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Diet, size and location as determinants of n-3 long-chain polyunsaturated fatty acid content in farmed Atlantic Salmon (*Salmo salar*)

Bailey C McMeans[1], Michael T Arts[2], Cory Dubetz[3] & Michael Ikonomou[3]

[1]Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

[2]Department of Chemistry and Biology, Ryerson University, Toronto, ON, Canada

[3]Institute of Ocean Sciences, Fisheries and Oceans Canada, Sidney, BC, Canada

Correspondence: B C McMeans, Department of Biology, University of Toronto Mississauga, 3359 Mississauga Road, Mississauga, ON, Canada. E-mail: bcmcmeans@gmail.com

Abstract

We explored how currently manufactured feeds, under real-world conditions and across geographically distinct locations, promoted flesh n-3 long-chain polyunsaturated fatty acid (LC-PUFA, i.e. 20:5n-3 + 22:6n-3) levels in various life stages of farmed Atlantic Salmon (*Salmo salar*). Potential effects on flesh LC-PUFA included: (1) diet and fish weight at one Canadian east coast farm, (2) diet and farm location across six east coast farms, and (3) diet and farm location between east and west coast farms. For objectives 1 and 2, salmon were fed a currently manufactured feed (labelled as feeds A, B or C) and harvested at 1, 3 and 5 kg. LC-PUFA levels in 5 kg (harvest size) fish were then compared to previously published values for west coast farmed Atlantic Salmon (Obj. 3). Combined results revealed that variability in LC-PUFA levels was better explained by diet than by fish weight or farm location. Fish size, however, was also important for two reasons. First, feeding a high LC-PUFA diet early in life appeared important for ensuring high LC-PUFA levels at harvest size. Second, salmon flesh LC-PUFA levels increased with fish size, but only when dietary LC-PUFA was provided above an apparent threshold value (~3000 mg per 100 g or 10% of total fatty acids) that likely promoted LC-PUFA incorporation and storage. Overall, our comparison makes new recommendations for feed manufacturers and demonstrates that farmed Atlantic Salmon reared under real-world conditions on currently available salmon feeds were good sources of n-3 LC-PUFA to consumers.

Keywords: feed quality, fish size, flesh quality, Canada

Introduction

The n-3 long-chain polyunsaturated fatty acids (LC-PUFA), notably eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), have been linked to a lowered risk of cardiovascular disease (Kris-Etherton, Harris & Appel 2002) and with enhanced cognitive performance in humans (Muldoon, Ryan, Sheu, Yao, Conklin & Manuck 2010; Carlson, Fallon, Kalish, Gura & Puder 2013). Overfishing has drastically depleted wild fish stocks in many parts of the world (Farrell, Friesen, Higgs & Ikonomou 2010) leading to an increased reliance on farmed fish as a progressively more important source of n-3 LC-PUFA (Arts, Ackman & Holub 2001; Henriques, Dick, Tocher & Bell 2014). Among current, farm-reared fishes, Atlantic Salmon (*Salmo salar*) has one of the highest n-3 LC-PUFA levels, due to their propensity for accumulating lipids in their flesh (% lipid is positively correlated with n-3 LC-PUFA amounts, Ikonomou, Higgs, Gibbs, Oakes, Skura, McKinley, Balfry, Jones, Withler & Dubetz 2007; Weaver, Ivester, Chilton, Wilson, Pandey & Chilton 2008). Worldwide, over 2 000 000 tonnes of farmed Atlantic Salmon are produced each year, and 90% of the global farmed salmon market is constituted by Atlantic Salmon (FAO, 2004-2013). Any factor that affects the n-3 LC-PUFA content and % lipid of Atlantic Salmon flesh is therefore of interest to farm managers, feed manufacturers and consumers.

Diet is widely considered as the most important determinant of n-3 LC-PUFA content in farmed Atlantic Salmon (e.g. Bell, McGhee, Campbell & Sargent 2003; Bell, Tocher, Henderson, Dick & Crampton 2003; Torstensen, Bell, Rosenlund, Henderson, Graff, Tocher, Lie & Sargent 2005; Arts, Browman, Jokinen & Skiftesvik 2010). However, non-dietary factors are also important. For example, temperature directly affects lipid digestibility (Bendiksen, Berg, Jobling, Arnesen & Måsøval 2003) as well as the fatty acid composition of cell membranes (Arts & Kohler 2009). In addition, both temperature and salmon size (Torstensen *et al.* 2005) influence fish nutrient requirements. It has also been suggested that dietary and environmental conditions experienced by juvenile salmon could influence resultant n-3 LC-PUFA levels of older fish at harvest size (Arts, Palmer, Skiftesvik, Jokinen & Browman 2012) and that the influence of temperature on fish n-3 LC-PUFA content depends on dietary PUFA sources (Olsen & Henderson 1997; Bendiksen *et al.* 2003). Thus, the dietary uptake and flesh deposition of n-3 LC-PUFA by farmed Atlantic Salmon depends on the independent as well as the interactive effects of diet, temperature and fish size (Bendiksen *et al.* 2003; Jobling & Bendiksen 2003; Torstensen *et al.* 2005; Bendiksen, Johnsen, Olsen & Jobling 2011; Bowyer, Qin & Stone 2013). Findings from controlled, experimental feeding studies (such as those described above) continue to provide crucial insight for the development of more sustainable salmon feeds, which includes replacing marine with terrestrial sources of lipid (Turchini, Torstensen & Ng 2009; Bowyer *et al.* 2013). However, few studies have explored how currently manufactured feeds are performing under real-world conditions, across geographically distinct locations, to promote and sustain flesh n-3 LC-PUFA levels in various life stages of farmed Atlantic Salmon.

