45.0 ± 4.70	37.5 ± 3.04

PI 200g	NC	0.5% DHA	1% DHA	1.5% DHA	2% DHA
C 14:0	0.5 ± 0.27	0.1 ± 0.10	0.1 ± 0.13	0.3 ± 0.30	1.1 ± 0.48
C 16:0	11.9 ± 2.76	9.7 ± 0.32	9.1 ± 1.83	9.9 ± 1.70	10.6 ± 0.92
C 18:0	19.6 ± 0.66	22.5 ± 1.49	21.4 ± 3.18	17.4 ± 2.35	23.4 ± 1.00
C 20:0	0.7 ± 0.09	0.0 ± 0.05	0.0 ± 0.00	0.2 ± 0.15	0.0 ± 0.00
C 22:0	1.8 ± 0.56	0.3 ± 0.08	0.1 ± 0.13	0.3 ± 0.03	0.6 ± 0.52
Sum N-0	36.6 ± 3.74	28.7 ± 1.01	28.9 ± 0.86	34.8 ± 5.58	32.3 ± 3.20
C 16:1 n-9	0.5 ± 0.13	0.5 ± 0.06	0.3 ± 0.00	0.6 ± 0.23	0.2 ± 0.12
C 18:1 n-9	10.5 ± 2.71	7.3 ± 0.03	6.7 ± 0.46	11.8 ± 2.72	8.0 ± 0.70
C 20:1 n-9	2.8 ± 0.60	1.6 ± 0.05	1.3 ± 0.29	1.7 ± 0.32	0.9 ± 0.45
C 20:3 n-9	3.9 ± 0.49	1.0 ± 0.09	0.5 ± 0.03	0.4 ± 0.28	1.4 ± 0.91
C 18:2 n-6	2.1 ± 0.77	0.6 ± 0.05	0.5 ± 0.05	1.6 ± 0.04	0.8 ± 0.24
C 20:2 n-6	0.3 ± 0.13	0.4 ± 0.06	0.3 ± 0.12	0.3 ± 0.00	0.0 ± 0.05
C 20:3 n-6	1.9 ± 0.36	2.2 ± 0.00	1.3 ± 0.21	1.0 ± 0.17	1.2 ± 0.40
C 20:4 n-6	11.1 ± 0.42	13.5 ± 0.41	10.8 ± 1.97	8.3 ± 2.42	10.8 ± 0.79
C 22:4 n-6	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 22:5 n-6	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Sum N-6	15.8 ± 2.02	11.4 ± 1.58	11.7 ± 0.32	9.1 ± 0.74	13.9 ± 2.00
C 18:3 n-3	0.5 ± 0.16	0.0 ± 0.00	0.0 ± 0.00	0.3 ± 0.19	0.0 ± 0.00
C 18:4 n-3	0.1 ± 0.09	0.3 ± 0.06	0.0 ± 0.00	0.2 ± 0.07	0.1 ± 0.06
C 20:3 n-3	0.9 ± 0.94	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 20:5 n-3	7.6 ± 1.40	15.4 ± 1.31	14.9 ± 2.54	12.3 ± 3.74	15.7 ± 0.79
C 22:5 n-3	1.7 ± 0.53	0.8 ± 0.48	1.8 ± 0.58	1.9 ± 0.14	1.1 ± 0.59
C 22:6 n-3	10.0 ± 1.84	14.5 ± 2.89	21.5 ± 3.34	23.8 ± 0.19	16.5 ± 2.14
Sum N-3	20.9 ± 5.36	39.2 ± 3.79	42.1 ± 1.02	36.4 ± 9.34	36.8 ± 4.65