The UK, Norway, Australia and Canada are among the world leaders in Atlantic Salmon aquaculture and in developing more sustainable (i.e. terrestrially based) fish feeds (Farrell *et al.* 2010; Henriques *et al.* 2014). Atlantic Salmon are farmed on both the east (Nova Scotia, New Brunswick, Newfoundland) and west (British Columbia) coasts of Canada, and nearby US states (e.g. Maine), where suitable locations and rearing temperatures exist (Surprenant 2010). Previous North American-based studies have reported n-3 LC-PUFA levels in (1) feeds and salmon flesh from Canadian west coast farms (Ikonomou *et al.* 2007; Friesen, Ikonomou, Higgs, Ang & Dubetz 2008), (2) between wild and farmed west coast salmonids (Ikonomou *et al.* 2007; Friesen *et al.* 2008), and (3) between east and west coast farmed Atlantic Salmon (Hamilton, Hites, Schwager, Foran, Knuth & Carpenter 2005). No previous study, however, has explored how flesh quality varies among different sizes of Atlantic Salmon reared on currently manufactured salmon feeds in east coast salmon farms. Possible differences in mean water temperature among salmon farming regions coupled with different diets and the potential for salmon to acquire and maintain n-3 LC-PUFA in a size-dependent manner are all potential sources of spatial variability in farmed Atlantic Salmon n-3 LC-PUFA contents that warrant further attention.

The goals of the present study were to investigate the influence of diet (three currently manufactured salmon feeds) and farm location as potential sources of variability in different weight classes (1 kg, 3 kg, 5 kg) of farmed Atlantic Salmon flesh n-3 LC-PUFA (EPA + DHA) levels. We asked three questions which progressively increased in scope and geographical scale. First, how do dietary lipids affect farmed Atlantic Salmon n-3 LC-PUFA levels across three size classes at one east coast farm (i.e. objective 1, diet and weight effects)? Second, how do Atlantic Salmon flesh n-3 LC-PUFA levels vary geographically among six farms located on the east coasts of the USA and Canada (i.e. objective 2, east coast farm comparison)? Third, how do flesh n-3 LC-PUFA levels differ between east vs. west coast farmed Atlantic Salmon (objective 3, east vs. west coast comparison)? Results from this study will lend insight into the performance of currently manufactured feeds under real-life conditions, the importance of dietary and non-dietary (fish weight, farm location) sources of variation in farmed Atlantic Salmon flesh quality, and will reveal how conditions experienced by younger life stages influence the n-3 LC-PUFA levels of market-size fish.

Materials and methods

Experimental design

For objective 1 (diet and weight effects), salmon at one east coast farm (Cooke Aquaculture Inc., Fairhaven, NB, Canada) were randomly allocated into one of three sea pens. Each pen received

(*ad libitum*) one of three currently manufactured salmon feeds that were arbitrarily labelled as feeds A, B and C to protect the manufacturers identity. Salmon from each of the three diet treatments were sampled at three different weight classes (1, 3, and 5 kg, Table 1) resulting in a 3 × 3 factorial design. The goal of sampling multiple weight classes was to determine how different diets fed to earlier salmon life stages (i.e. 1 and 3 kg) influenced the n-3 LC-PUFA levels of market-sized fish (optimum market size for Atlantic Salmon = 77 cm fork length or ~ 5 kg, Friesen *et al.* 2008).

For objective 2 (east coast farm comparison), Atlantic Salmon were reared on one of two diets at six different salmon farms along the east coast of Canada and the USA (Table 2). Fish at Benson (Benson Aquaculture Ltd, Grand Manan, NB, Canada), Pot Harbour (Cooke Aquaculture Inc., Hermitage Bay, NF, Canada) and Sand (Phoenix Salmon US, Inc., Sand Cove, Beals, ME, USA) farms were fed Feed B and fish at Cutler (Phoenix Salmon US, Inc., Machias Bay, Cutler, ME, USA), Foley (Foley's Cove, NB, Canada) and Seeley (Seeley's Cove, NB, Canada) farms were reared on Feed C (see Table 3 for feed composition information). As in objective 1, each treatment consisted of salmon contained within one sea pen that were fed one diet, and fish were sampled at three target weight classes (1, 3 and 5 kg). Only one feed formula from a single feed manufacturer was fed per farm with the exception of Seeley farm, where salmon in two sea pens received two different regimes of Feed C: one pen received formula 2 at all three weight classes and a second pen received formula 2 at 1 kg (these fish were not sampled, however, and presumably similar to the sampled 1 kg formula 2 fish) were switched to formula 1 at 3 kg and then switch back to formula 2 at 5 kg (Table 2). For objective 2, the goal of sampling multiple weight classes was to explore a potential farm location effect on n-3 LC-PUFA levels of different sized salmon. The goal of feeding two different diets across the six farms was to determine whether a farm location effect was dependent on dietary n-3 LC-PUFA levels.

Differences in farmed Atlantic Salmon n-3 LC-PUFA levels at a broader geographical scale (objective 3) were assessed by comparing east coast data from the present study to previously published data for west coast farmed Atlantic Salmon reared on either a traditional, marine oil diet (Ikonomou *et al.* 2007) or on a terrestrial oil-subsidized diet (i.e. Feed C, Friesen *et al.* 2008). We focused on market-size salmon for this comparison because these data were reported by Ikonomou *et al.* 2007 and have the most immediate relevance for consumers of farmed salmon.

Atlantic Salmon sampling

All Atlantic Salmon in the present study were likely of the same genetic stock (St. John River) as is typical for Atlantic Canada (Atlantic Canada Fish Farmers Association, 2010), although this information was unable to be confirmed by farm managers at Cutler or Pot Harbour. Salmon for objective 1 were sampled at the three target weight classes during February 2007, August

Table 1 Sample size (n), % lipid (wet weight basis) and % n-3 LC-PUFA [all mean (standard deviation)] of Atlantic Salmon diet and skinless fillets from one farm (Cooke Aqua) in Fairhaven, New Brunswick. Salmon were fed one of three diets that each consisted of different weight class-specific formulae. For all diet treatments, fish were sampled at three target weight classes (fork lengths also shown): at 1 kg, 3 kg and 5 kg on February 2007, August 2007 and April 2008 respectively

Feed type	Weight class	Diet			Salmon				
		n	% lipid	% n-3 LC- PUFA	*n*	Weight (kg)	Length (cm)	% lipid	% n-3 LC- PUFA
A	1 kg	1.0	30.9	3.9	3.0	1.3 (0.1)	49.7 (2.1)	7.1 (0.6)	8.1 (1.2)
	3 kg	1.0	26.2	6.2	3.0	2.4 (0.5)	56.7 (4.7)	9.9 (3.4)	9.2 (1.6)
	5 kg	1.0	32.8	14.4	3.0	4.1 (0.5)	71.0 (1)	8.9 (0.6)	10.9 (0.4)
B	1 kg	1.0	27.4	15.1	3.0	1.5 (0.3)	52.7 (1.2)	7.4 (3.2)	14.5 (4.7)
	3 kg	1.0	26.7	9.4	3.0	2.4 (1.6)	59.3 (3.5)	9.2 (1.1)	12.9 (0.3)
	5 kg	1.0	36.1	5.7	3.0	4.7 (0.3)	76.3 (1.5)	7.5 (0.3)	14.1 (0.7)
C	1 kg	1.0	35.3	12.0	3.0	1.4 (0.3)	48.7 (5.5)	7.2 (2.6)	14.3 (2.4)
	3 kg	1.0	26.0	16.0	3.0	3.1 (0.5)	64.7 (3.2)	10.4 (1.6)	18.8 (0.4)
	5 kg	1.0	31.0	8.2	3.0	5.2 (1.0)	76.8 (5.5)	9.2 (1.4)	16 (2.8)

Table 2 Sample size (n), % lipid and % n-3 LC-PUFA [mean(SD)] of Atlantic Salmon diet and skinless fillets. Salmon total weight and fork length are also provided, as are monthly composites of satellite sea surface temperature data (Aqua MODIS) from each sampling month at each of the six east coast farms. Fish were sampled at three different target weight classes (WC: 1, 3 and 5 kg, sample dates provided). All fish at each farm were sampled from a single pen except for Sand* and Seeley†

					Diet				Salmon				
Diet regime	Farm	WC	Date	Water Temp. (°C)	Feed type	*n*	% lipid	% n-3 LC- PUFA	*n*	Weight (kg)	Length (cm)	% lipid	% n-3 LC- PUFA
B	Benson (NB)	1 kg	July 2009	14.1	B	1	30.6	16.5	3	1.8 (0.5)	51.2 (2.8)	6.8 (1.8)	15.2 (4.4)
		3 kg	February 2010	3.6	B	1	34.6	22.7	2	5.1 (0.9)	76.8 (2.1)	11.9 (1.9)	12.6 (0.7)
		5 kg	May 2010	7.5	B	1	34.5	10.2	2	7.0 (0.9)	76.6 (5.3)	12.5 (0.7)	14.8 (0.4)
	Pott (NF)	1 kg	ND	ND	B	1	26.5	7.0	3	ND	42.1 (2.6)	5.8 (0.7)	16.9 (1.7)
		3 kg	February 2010	1.5	B	1	32.8	21.9	2	3.3 (0.4)	63.3 (3.9)	9.1 (0.6)	11.2 (0.5)
		5 kg	August 2010	17.6	B	1	31	9.0	2	5.4 (1.1)	72.6 (4.2)	10 (1.6)	13.1 (0.9)
	Sand* (ME)	1 kg	June 2009	9.7	B	1	31.8	4.7	3	1.1 (2.1)	46.1 (1.8)	2.9 (2.1)	20.3 (5.4)
		3 kg	February 2010	3.5	B	1	35	22.1	4	2.7 (1.3)†	56.1 (10.5)	6.4 (2.8)	14.7 (3.6)
		5 kg	February 2010	3.5	B	1	35	22.1	2	5.1 (0.7)†	73.3	9.4 (0.1)	11.8 (0.2)
C	Cutler (ME)	1 kg	ND	ND	C formula 1	1	30.4	23.9	3	1.3 (0.2)	48 (3)	5.5 (1.9)	19.4 (3.8)
		3 kg	ND	ND	C formula 1	ND	ND	NA	2	3.2 (0.9)	63 (1)	7.7 (1)	20.4 (0.8)
		5 kg	August 2010	13.3	C formula 1	ND	ND	NA	2	3.0 (0.4)‡	ND	11.1 (0.9)	16.6 (0.7)
	Foley (NB)	1 kg	July 2009	13.7	C formula 1	1	33.5	18.0	3	0.9 (0.2)	41.8 (2.9)	3.4 (0.9)	19.5 (3.3)
		3 kg	January 2010	4.2	C formula 2	1	33.4	11.8	2	3.1 (1.4)	58.7 (6.6)	7.4 (3.1)	20.1 (2)
		5 kg	June 2010	11.0	C formula 2	1	34.9	11.1	2	4.4 (0.5)	66.7 (2.1)	12.4 (0.7)	15 (0.2)
	Seeley§ (NB)												18.8 (2.3)
		1 kg	July 2009	13.5	C formula 2	1	35.4	25.3	3	1.6 (0.3)	50.8 (0.4)	4.3 (2.1)	20.7 (1.8)
	Pen 1	3 kg	March 2010	3.0	C formula 2	1	36.7	12.1	2	4.4 (1.1)	66.9 (5.8)	8.1 (1.1)	22.3 (0.2)
		5 kg	May 2010	7.7	C formula 2	1	34.9	11.1	2	5.4 (0.5)	70.4 (4.4)	10.0 (0.1)	19.5 (0.1)
	Pen 2	3 kg	March 2010	3.0	C formula 1	1	31.5	12.8	2	4.7 (0.6)	73 (4.2)	7.9 (1.1)	19.1 (0.1)
		5 kg	May 2010	7.7	C formula 2	1	35.6	11.1	2	4.8 (0.5)	70.9 (2.6)	8.1 (1.9)	18.5 (0.4)

'ND', no data available; NB, New Brunswick; NF, Newfoundland; ME, Maine.