PI 200g	NC	0.5% EPA+DHA	1% EPA+DHA	1.5% EPA+DHA	2% EPA+DHA	CC
C 14:0	0.5 ± 0.27	0.4 ± 0.09	0.2 ± 0.05	0.4 ± 0.04	0.4 ± 0.34	0.6 ± 0.53
C 16:0	11.9 ± 2.76	11.6 ± 2.45	8.9 ± 0.17	7.2 ± 1.81	9.0 ± 1.32	12.8 ± 1.18
C 18:0	19.6 ± 0.66	18.8 ± 0.63	20.1 ± 0.03	21.0 ± 2.07	21.9 ± 1.46	22.3 ± 1.17
C 20:0	0.7 ± 0.09	0.1 ± 0.08	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.14
C 22:0	1.8 ± 0.56	0.3 ± 0.05	0.3 ± 0.02	0.3 ± 0.31	0.1 ± 0.05	0.2 ± 0.15
Sum N-0	36.6 ± 3.74	29.3 ± 2.19	24.3 ± 4.32	29.9 ± 6.35	36.0 ± 5.08	30.0 ± 2.58
C 16:1 n-9	0.5 ± 0.13	0.5 ± 0.15	0.3 ± 0.03	0.2 ± 0.06	0.5 ± 0.41	0.4 ± 0.25
C 18:1 n-9	10.5 ± 2.71	11.3 ± 0.98	7.5 ± 0.80	6.5 ± 0.98	6.2 ± 0.30	7.9 ± 0.38
C 20:1 n-9	2.8 ± 0.60	1.7 ± 0.34	1.3 ± 0.03	1.2 ± 0.24	1.2 ± 0.22	2.3 ± 0.63
C 20:3 n-9	3.9 ± 0.49	0.3 ± 0.03	0.2 ± 0.08	0.6 ± 0.13	0.7 ± 0.36	0.7 ± 0.30
C 18:2 n-6	2.1 ± 0.77	1.9 ± 0.28	0.7 ± 0.31	0.9 ± 0.49	0.8 ± 0.29	0.3 ± 0.18
C 20:2 n-6	0.3 ± 0.13	0.2 ± 0.11	0.2 ± 0.01	0.3 ± 0.34	0.2 ± 0.16	0.2 ± 0.10
C 20:3 n-6	1.9 ± 0.36	1.5 ± 0.04	1.4 ± 0.13	0.7 ± 0.36	0.6 ± 0.29	0.6 ± 0.29
C 20:4 n-6	11.1 ± 0.42	9.6 ± 0.81	9.6 ± 0.60	7.8 ± 0.36	9.2 ± 0.75	7.6 ± 0.97
C 22:4 n-6	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.3 ± 0.35	0.0 ± 0.00
C 22:5 n-6	0.0 ± 0.00	0.1 ± 0.12	0.1 ± 0.09	0.0 ± 0.00	0.1 ± 0.07	0.0 ± 0.00
Sum N-6	15.8 ± 2.02	15.3 ± 2.09	9.3 ± 4.37	6.0 ± 0.72	11.0 ± 2.84	12.9 ± 1.60
C 18:3 n-3	0.5 ± 0.16	0.4 ± 0.40	0.1 ± 0.13	0.0 ± 0.00	0.0 ± 0.03	0.0 ± 0.03
C 18:4 n-3	0.1 ± 0.09	0.2 ± 0.02	0.2 ± 0.01	0.1 ± 0.15	0.0 ± 0.05	0.1 ± 0.07
C 20:3 n-3	0.9 ± 0.94	0.0 ± 0.00	0.0 ± 0.00	0.2 ± 0.25	0.1 ± 0.05	0.0 ± 0.03
C 20:5 n-3	7.6 ± 1.40	11.4 ± 0.97	14.2 ± 0.01	14.0 ± 1.84	17.2 ± 1.65	15.1 ± 1.39
C 22:5 n-3	1.7 ± 0.53	2.2 ± 0.96	2.5 ± 0.01	3.2 ± 1.78	2.6 ± 0.60	2.0 ± 0.38
C 22:6 n-3	10.0 ± 1.84	18.6 ± 2.33	24.8 ± 0.85	27.8 ± 1.44	22.4 ± 1.82	18.6 ± 2.09
Sum N-3	20.9 ± 5.36	38.6 ± 6.00	52.0 ± 9.97	49.0 ± 8.52	37.6 ± 7.96	35.4 ± 6.75

Figure 5.5 Fatty acid compositions (% of total fatty acids) of PI at fish body weight (BW) 200g. PI: Phosphatidylinositol; NC: negative control (0% EPA+DHA); CC: commercial control (2.2% EPA+DHA); EPA: Eicosapentaenoic acid (20:5 n-3); DHA: Docosahexaenoic acid (22:5 n-3). Data are expressed as mean (n=1, 2 or 3) ± standard error of mean (SEM). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish.

PI 400g	NC	0.5% EPA	1% EPA	1.5% EPA	2% EPA
C 14:0	0.1 ± 0.05	0.1 ± 0.12	0.3 ± 0.13	0.2 ± 0.20	0.6 ± 0.41
C 16:0	6.2 ± 0.20	7.1 ± 0.67	7.8 ± 1.96	8.1 ± 0.61	10.0 ± 1.51
C 18:0	19.7 ± 1.62	18.6 ± 0.31	19.3 ± 1.64	22.5 ± 0.88	16.2 ± 2.34
C 20:0	0.1 ± 0.03	0.1 ± 0.05	0.0 ± 0.00	0.1 ± 0.05	0.2 ± 0.13
C 22:0	0.3 ± 0.02	0.2 ± 0.04	0.3 ± 0.09	0.1 ± 0.03	0.2 ± 0.09
Sum N-0	22.9 ± 2.51	27.2 ± 0.74	28.7 ± 0.83	25.9 ± 2.08	28.0 ± 1.13
C 16:1 n-9	0.7 ± 0.42	0.3 ± 0.03	0.6 ± 0.12	0.3 ± 0.08	1.1 ± 0.70
C 18:1 n-9	10.0 ± 2.08	7.0 ± 0.43	8.6 ± 1.61	8.1 ± 1.59	14.7 ± 2.53
C 20:1 n-9	1.3 ± 0.11	1.1 ± 0.06	1.4 ± 0.21	1.3 ± 0.21	1.8 ± 0.74
C 20:3 n-9	0.4 ± 0.13	0.4 ± 0.36	0.8 ± 0.40	0.8 ± 0.39	0.4 ± 0.11
C 18:2 n-6	0.6 ± 0.03	0.6 ± 0.22	0.6 ± 0.22	0.8 ± 0.36	0.4 ± 0.11
C 20:2 n-6	0.4 ± 0.09	0.3 ± 0.01	0.2 ± 0.04	0.3 ± 0.05	0.2 ± 0.13
C 20:3 n-6	2.0 ± 0.12	1.5 ± 0.05	1.1 ± 0.19	0.8 ± 0.08	0.5 ± 0.05
C 20:4 n-6	9.1 ± 0.58	8.2 ± 0.52	7.5 ± 0.05	8.8 ± 0.28	2.9 ± 0.87
C 22:4 n-6	0.0 ± 0.00	0.4 ± 0.00	0.0 ± 0.00	0.1 ± 0.12	0.1 ± 0.11
C 22:5 n-6	0.7 ± 0.36	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.12	0.0 ± 0.00
Sum N-6	9.0 ± 0.94	7.5 ± 0.83	9.5 ± 1.12	7.6 ± 2.73	10.5 ± 0.22
C 18:3 n-3	0.1 ± 0.01	0.2 ± 0.08	0.1 ± 0.11	0.2 ± 0.05	0.1 ± 0.11
C 18:4 n-3	0.1 ± 0.05	0.1 ± 0.06	0.1 ± 0.06	0.1 ± 0.06	0.1 ± 0.04
C 20:3 n-3	0.1 ± 0.05	0.1 ± 0.05	0.1 ± 0.06	0.1 ± 0.05	0.1 ± 0.04
C 20:5 n-3	4.1 ± 0.38	9.5 ± 0.57	12.3 ± 0.94	15.3 ± 0.93	4.7 ± 0.33
C 22:5 n-3	5.6 ± 0.48	5.7 ± 0.16	4.7 ± 1.73	4.2 ± 1.61	5.3 ± 1.03
C 22:6 n-3	28.4 ± 1.53	31.7 ± 1.88	25.6 ± 2.43	21.1 ± 2.53	26.6 ± 2.78
Sum N-3	36.7 ± 4.51	51.5 ± 1.04	48.1 ± 0.04	41.0 ± 1.56	45.9 ± 2.32