*5 kg fish sampled from a different pen and simultaneously with 3 kg fish (both cages received same diet).

†Estimated from gutted fish weight (see text).

‡Estimated from fillet weight (see text).

§Two pens sampled for 3 and 5 kg fish.

Table 3 Composition of salmon feeds used in the present study provided by the feed manufacturer. Ranges represent minimum and maximum values across all individual weight class formulae within a given feed type

Component	A	B	C
Protein (min)	45–48%	39–43%	37–41%
Fat (min)	18–20%	24–29%	28–33%
Fibre (max)	not provided	4%	1.50%

2007 and April 2008 respectively. As these are 'target' weight classes, however, some fish were heavier or lighter than the target values (mean and standard deviation of fish fork length and weight from each sampling event provided in Table 1). Six salmon were collected at each target weight class from each diet treatment. An attempt was made to ensure that these six fish were close to each other in weight and size.

For objective 2, six salmon were sampled from each target weight class from one sea pen (diet treatment) at each farm on three different sampling occasions (Table 2). One exception is at Sand, where the 5 kg salmon were sampled simultaneously with the 3 kg salmon (both of which were consuming Feed B), but from a different sea pen. All six fish collected in each sampling event were similar in size and weight (Table 2). The dates of the three target weight class sampling events varied among the farms depending on the time it took the salmon to reach the target weight class, as judged by the farm managers (Table 2). Actual fish weight corresponded well with target weight class values, except for at Benson, where actual fish weights were ~2 kg heavier than target 3 and 5 kg values and at Cutler where fish sampled for the 5 kg target weight class actually weighed only 3 kg (Table 2).

For both objective 1 (Table 1) and objective 2 (Table 2), fish sex, weight (kg) and fork length (cm) measurements were taken at the farm. Fish were then immediately frozen on site either whole or as the skinless left fillets (with belly fat removed) and shipped frozen, on ice, to the Institute of Ocean Sciences (IOS), Sidney, BC. Samples were subsequently stored at −20°C until analysis. In some instances (i.e. 5 kg fish sampled at Sand and 3 and 5 kg fish sampled at Cutler), fish weights were not recorded at the farm and had to be estimated at IOS from gutted fish weights (fish weight = gutted fish weight * 1.125) or from a single fillet weight (fish weight = fillet weight * 2 * 1.3).

Experimental diets

Feed A had higher % protein and lower % lipid compared to Feed B and Feed C (Table 3). All three salmon feeds are compositionally tailored to maximize fish growth and health as a function of fish size and temperature. Thus, there were 3 size-specific formulae for each of the 3 feeds. The salmon were switched to the appropriate formulae at a time judged by the farm manager. A sample of each formula from all feed types and all farms was provided for fatty acid analysis, except for the 5 kg formula from Cutler and Sand, which was not provided.

Fatty acid analysis

Each group of six salmon sampled at each weight class from each diet treatment was reduced to three composites (two fish per composite) for objective 1. For objective 2, three composites were prepared for each group of 1 kg fish and two composites were prepared from both the 3 and 5 kg weight class fish (three fish per composite). Each composite was created by partially thawing the left skinless fillets (with belly fat removed) and homogenizing them together in a commercial Hobart meat grinder. Samples were kept cool (below 5°C) during this processes. Subsamples (20 g) were removed from the homogenate, blown with N_2, capped and stored at −20°C until analysis.

Salmon homogenates and salmon feed samples were freeze-dried (dry weight = DW) for 48 h and weighed to the nearest microgram. Samples were homogenized in 2:1 chloroform: methanol to extract lipids and fatty acid methyl esters (FAME) were generated from the total lipid extract, analysed on a Hewlett Packard 6890 GC and quantified using known standards as described in McMeans, Arts, Rush and Fisk (2012). Values of % lipid are reported on a wet weight basis for both feed and fish samples. To maintain consistency with previous studies (Ikonomou *et al.* 2007; Friesen *et al.* 2008), individual FAME, reported as a percentage of the total identifiable fatty acids, were converted into estimates of absolute fatty acid quantities (mg FA·100 g^{-1} skinless flesh, wet weight) using determined lipid contents (reported on a wet weight basis) as follows:

$$\text{mg FA} \cdot 100g^{-1}\text{skinless flesh} = \left(\%\text{lipid} * \frac{\%\text{FA}}{100} * \text{correction factor}\right) * 100 \quad (1)$$

The 'correction factor' is the proportion of total lipids present as triacylglycerides and is equal to 0.851 for feed and 0.889 for salmon as previously described (Ikonomou *et al.* 2007).

Statistical analyses

All analyses were performed on n-3 LC-PUFA (i.e. EPA + DHA) mg·100 g^{-1} wet weight calculated in an identical manner (see preceding paragraph) to previous studies (Ikonomou *et al.* 2007; Friesen *et al.* 2008). Values of n-3 LC-PUFA were log10-transformed, when necessary, to meet the assumptions of normality (confirmed via Shapiro–Wilk's tests, $P > 0.05$) and homoscedasticity (confirmed via Levene's tests, $P > 0.05$) prior to analyses. For objective 1, a two-way factorial ANOVA was used to test for the effects of diet and weight class (and their interaction) on salmon n-3 LC-PUFA levels. To test for significant differences in fish n-3 LC-PUFA levels among salmon farms (that fed the same diets) in objective 2, one ANOVA was performed to compare among Feed B farms (Benson, Pot and Sand) and one ANOVA to compare among Feed C farms (Cutler, Seeley and Foley). Although we were interested in potential farm location effects at different weight classes, the low sample size ($n = 2$) for the 3 and 5 kg salmon sampled at each farm for objective 2 necessitated that data across all three weight classes be pooled for these comparisons. By restricting statistical comparisons to farms that fed the same diet, however, dietary effects should be minimized and a potential affect of farm environment will still be revealed, albeit across all weight classes combined. For objectives 1 and 2, Pearson's correlation coefficients were used to explore relationships between individual fish weight (continuous weight variable) and salmon n-3 LC-PUFA for each of the three diets (objective 1) and each of the six sampled farms (objective 2). For objective 3, the n-3 LC-PUFA of the 5 kg target weight class salmon (i.e. harvest weight) from objectives 1 and 2 were first grouped by the three diet types: (1) Feed A (only objective 1 fish), Feed B (objective 1 and objective 2 fish) and Feed C (objective 1 and objective 2 fish). Using ANOVA, these three separate groups were then compared to previously published n-3 LC-PUFA values of west coast harvest size farmed Atlantic Salmon fed: (1) marine oil-based diets (Ikonomou *et al.* 2007) and (2) Feed C (Friesen *et al.* 2008). Salmon included in Ikonomou *et al.* (2007) were all harvest size fish, but because Friesen *et al.* (2008) included different sized salmon, only individual fish that were within the length and weight range for the 5 kg fish from the present study were included in this comparison (sample size and weight of the fish included in the present study are provided in Table 4). Values of n-3 LC-PUFA that were previously published in Ikonomou *et al.* (2007) from the following wild Pacific salmonids were also provided for comparison to farmed Atlantic Salmon: Sockeye (*Oncorhynchus nerka*), Chinook (*O. tshawytscha*), Pink (*O. gorbuscha*), Coho (*O. kisutch*) and Chum (*O. keta*). These data for other salmonid species were treated in an identical manner to Atlantic Salmon data (see preceding paragraph) and are thus comparable. All statistical analyses were performed in R (R Development Core Team 2010), the significance level was set at 0.05, and values are provided as means (±SD).

Results and discussion

Objective 1 – Diet and weight effects at one east coast farm

Variability existed in the n-3 LC-PUFA content both among (i.e. Feed A vs. B vs. C) and within each of the three diet treatments (i.e. among weight class formulae of a given feed type, Fig. 1b). Feed A varied in n-3 LC-PUFA content by almost fourfold, from a low of 1020 mg·100 g^{-1} in the 1 kg formula to a high of 4011 mg·100 g^{-1} in the 5 kg formula (Fig. 1b). Reported on a proportion of total fatty acids basis, the range of n-3 LC-PUFA for Feed A formula was 8.1–10.9% (Table 1). The Feed B and Feed C formulae varied less, but still by twofold in Feed B (1749 in 5 kg to 3, 519.9 mg·100 g^{-1} in 1 kg formulae, Fig. 1), and by 1.7-fold in Feed C (2153 in 5 kg to 3612.6 in 1 kg mg·100 g^{-1}, Fig. 1). Proportionally, the range for Feed B was 12.9–14.5% and 14.3–18.8% n-3 LC-PUFA for Feed C (Table 1). The n-3 LC-PUFA content of Feed A increased from the 1 to 3 to 5 kg formula, whereas Feed B and Feed C n-3 LC-PUFA contents exhibited the opposite trend (Fig. 1b).

Table 4 East and west coast farmed Atlantic Salmon weight and feed and flesh % lipid and % n-3 LC-PUFA [mean (SD)]. Feed data are pooled across weight class formulae and farmed Atlantic Salmon data are for market-sized individuals only. Farmed Atlantic Salmon were reared at either east (present study) or west coasts and data for wild salmonids are from the west coast (previously published in Ikonomou *et al.* 2007 (a) and Friesen *et al.* 2008 (b)). No diet samples existed for the wild fish

		Diet				Salmon				
Region	Species	Feed type	*n*	% Lipid	% n-3 LC-PUFA	*n*	Weight (kg)	% Lipid	% n-3 LC-PUFA	References
East Coast										
Farmed	Atlantic	C	13	33.1 (2.9)	14.3 (5.2)	10	4.8 (0.9)	9.9 (1.8)	17.1 (2.2)	This study
		B	12	31.8 (3.4)	13.9 (7.0)	9	5.5 (1.1)	9.6 (2.1)	13.5 (1.2)	This study
		A	3	30.0 (3.4)	8.1 (5.5)	3	4.1 (0.5)	8.9 (0.6)	10.9 (0.4)	This study
West Coast										
Farmed	Atlantic	Marine fish oil	4	31.7 (2.1)	18.7 (7.7)	66	4.6 (1.0)	13.3 (2.8)	19.8 (2.8)	a
		C	1	25.6	13.4	6	4.1 (0.5)	12.3 (1.1)	12.3 (1.8)	b
Wild	Sockeye					12	3.0 (0.7)	4.0 (1.9)	24.9 (8.8)	a
	Chinook					23	4.1 (1.9)	4.2 (2.3)	23.2 (7.5)	a
	Pink					12	1.9 (0.2)	3.2 (0.8)	31.0 (11.1)	a
	Coho					37	3.4 (1.5)	2.9 (1.5)	33.9 (9.7)	a
	Chum					12	4.0 (1.1)	1.6 (0.5)	28.0 (6.9)	a