PI 400g	NC	0.5% DHA	1% DHA	1.5% DHA	2% DHA
C 14:0	0.1 ± 0.05	0.1 ± 0.12	0.2 ± 0.04	0.1 ± 0.06	0.2 ± 0.02
C 16:0	6.2 ± 0.20	7.4 ± 0.49	8.0 ± 0.91	6.5 ± 0.98	7.7 ± 0.02
C 18:0	19.7 ± 1.62	14.6 ± 2.07	15.5 ± 2.11	20.3 ± 0.63	15.4 ± 3.51
C 20:0	0.1 ± 0.03	0.1 ± 0.08	0.0 ± 0.00	0.2 ± 0.10	0.0 ± 0.03
C 22:0	0.3 ± 0.02	0.2 ± 0.05	0.5 ± 0.22	0.2 ± 0.15	0.1 ± 0.11
Sum N-0	22.9 ± 2.51	23.4 ± 3.29	27.9 ± 0.49	26.2 ± 1.31	27.5 ± 1.67
C 16:1 n-9	0.7 ± 0.42	1.4 ± 0.82	1.3 ± 0.88	0.3 ± 0.01	1.3 ± 0.77
C 18:1 n-9	10.0 ± 2.08	18.4 ± 1.02	13.6 ± 0.64	6.9 ± 0.66	12.6 ± 2.41
C 20:1 n-9	1.3 ± 0.11	1.6 ± 0.14	1.7 ± 0.19	0.7 ± 0.13	1.4 ± 0.20
C 20:3 n-9	0.4 ± 0.13	0.1 ± 0.00	0.7 ± 0.60	0.2 ± 0.15	0.5 ± 0.34
C 18:2 n-6	0.6 ± 0.03	0.9 ± 0.19	0.8 ± 0.10	0.4 ± 0.01	0.7 ± 0.06
C 20:2 n-6	0.4 ± 0.09	0.3 ± 0.27	0.3 ± 0.02	0.3 ± 0.27	0.3 ± 0.02
C 20:3 n-6	2.0 ± 0.12	0.8 ± 0.07	0.8 ± 0.27	0.9 ± 0.01	0.6 ± 0.21
C 20:4 n-6	9.1 ± 0.58	2.7 ± 1.28	7.3 ± 0.46	7.6 ± 1.24	6.0 ± 0.83
C 22:4 n-6	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 22:5 n-6	0.7 ± 0.36	0.2 ± 0.23	0.0 ± 0.00	0.2 ± 0.00	0.0 ± 0.00
Sum N-6	9.0 ± 0.94	2.9 ± 0.57	3.5 ± 0.34	7.8 ± 0.77	8.2 ± 1.71
C 18:3 n-3	0.1 ± 0.01	0.1 ± 0.07	0.1 ± 0.09	0.5 ± 0.49	0.1 ± 0.04
C 18:4 n-3	0.1 ± 0.05	0.3 ± 0.12	0.1 ± 0.08	0.1 ± 0.08	0.1 ± 0.04
C 20:3 n-3	0.1 ± 0.05	0.1 ± 0.07	0.1 ± 0.10	0.2 ± 0.06	0.1 ± 0.06
C 20:5 n-3	4.1 ± 0.38	2.3 ± 0.30	10.0 ± 0.77	5.8 ± 0.73	9.7 ± 0.77
C 22:5 n-3	5.6 ± 0.48	2.7 ± 1.06	1.9 ± 0.18	3.2 ± 0.12	2.3 ± 0.38
C 22:6 n-3	28.4 ± 1.53	28.9 ± 2.71	22.6 ± 0.67	34.8 ± 1.74	26.1 ± 3.14
Sum N-3	36.7 ± 4.51	22.0 ± 0.76	39.3 ± 0.20	42.1 ± 1.75	43.5 ± 4.64

PI 400g	NC	0.5% EPA+DHA	1% EPA+DHA	1.5% EPA+DHA	2% EPA+DHA	CC
C 14:0	0.1 ± 0.05	0.1 ± 0.01	0.1 ± 0.15	0.1 ± 0.06	0.2 ± 0.08	0.1 ± 0.09
C 16:0	6.2 ± 0.20	6.3 ± 0.03	5.8 ± 1.19	8.0 ± 0.77	6.8 ± 1.26	7.0 ± 1.56
C 18:0	19.7 ± 1.62	20.9 ± 0.02	15.8 ± 1.36	22.2 ± 1.20	17.2 ± 2.88	20.9 ± 1.42
C 20:0	0.1 ± 0.03	0.1 ± 0.01	0.0 ± 0.04	0.0 ± 0.00	0.2 ± 0.11	0.1 ± 0.03
C 22:0	0.3 ± 0.02	0.1 ± 0.00	0.1 ± 0.10	0.1 ± 0.13	0.6 ± 0.25	0.3 ± 0.14
Sum N-0	22.9 ± 2.51	27.4 ± 0.57	31.9 ± 0.96	30.6 ± 4.15	30.8 ± 0.50	20.6 ± 1.60
C 16:1 n-9	0.7 ± 0.42	0.4 ± 0.19	1.3 ± 1.09	0.3 ± 0.04	0.6 ± 0.15	0.2 ± 0.08
C 18:1 n-9	10.0 ± 2.08	8.9 ± 2.41	13.4 ± 2.19	5.5 ± 0.45	10.6 ± 2.34	7.1 ± 0.62
C 20:1 n-9	1.3 ± 0.11	1.1 ± 0.15	1.2 ± 0.43	1.2 ± 0.27	1.8 ± 0.39	1.1 ± 0.28
C 20:3 n-9	0.4 ± 0.13	0.4 ± 0.03	0.2 ± 0.02	1.0 ± 0.07	0.6 ± 0.37	0.8 ± 0.18
C 18:2 n-6	0.6 ± 0.03	0.5 ± 0.06	0.5 ± 0.16	0.6 ± 0.16	1.0 ± 0.26	0.5 ± 0.14
C 20:2 n-6	0.4 ± 0.09	0.3 ± 0.06	0.4 ± 0.07	0.1 ± 0.11	0.2 ± 0.08	0.3 ± 0.07
C 20:3 n-6	2.0 ± 0.12	1.3 ± 0.04	0.7 ± 0.08	1.2 ± 0.02	0.5 ± 0.32	0.7 ± 0.08
C 20:4 n-6	9.1 ± 0.58	6.3 ± 1.64	3.8 ± 0.48	10.6 ± 1.49	4.5 ± 0.29	6.6 ± 1.62
C 22:4 n-6	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.13	0.1 ± 0.11	0.0 ± 0.00
C 22:5 n-6	0.7 ± 0.36	0.2 ± 0.19	0.2 ± 0.17	0.0 ± 0.00	0.1 ± 0.07	0.0 ± 0.04
Sum N-6	9.0 ± 0.94	11.7 ± 0.27	14.8 ± 1.00	15.4 ± 2.46	13.9 ± 0.64	8.5 ± 1.41
C 18:3 n-3	0.1 ± 0.01	0.0 ± 0.04	0.1 ± 0.01	0.7 ± 0.47	0.2 ± 0.11	0.1 ± 0.06
C 18:4 n-3	0.1 ± 0.05	0.1 ± 0.08	0.1 ± 0.01	0.1 ± 0.07	0.0 ± 0.03	0.1 ± 0.05
C 20:3 n-3	0.1 ± 0.05	0.1 ± 0.03	0.1 ± 0.01	0.1 ± 0.07	0.6 ± 0.56	0.1 ± 0.04
C 20:5 n-3	4.1 ± 0.38	7.3 ± 1.51	3.0 ± 0.92	16.7 ± 0.51	7.0 ± 0.54	10.7 ± 1.21
C 22:5 n-3	5.6 ± 0.48	4.7 ± 0.05	4.0 ± 1.46	2.8 ± 0.60	3.4 ± 0.42	3.7 ± 0.93
C 22:6 n-3	28.4 ± 1.53	34.5 ± 0.04	32.0 ± 2.25	18.0 ± 2.78	23.1 ± 2.55	28.9 ± 2.04
Sum N-3	36.7 ± 4.51	46.3 ± 0.62	34.0 ± 0.65	36.5 ± 2.23	37.5 ± 0.98	38.8 ± 6.00

Figure 5.6 Fatty acid compositions (% of total fatty acids) of PI at fish body weight (BW) 400g. PI: Phosphatidylinositol; NC: negative control (0% EPA+DHA); CC: commercial control (2.2% EPA+DHA); EPA: Eicosapentaenoic acid (20:5 n-3); DHA: Docosahexaenoic acid (22:5 n-3). Data are expressed as mean (n=1, 2 or 3) ± standard error of mean (SEM). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish.

PE 200g	NC	0.5% EPA	1% EPA	1.5% EPA	2% EPA
C 14:0	0.0 ± 0.00	0.2 ± 0.06	0.2 ± 0.04	0.2 ± 0.06	0.3 ± 0.16
C 15:0	1.1 ± 0.09	0.8 ± 0.11	0.7 ± 0.21	0.7 ± 0.12	0.7 ± 0.06
C 16:0	6.2 ± 0.05	7.5 ± 0.11	7.5 ± 0.04	7.9 ± 0.05	8.4 ± 0.76
C 18:0	7.9 ± 0.11	7.8 ± 0.45	7.7 ± 0.06	7.8 ± 0.26	7.8 ± 0.20
Sum N-0	15.2 ± 0.21	16.3 ± 0.17	16.2 ± 0.23	16.6 ± 0.03	17.1 ± 0.73
C 16:1 n-9	1.7 ± 0.13	1.8 ± 0.14	1.8 ± 0.21	1.9 ± 0.04	1.6 ± 0.05
C 18:1 n-9	18.1 ± 0.26	18.5 ± 0.00	17.4 ± 0.04	17.9 ± 0.01	18.6 ± 1.73
C 20:1 n-9	2.4 ± 0.04	2.0 ± 0.14	1.9 ± 0.04	1.9 ± 0.01	1.7 ± 0.25
C 20:3 n-9	0.4 ± 0.14	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.04
C 16:2 n-6	4.0 ± 0.13	4.5 ± 0.15	4.2 ± 0.18	4.7 ± 0.22	4.3 ± 0.26
C 18:2 n-6	1.5 ± 0.09	1.1 ± 0.15	1.1 ± 0.03	1.0 ± 0.03	1.4 ± 0.08
C 18:3 n-6	0.1 ± 0.06	0.0 ± 0.03	0.1 ± 0.14	0.1 ± 0.06	0.1 ± 0.03
C 20:2 n-6	0.6 ± 0.04	0.5 ± 0.02	0.5 ± 0.06	0.4 ± 0.01	0.4 ± 0.05
C 20:3 n-6	1.2 ± 0.08	0.5 ± 0.24	0.5 ± 0.00	0.4 ± 0.01	0.3 ± 0.00
C 20:4 n-6	4.4 ± 0.15	1.4 ± 0.35	2.0 ± 0.00	1.4 ± 0.02	1.3 ± 0.07
C 22:5 n-6	0.4 ± 0.19	0.2 ± 0.17	0.3 ± 0.01	0.0 ± 0.00	0.1 ± 0.06
Sum N-6	12.1 ± 0.41	8.1 ± 0.05	8.8 ± 0.04	8.0 ± 0.29	7.8 ± 0.29
C 18:3 n-3	0.1 ± 0.06	0.2 ± 0.04	0.2 ± 0.03	0.2 ± 0.02	0.3 ± 0.12
C 18:4 n-3	0.1 ± 0.07	0.2 ± 0.02	0.2 ± 0.00	0.2 ± 0.01	0.2 ± 0.03
C 20:3 n-3	0.1 ± 0.07	0.2 ± 0.02	0.3 ± 0.01	0.2 ± 0.00	0.2 ± 0.01
C 20:5 n-3	4.4 ± 0.14	5.5 ± 0.14	6.6 ± 0.32	6.1 ± 0.09	6.0 ± 0.34
C 22:5 n-3	2.2 ± 0.70	2.7 ± 0.49	4.0 ± 0.28	3.6 ± 0.05	3.8 ± 0.41
C 22:6 n-3	25.5 ± 0.77	28.1 ± 1.87	26.6 ± 0.01	26.6 ± 0.43	27.3 ± 2.70
Sum N-3	34.8 ± 1.60	39.4 ± 0.93	40.6 ± 0.76	39.8 ± 0.30	40.4 ± 3.39