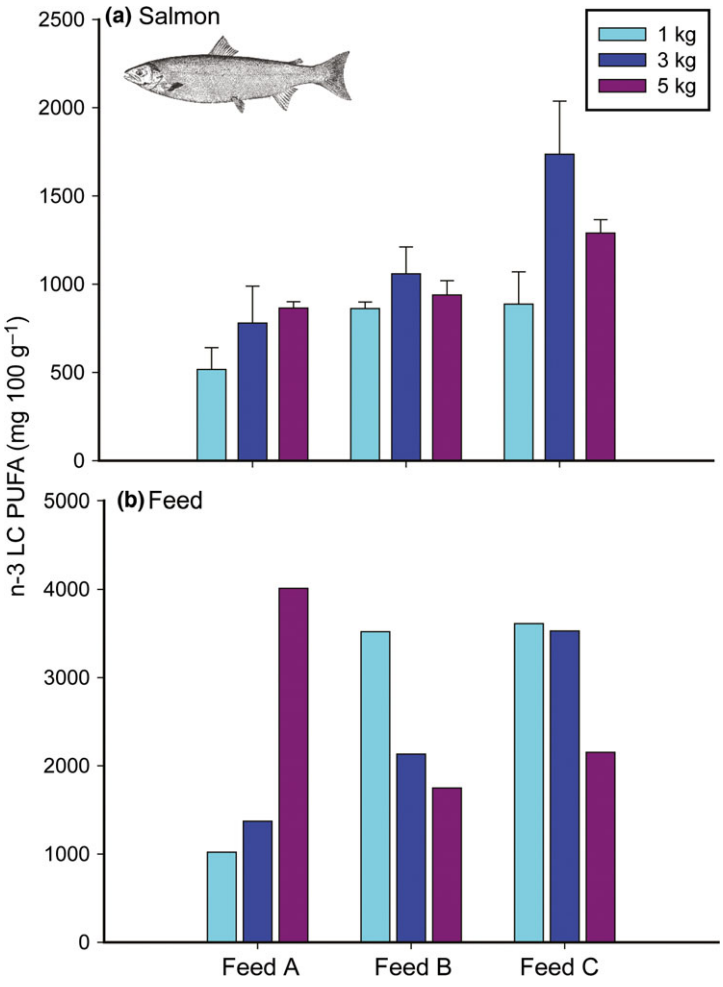

Figure 1 Farmed Atlantic Salmon (a) and feed (b) n-3 LC-PUFA at one east coast farm (Cooke Aqua). Fish were fed one of three diets (A, B and C) and sampled at three target weight classes (1 kg, 3 kg and 5 kg).

Weight class and feed type had a significant effect on salmon n-3 LC-PUFA levels based on ANOVA (Table 5). The 1 kg fish had significantly lower n-3 LC-PUFA than the 3 or 5 kg fish (Table 5, Fig. 1a) and significant differences among fish from the three diet treatments were in the order: Feed A < Feed B < Feed C (Table 5). The interaction between weight class and diet was also significant (Table 5). Feed A salmon had significantly lower n-3 LC-PUFA levels than Feed B and Feed C salmon at 1 kg (Table 5, Fig. 1a). Both Feed A and Feed B salmon had lower n-3 LC-PUFA levels than Feed C fish at 3 kg, but by 5 kg, there were no significant differences in salmon reared at Cooke Aqua on the three different diets (Table 5). Thus, irrespective of the observed variability within and among the different feeds (Fig. 1b), all salmon had similar mean n-3 LC-PUFA levels by harvest size (Fig. 1a).

Diet was, however, more important than weight class in terms of explaining the variability in n-3 LC-PUFA levels among salmon based on the higher F statistic for diet (Table 5) and the observation that salmon weight classes generally tracked dietary changes among the different feed formulae (Fig. 1a,b). Specifically, fish consuming Feed A exhibited increasing n-3 LC-PUFA levels from 1 to 3 to 5 kg that mirrored the increasing values in the feed formulae (Fig. 1a,b). For feeds B and C, the 3 kg salmon had higher n-3 LC-PUFA than the 5 kg salmon, which is consistent with the observation that n-3 LC-PUFA levels in feeds B and C decreased from the 1 and 3 kg formulae to the 5 kg formula (Fig. 1a,b).

These patterns in salmon flesh n-3 LC-PUFA levels among categorical, target weight classes (Fig. 1a) were also reflected in the individual fish weight data because only Feed A fish exhibited a significant, positive correlation between fish weight and flesh n-3 LC-PUFA values (Pearson's $r = 0.71$, Table 6). If weight alone were influencing the propensity for Atlantic Salmon to accumulate n-3 LC-PUFA, we would have expected increasing n-3 LC-PUFA with increasing fish weight regardless of dietary n-3 LC-PUFA values. The fact that neither Feed B nor Feed C fish exhibited a positive correlation with fish weight suggests that increasing n-3 LC-PUFA with weight observed in the Feed A fish was not attributed to a greater physiological need or ability to retain n-3 LC-PUFA with size and age, but to increasing n-3 LC-PUFA in the Feed A formulae (i.e. from 1 to 3 to 5 kg). Our findings are therefore consistent with previous studies (Arts *et al.* 2010) in suggesting that diet was a more important determinant of salmon n-3 LC-PUFA than fish size.

Although there were no significant differences among the 5 kg salmon from the different diet treatments, it is notable that the n-3 LC-PUFA level in the 5 kg salmon consuming Feed A (865 ± 36 mg·100 g^{-1}) was lower than Feed B fish (939 ± 81 mg·100 g^{-1}) and Feed C fish (1289 ± 76 mg·100 g^{-1}). The target 5 kg fish harvested from the Feed C treatment were also heavier than fish from the Feed B and Feed A diet treatments (5.2 versus 4.7 and 4.1 kg respectively), even though all fish were the same age and were harvested on the same date. These differences among diet treatments could be attributed to the lower n-3 LC-PUFA level of the 1 kg Feed A formulation, relative to the other two 1 kg feeds

Table 5 Results of two-way factorial ANOVA (and Tukey's HSD post hoc comparisons) performed to determine the effects of weight class (1 kg, 3 kg, 5 kg), diet (feeds A, B or C) and the weight class: diet interaction on east coast farmed Atlantic Salmon flesh n-3 LC-PUFA sampled from one farm (objective 1).

Factor	d.f.	*F*	*P*	Tukey's HSD comparisons
Weight class	2	17.27	<0.001	1 kg < 3 kg = 5 kg
Diet	2	29.67	<0.001	A < B < C
Weight class: Diet	4	2.93	0.049	1 kg: A < B = C 3 kg: A = B < C 5 kg: A = B = C

Table 6 Results of correlation analyses (Pearson's r) performed between fish weight (g) and Atlantic Salmon n-3 LC-PUFA (mg·100 g^{-1})

Farm	Diet	*r*	*t*	*P*	d.f.
Objective 1					
Cooke Aqua	A	0.74*	2.88	0.03	7
	B	0.15	0.39	0.71	7
	C	0.43	1.26	0.25	7
Objective 2					
Benson	B	0.97*	9.52	0.002	5
Pott	B	0.99*	9.64	0.01	2
Sand	B	0.84*	43	0.01	7
Foley	C	0.99*	21.57	<0.001	5
Cutler	C	0.71	2.26	0.07	5
Seeley	C	0.91*	6.62	<0.001	9

Significant correlations at $P < 0.05$ are denoted with '*'.