PE 200g	NC	0.5% DHA	1% DHA	1.5% DHA	2% DHA
C 14:0	0.0 ± 0.00	0.2 ± 0.00	0.4 ±	0.2 ± 0.01	0.4 ± 0.24
C 15:0	1.1 ± 0.09	0.8 ± 0.03	0.4 ±	0.7 ± 0.06	0.7 ± 0.31
C 16:0	6.2 ± 0.05	7.2 ± 0.08	8.6 ±	7.2 ± 0.02	8.4 ± 0.34
C 18:0	7.9 ± 0.11	7.4 ± 0.32	7.3 ±	7.5 ± 0.33	8.0 ± 0.60
Sum N-0	15.2 ± 0.21	15.6 ± 0.37	16.6 ±	15.6 ± 0.28	17.5 ± 0.33
C 16:1 n-9	1.7 ± 0.13	1.9 ± 0.02	1.9 ±	1.9 ± 0.02	1.7 ± 0.12
C 18:1 n-9	18.1 ± 0.26	19.0 ± 0.13	20.4 ±	20.3 ± 0.22	19.1 ± 0.81
C 20:1 n-9	2.4 ± 0.04	1.7 ± 0.37	2.2 ±	2.1 ± 0.06	2.2 ± 0.60
C 20:3 n-9	0.4 ± 0.14	0.0 ± 0.00	0.0 ±	0.0 ± 0.00	0.1 ± 0.13
C 16:2 n-6	4.0 ± 0.13	4.8 ± 0.01	4.1 ±	4.9 ± 0.04	4.1 ± 0.26
C 18:2 n-6	1.5 ± 0.09	1.2 ± 0.03	1.3 ±	1.0 ± 0.01	1.3 ± 0.18
C 18:3 n-6	0.1 ± 0.06	0.1 ± 0.04	0.0 ±	0.0 ± 0.02	0.0 ± 0.00
C 20:2 n-6	0.6 ± 0.04	0.5 ± 0.00	0.5 ±	0.4 ± 0.07	0.4 ± 0.02
C 20:3 n-6	1.2 ± 0.08	0.6 ± 0.05	0.4 ±	0.3 ± 0.03	0.4 ± 0.14
C 20:4 n-6	4.4 ± 0.15	2.7 ± 0.26	1.8 ±	1.8 ± 0.16	1.7 ± 0.27
C 22:5 n-6	0.4 ± 0.19	0.2 ± 0.01	0.2 ±	0.2 ± 0.05	0.0 ± 0.00
Sum N-6	12.1 ± 0.41	10.1 ± 0.34	8.3 ±	8.8 ± 0.32	7.9 ± 0.36
C 18:3 n-3	0.1 ± 0.06	0.2 ± 0.02	0.2 ±	0.2 ± 0.00	0.2 ± 0.13
C 18:4 n-3	0.1 ± 0.07	0.2 ± 0.01	0.2 ±	0.1 ± 0.03	0.0 ± 0.00
C 20:3 n-3	0.1 ± 0.07	0.2 ± 0.01	0.2 ±	0.2 ± 0.03	0.0 ± 0.03
C 20:5 n-3	4.4 ± 0.14	4.9 ± 0.13	4.6 ±	5.1 ± 0.18	4.7 ± 0.17
C 22:5 n-3	2.2 ± 0.70	1.9 ± 0.00	1.8 ±	1.8 ± 0.08	2.3 ± 0.28
C 22:6 n-3	25.5 ± 0.77	27.2 ± 0.64	26.8 ±	26.2 ± 0.91	27.4 ± 1.41
Sum N-3	34.8 ± 1.60	37.7 ± 0.47	36.4 ±	37.0 ± 0.49	37.4 ± 1.98

PE 200g	NC	0.5% EPA+DHA	1% EPA+DHA	1.5% EPA+DHA	2% EPA+DHA	CC
C 14:0	0.0 ± 0.00	0.2 ± 0.07	0.2 ± 0.02	0.2 ± 0.04	0.2 ± 0.02	0.3 ± 0.09
C 15:0	1.1 ± 0.09	0.7 ± 0.14	0.6 ± 0.00	0.7 ± 0.11	0.8 ± 0.08	0.8 ± 0.19
C 16:0	6.2 ± 0.05	7.6 ± 0.06	7.4 ± 0.11	7.5 ± 0.30	7.8 ± 0.21	8.1 ± 0.26
C 18:0	7.9 ± 0.11	7.8 ± 0.51	7.6 ± 0.04	7.8 ± 0.32	7.9 ± 0.13	8.1 ± 0.58
Sum N-0	15.2 ± 0.21	16.3 ± 0.24	15.8 ± 0.14	16.1 ± 0.13	16.7 ± 0.36	17.2 ± 0.95
C 16:1 n-9	1.7 ± 0.13	1.8 ± 0.05	1.9 ± 0.03	1.7 ± 0.10	1.8 ± 0.01	1.7 ± 0.03
C 18:1 n-9	18.1 ± 0.26	18.5 ± 0.42	19.6 ± 0.18	17.9 ± 0.34	18.3 ± 0.37	17.9 ± 0.59
C 20:1 n-9	2.4 ± 0.04	2.2 ± 0.03	2.1 ± 0.05	1.6 ± 0.26	1.8 ± 0.06	2.0 ± 0.06
C 20:3 n-9	0.4 ± 0.14	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.10	0.1 ± 0.05	0.2 ± 0.21
C 16:2 n-6	4.0 ± 0.13	4.6 ± 0.25	5.0 ± 0.02	4.3 ± 0.04	4.4 ± 0.07	4.1 ± 0.28
C 18:2 n-6	1.5 ± 0.09	1.2 ± 0.05	1.1 ± 0.01	1.1 ± 0.05	0.9 ± 0.05	1.1 ± 0.22
C 18:3 n-6	0.1 ± 0.06	0.1 ± 0.07	0.2 ± 0.01	0.1 ± 0.14	0.0 ± 0.00	0.1 ± 0.07
C 20:2 n-6	0.6 ± 0.04	0.6 ± 0.00	0.5 ± 0.02	0.3 ± 0.09	0.4 ± 0.03	0.4 ± 0.02
C 20:3 n-6	1.2 ± 0.08	0.7 ± 0.03	0.5 ± 0.03	0.3 ± 0.05	0.2 ± 0.02	0.3 ± 0.01
C 20:4 n-6	4.4 ± 0.15	2.6 ± 0.06	2.0 ± 0.19	1.5 ± 0.04	1.4 ± 0.10	1.3 ± 0.06
C 22:5 n-6	0.4 ± 0.19	0.3 ± 0.09	0.2 ± 0.05	0.1 ± 0.11	0.0 ± 0.05	0.0 ± 0.00
Sum N-6	12.1 ± 0.41	10.1 ± 0.23	9.4 ± 0.16	7.9 ± 0.06	7.4 ± 0.19	7.3 ± 0.15
C 18:3 n-3	0.1 ± 0.06	0.2 ± 0.02	0.2 ± 0.01	0.4 ± 0.19	0.2 ± 0.01	0.3 ± 0.07
C 18:4 n-3	0.1 ± 0.07	0.2 ± 0.02	0.2 ± 0.00	0.1 ± 0.09	0.2 ± 0.01	0.1 ± 0.05
C 20:3 n-3	0.1 ± 0.07	0.2 ± 0.03	0.2 ± 0.02	0.3 ± 0.01	0.2 ± 0.01	0.3 ± 0.04
C 20:5 n-3	4.4 ± 0.14	5.2 ± 0.01	5.6 ± 0.16	5.5 ± 0.19	5.7 ± 0.18	5.6 ± 0.17
C 22:5 n-3	2.2 ± 0.70	2.5 ± 0.25	2.3 ± 0.04	2.7 ± 0.19	2.6 ± 0.07	2.4 ± 0.05
C 22:6 n-3	25.5 ± 0.77	26.7 ± 0.71	25.5 ± 0.21	29.0 ± 0.31	29.2 ± 0.57	28.7 ± 0.94
Sum N-3	34.8 ± 1.60	37.8 ± 0.77	37.2 ± 0.03	41.1 ± 0.78	40.7 ± 0.27	40.1 ± 0.43

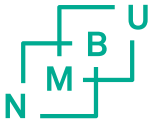
Figure 5.7 Fatty acid compositions (% of total fatty acids) of PE at fish body weight (BW) 200g. PE: Phosphatidylethanolamine; NC: negative control (0% EPA+DHA); CC: commercial control (2.2% EPA+DHA); EPA: Eicosapentaenoic acid (20:5 n-3); DHA: Docosahexaenoic acid (22:5 n-3). Data are expressed as mean (n=1, 2 or 3) ± standard error of mean (SEM). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish.