(Fig. 1b), suggesting that the Feed A fish were unable to catch up to the n-3 LC-PUFA levels exhibited by the 5 kg Feed B and Feed C fish. Previous studies confirm that salmon have lower n-3 LC-PUFA levels at harvest time if they are reared on diets containing lower n-3 LC-PUFA (Bell, McGhee *et al.* 2003; Bell, Tocher *et al.* 2003). However, results from one study indicate that a 12-week 'washout' period or 'finishing diet' of fish oil was sufficient to restore the n-3 LC-PUFA that were lost by rearing fish on vegetable oil-based diets (Bell, McGhee *et al.* 2003). In contrast, a separate study found that only 80% of n-3 LC-PUFA of fish reared on 100% vegetable oil-based diets were recovered after 20 weeks on a finishing diet compared to fish reared on a 100% fish oil (Bell, Tocher *et al.* 2003). Previous research (e.g. Arts *et al.* 2012), suggests that the acquisition of sufficient n-3 LC-PUFA early in life could significantly affect maximum n-3 LC-PUFA accumulation later in life, which could reconcile these apparently conflicting findings.

Environmental effects – differences among east coast farms

Similar to the results for objective 1, significant variability existed in feed n-3 LC-PUFA levels within a given diet treatment and among the different weight class feed formulae (Fig. 2b). Feed variability was most apparent for the 1 kg Feed B formulations which were approximately 3X lower at Sand (1260 mg·100 g^{-1}) and Pot (1570 mg·100 g^{-1}) than at Benson (4288 mg·100 g^{-1}, Fig. 2b) which corresponded to proportional feed n-3 LC-PUFA values of 4.7, 7.0 and 16.5% at Sand, Pot and Benson respectively (Table 2). The reason for this discrepancy is unknown but improper storage conditions or among-batch variability could have reduced the n-3 LC-PUFA levels of the 1 kg Feed B formulations at Sand and Pot compared to 1 kg formulations at Benson (Fig. 2b) and Cooke Aqua (objective 1, Fig. 1b). In comparison, the variability among each feed weight class formulations of Feed C was lower (maximum of

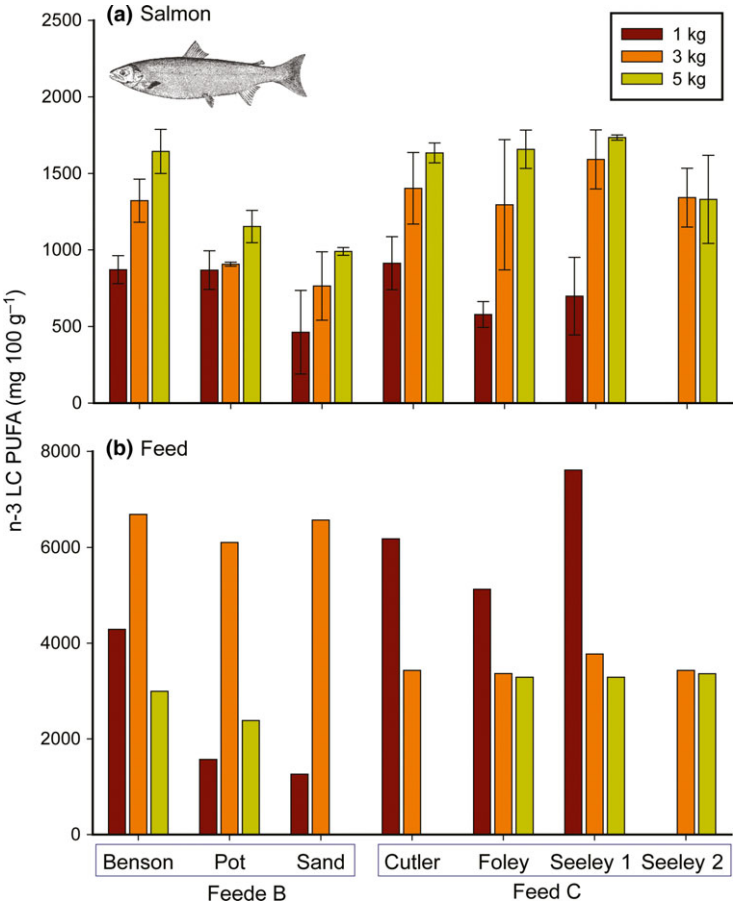

Figure 2 Values of n-3 LC-PUFA in Atlantic Salmon flesh (a) and feed (b) from six east coast farms. Fish were fed Feed B at Benson, Pot and Sand, and Feed C at Cutler, Foley and Seeley.

1.5-fold, Fig. 2b). At Seeley, the only farm that fed two different Feed C formulations to one weight class (3 kg fish received one of two separate formulae), there was minimal variability between these two diets (Fig. 2b). Some general trends were apparent in feeds across all six farms sampled for objective 2, however; both Feed B and Feed C formulations had lower n-3 LC-PUFA content at 5 than at 3 kg (Fig. 2b), which agrees with trends from these feeds used at the Cooke Aquaculture facility in objective 1 (Fig. 1b).