PE 400g	NC	0.5% EPA	1% EPA	1.5% EPA	2% EPA
C 14:0	0.2 ± 0.03	0.4 ± 0.19	0.3 ± 0.06	0.3 ± 0.09	0.7 ± 0.16
C 15:0	1.8 ± 0.42	1.9 ± 0.72	0.8 ± 0.19	1.5 ± 0.41	2.2 ± 0.06
C 16:0	7.5 ± 0.23	8.0 ± 0.74	7.5 ± 0.21	8.3 ± 0.29	8.2 ± 1.20
C 18:0	8.3 ± 0.23	8.0 ± 0.03	8.3 ± 0.63	8.4 ± 0.22	8.5 ± 0.55
Sum N-0	17.8 ± 0.48	18.2 ± 1.69	16.9 ± 0.29	18.5 ± 1.00	19.6 ± 0.87
C 16:1 n-9	3.2 ± 0.51	3.2 ± 1.19	2.6 ± 0.48	2.6 ± 0.56	3.0 ± 1.09
C 18:1 n-9	19.6 ± 0.89	19.5 ± 0.49	19.3 ± 1.23	18.6 ± 1.46	18.1 ± 0.18
C 20:1 n-9	1.8 ± 0.09	1.8 ± 0.03	1.6 ± 0.06	1.6 ± 0.02	2.1 ± 0.37
C 20:3 n-9	0.0 ± 0.00	0.1 ± 0.06	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 16:2 n-6	5.4 ± 1.31	3.5 ± 1.32	5.2 ± 0.32	4.1 ± 0.66	5.4 ± 0.14
C 18:2 n-6	1.3 ± 0.02	1.1 ± 0.06	1.1 ± 0.05	0.9 ± 0.01	0.9 ± 0.09
C 18:3 n-6	0.1 ± 0.03	0.1 ± 0.00	0.2 ± 0.05	0.0 ± 0.05	0.1 ± 0.10
C 20:2 n-6	0.4 ± 0.05	0.4 ± 0.02	0.2 ± 0.23	0.3 ± 0.00	0.3 ± 0.02
C 20:3 n-6	1.4 ± 0.05	0.9 ± 0.03	0.5 ± 0.05	0.4 ± 0.00	0.3 ± 0.03
C 20:4 n-6	4.9 ± 0.15	2.6 ± 0.10	2.0 ± 0.13	1.5 ± 0.01	1.5 ± 0.21
C 22:5 n-6	0.4 ± 0.19	0.3 ± 0.03	0.2 ± 0.01	0.2 ± 0.02	0.1 ± 0.06
Sum N-6	14.0 ± 1.33	9.0 ± 1.54	9.3 ± 0.10	7.6 ± 0.74	8.7 ± 0.13
C 18:3 n-3	0.1 ± 0.02	0.1 ± 0.00	0.2 ± 0.11	0.1 ± 0.04	0.2 ± 0.03
C 18:4 n-3	0.2 ± 0.01	0.2 ± 0.01	0.3 ± 0.02	0.1 ± 0.02	0.2 ± 0.01
C 20:3 n-3	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.04	0.2 ± 0.01	0.2 ± 0.01
C 20:5 n-3	3.9 ± 0.13	5.0 ± 0.11	5.0 ± 0.69	5.6 ± 0.05	5.2 ± 0.73
C 22:5 n-3	2.5 ± 0.21	3.1 ± 0.05	2.5 ± 1.12	3.7 ± 0.20	3.4 ± 0.41
C 22:6 n-3	20.8 ± 2.40	24.5 ± 0.26	25.9 ± 0.80	25.7 ± 1.56	24.3 ± 1.65
Sum N-3	30.6 ± 1.94	35.1 ± 1.26	38.1 ± 2.01	37.9 ± 1.79	36.3 ± 3.69

PE 400g	NC	0.5% DHA	1% DHA	1.5% DHA	2% DHA
C 14:0	0.2 ± 0.03	0.1 ± 0.04	0.2 ± 0.03	0.2 ± 0.00	0.3 ± 0.04
C 15:0	1.8 ± 0.42	1.4 ± 0.83	1.0 ± 0.11	1.1 ± 0.88	1.1 ± 0.27
C 16:0	7.5 ± 0.23	6.9 ± 0.30	7.7 ± 0.08	9.0 ± 0.31	7.9 ± 0.14
C 18:0	8.3 ± 0.23	8.3 ± 0.00	7.7 ± 0.21	10.5 ± 0.14	8.4 ± 0.04
Sum N-0	17.8 ± 0.48	16.8 ± 0.50	16.7 ± 0.37	20.7 ± 0.71	17.6 ± 0.33
C 16:1 n-9	3.2 ± 0.51	1.7 ± 0.15	2.1 ± 0.06	2.6 ± 0.67	2.7 ± 0.74
C 18:1 n-9	19.6 ± 0.89	17.8 ± 1.31	17.9 ± 0.73	19.9 ± 0.57	18.4 ± 0.99
C 20:1 n-9	1.8 ± 0.09	1.7 ± 0.15	1.8 ± 0.23	1.9 ± 0.07	1.6 ± 0.03
C 20:3 n-9	0.0 ± 0.00	0.1 ± 0.13	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 16:2 n-6	5.4 ± 1.31	5.1 ± 0.75	4.4 ± 0.61	3.1 ± 2.78	4.3 ± 0.15
C 18:2 n-6	1.3 ± 0.02	1.2 ± 0.01	1.0 ± 0.09	1.5 ± 0.09	1.0 ± 0.05
C 18:3 n-6	0.1 ± 0.03	0.1 ± 0.02	0.1 ± 0.08	0.2 ± 0.02	0.1 ± 0.07
C 20:2 n-6	0.4 ± 0.05	0.4 ± 0.06	0.5 ± 0.06	0.4 ± 0.03	0.4 ± 0.02
C 20:3 n-6	1.4 ± 0.05	0.7 ± 0.01	0.2 ± 0.23	0.5 ± 0.09	0.3 ± 0.01
C 20:4 n-6	4.9 ± 0.15	2.7 ± 0.03	1.8 ± 0.04	1.8 ± 0.02	1.7 ± 0.07
C 22:5 n-6	0.4 ± 0.19	0.3 ± 0.00	0.1 ± 0.12	0.0 ± 0.02	0.2 ± 0.06
Sum N-6	14.0 ± 1.33	10.4 ± 0.85	8.1 ± 0.34	7.4 ± 2.65	7.9 ± 0.09
C 18:3 n-3	0.1 ± 0.02	0.2 ± 0.04	0.1 ± 0.04	0.1 ± 0.06	0.1 ± 0.05
C 18:4 n-3	0.2 ± 0.01	0.1 ± 0.04	0.2 ± 0.06	0.1 ± 0.07	0.1 ± 0.05
C 20:3 n-3	0.2 ± 0.01	0.2 ± 0.00	0.2 ± 0.02	0.2 ± 0.01	0.2 ± 0.01
C 20:5 n-3	3.9 ± 0.13	3.8 ± 0.09	5.1 ± 0.70	4.4 ± 0.10	4.6 ± 0.09
C 22:5 n-3	2.5 ± 0.21	2.3 ± 0.50	2.4 ± 0.58	1.7 ± 0.17	2.2 ± 0.23
C 22:6 n-3	20.8 ± 2.40	29.5 ± 1.68	29.6 ± 2.89	29.2 ± 0.66	29.5 ± 1.64
Sum N-3	30.6 ± 1.94	39.0 ± 1.97	40.0 ± 1.25	36.6 ± 0.17	39.2 ± 1.69