Rearing temperatures varied among farms based on monthly composites of satellite sea surface temperature data (Aqua MODIS). At the time of the 1 kg sampling (June to July), temperatures ranged from 9.7 (Sand, Maine) to 14.1°C (Benson, New Brunswick) and at the 3 kg sampling (January to February) from 1.5 (Pott, Newfoundland) to 4.2°C (Foley, New Brunswick, Table 2). However, salmon n-3 LC-PUFA levels did not significantly differ among farms feeding Feed C based on ANOVA (Table 7, Fig. 2a). At Feed B farms, only Sand fish were significantly lower than Benson fish (Pott fish did not significantly differ from fish at Sand or Benson, Table 7). Benson fish clearly grew better than Sand fish because Benson fish actual weight was 2 kg heavier than Sand fish when harvested for the 3 kg target weight class (Table 7). Because temperatures at Benson were the same as Sand during February (Table 2), dietary differences most likely explain observed between-farm differences in fish flesh quality (Fig. 2a) because n-3 LC-PUFA levels of the Feed B formulations were lower at Sand than Benson (Fig. 2b). This conclusion supports previous studies (Jobling & Bendiksen 2003) which found that diet had a greater impact on Atlantic Salmon flesh FA than external, environmental (temperature) differences. Although temperature is known to influence Atlantic Salmon PUFA uptake and retention in experimental studies (Arts *et al.* 2012), often in a diet-dependent manner (Jobling & Bendiksen 2003), the large variability in n-3 LC-PUFA levels within and among salmon feeds observed in the present study (Fig. 2b) likely precluded the detection of any subtle effects on salmon flesh quality arising from environmental differences (e.g. temperature) among salmon farms. Importantly, based on the present study, farm location does not introduce any major variability into Atlantic Salmon flesh quality bound for market. This conclusion agrees with findings from objective 1 where diet was the most important factor driving observed n-3 LC-PUFA levels.

Table 7 Results of two separate ANOVAs performed to compare salmon flesh n-3 LC-PUFA among east coast salmon farms by pooling data across the three weight classes. Values were compared among farms that fed salmon either: (1) Feed B or (2) Feed C. Mean and standard deviation (in parentheses) of n-3 LC-PUFA (mg·100 g^{-1}) of skinless Atlantic Salmon fillets (pooled across 1, 3 and 5 kg weight classes) are shown

Diet Regime	Farm	n-3 LC-PUFA	ANOVA result
Feed B	Benson	1220.7 (365.5)a	$F = 6.32$, $P < 0.01$
	Pott	960.8 (157.4)ab	
	Sand	714.2 (285.6)b	
Feed C	Cutler	1259.2 (364.9)a	$F = 0.41$, $P > 0.05$
	Foley	1091.6 (536.1)a	
	Seeley	1280.9 (438.3)a	

Significantly different values based on ANOVA do not share the same letter.

Contrary to findings from objective 1, changes in n-3 LC-PUFA levels among objective 2 salmon weight classes did not track dietary changes (Fig. 2a,b). At all farms sampled, salmon n-3 LC-PUFA (Fig. 2a) and % lipid (Table 2) increased from 1 to 3 to 5 kg, even though feed formulations (from both Feed B and Feed C) declined from 3 to 5 kg (Fig. 2b). Furthermore, when fish weights were considered on an individual, instead of on a categorical weight class basis, significant positive correlations between fish weight and n-3 LC-PUFA were observed for fish sampled from all east coast farms except for Cutler (Table 6). Because dietary values did not consistently increase in either % lipid, % n-3 LC-PUFA (Table 2) or mg·100 g^{-1} n-3 LC-PUFA (Fig. 2b) across weight class formulae, results of objective 2 suggest an effect of fish weight on flesh n-3 LC-PUFA that was independent of dietary values. Larger fish in objective 2 appeared able to accumulate higher levels of % lipid and n-3 LC-PUFA per 100 g wet weight of fillet than smaller fish, which agrees with previous reports of significant, positive correlations between fish weight and flesh % lipid (Hemre & Sandnes 1999). Thus, based on findings from objective 2, both diet (the observed differences between Sand and Benson farms) and fish weight (increasing flesh quality with increasing fish weight at all farms) significantly influence farmed Atlantic Salmon n-3 LC-PUFA.

Evidence for a dietary threshold effect on Atlantic Salmon n-3 LC-PUFA

A possible explanation for the observation that salmon flesh n-3 LC-PUFA increased with fish weight in objective 2, but mirrored dietary changes in objective 1, is that objective 2 fish were receiving sufficient dietary n-3 LC-PUFA to support a near maximum rate of PUFA incorporation. As a result, PUFA accumulation may have been limited by and be a function of fish weight and flesh capacity to store lipids, not solely by dietary supply. On the other hand, objective 1 fish could have been receiving dietary n-3 LC-PUFA below this 'threshold' of maximum incorporation, such that the accumulation of n-3 LC-PUFA by these salmon was limited by dietary supply, not fish size. This suggestion is supported by the fact that the feeds used in objective 2 (Fig. 2b, Table 2) were generally higher in n-3 LC-PUFA than the feeds used in objective 1 (Fig. 1b, Table 1). Among-batch variability is a likely explanation for this observation because objective 1 was performed earlier (2007–2008) than objective 2 (2009–2010), which is especially relevant for Feed B that changed owners in August 2007. Regardless, inspection of the data provides some insight into this possible dietary threshold level. In objective 1, when Feed A and fish increased from 1 to 3 kg and when Feed B and Feed C feed and fish decreased from 3 to 5 kg, the 1 and 3 kg Feed A and 5 kg Feed B and Feed C formulae were all below 2200 mg·100 g^{-1} (Fig. 1b), which corresponds to 9% n-3 LC-PUFA of total fatty acids (Table 1). Feeds from objective 2 were only below 2200 mg·100 g^{-1} (Fig. 2b) and 9% (Table 2) in the 1 kg Feed B formulation from Sand and Pot farms.

Our findings could therefore suggest that when feed values are below ~2200 mg·100 g^{-1} or 9% n-3 LC-PUFA, which corresponded to ~1400 and 800 mg·100 g^{-1} of EPA and DHA, respectively, salmon flesh changes proportionally (increases or decreases) with changes in feed (Fig. 2). When feed values are above 2200 mg·100 g^{-1} and 9%, on the other hand, salmon n-3