PE 400g	NC	0.5% EPA+DHA	1% EPA+DHA	1.5% EPA+DHA	2% EPA+DHA	CC
C 14:0	0.2 ± 0.03	0.2 ± 0.08	0.2 ± 0.01	0.3 ± 0.06	0.3 ± 0.09	0.3 ± 0.03
C 15:0	1.8 ± 0.42	1.2 ± 0.33	1.7 ± 0.67	1.3 ± 0.33	1.6 ± 0.37	1.7 ± 0.18
C 16:0	7.5 ± 0.23	7.8 ± 0.78	7.8 ± 0.42	8.2 ± 0.41	7.7 ± 0.31	7.9 ± 0.35
C 18:0	8.3 ± 0.23	8.5 ± 0.31	9.3 ± 0.70	8.1 ± 0.37	8.5 ± 0.31	8.7 ± 0.67
Sum N-0	17.8 ± 0.48	17.8 ± 1.50	18.9 ± 1.78	17.8 ± 1.18	18.1 ± 0.56	18.5 ± 0.50
C 16:1 n-9	3.2 ± 0.51	3.2 ± 0.19	2.9 ± 0.44	2.5 ± 0.47	2.2 ± 0.32	2.8 ± 0.82
C 18:1 n-9	19.6 ± 0.89	17.9 ± 0.56	18.9 ± 0.74	18.2 ± 0.63	18.8 ± 0.30	19.2 ± 0.68
C 20:1 n-9	1.8 ± 0.09	1.7 ± 0.13	1.7 ± 0.11	1.6 ± 0.10	1.6 ± 0.02	1.7 ± 0.08
C 20:3 n-9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.05	0.0 ± 0.00	0.0 ± 0.00
C 16:2 n-6	5.4 ± 1.31	4.7 ± 0.64	5.9 ± 1.13	4.3 ± 0.63	4.9 ± 0.61	4.9 ± 0.86
C 18:2 n-6	1.3 ± 0.02	1.2 ± 0.07	1.0 ± 0.00	1.0 ± 0.02	0.9 ± 0.03	1.2 ± 0.46
C 18:3 n-6	0.1 ± 0.03	0.1 ± 0.00	0.1 ± 0.07	0.1 ± 0.02	0.0 ± 0.02	0.2 ± 0.05
C 20:2 n-6	0.4 ± 0.05	0.5 ± 0.00	0.4 ± 0.00	0.4 ± 0.02	0.2 ± 0.12	0.6 ± 0.34
C 20:3 n-6	1.4 ± 0.05	0.8 ± 0.04	0.5 ± 0.02	0.4 ± 0.00	0.3 ± 0.02	0.2 ± 0.02
C 20:4 n-6	4.9 ± 0.15	2.6 ± 0.27	2.1 ± 0.04	1.6 ± 0.01	1.5 ± 0.08	1.1 ± 0.03
C 22:5 n-6	0.4 ± 0.19	0.3 ± 0.01	0.3 ± 0.12	0.2 ± 0.00	0.1 ± 0.06	0.1 ± 0.06
Sum N-6	14.0 ± 1.33	10.2 ± 0.89	10.4 ± 0.96	7.9 ± 0.63	8.0 ± 0.49	8.3 ± 1.70
C 18:3 n-3	0.1 ± 0.02	0.0 ± 0.04	0.1 ± 0.00	0.1 ± 0.03	0.1 ± 0.02	0.6 ± 0.40
C 18:4 n-3	0.2 ± 0.01	0.2 ± 0.01	0.1 ± 0.01	0.1 ± 0.03	0.2 ± 0.05	0.1 ± 0.01
C 20:3 n-3	0.2 ± 0.01	0.2 ± 0.02	0.2 ± 0.00	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01
C 20:5 n-3	3.9 ± 0.13	4.4 ± 0.40	5.3 ± 0.27	4.8 ± 0.12	5.3 ± 0.12	5.0 ± 0.16
C 22:5 n-3	2.5 ± 0.21	2.8 ± 0.11	2.1 ± 0.27	2.6 ± 0.26	2.6 ± 0.20	2.2 ± 0.29
C 22:6 n-3	20.8 ± 2.40	26.3 ± 0.56	24.7 ± 1.57	28.5 ± 1.12	27.8 ± 0.26	25.1 ± 1.56
Sum N-3	30.6 ± 1.94	36.8 ± 0.06	35.7 ± 2.63	38.8 ± 0.78	39.0 ± 0.30	36.2 ± 1.35

Figure 5.8 Fatty acid compositions (% of total fatty acids) of PE at fish body weight (BW) 400g. PE: Phosphatidylethanolamine; NC: negative control (0% EPA+DHA); CC: commercial control (2.2% EPA+DHA); EPA: Eicosapentaenoic acid (20:5 n-3); DHA: Docosahexaenoic acid (22:5 n-3). Data are expressed as mean (n=1, 2 or 3) ± standard error of mean (SEM). Each group includes 2 or 3 tanks, whereby every tank represents a pooled sample of 5 fish.



Norges miljø- og biovitenskapelig universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway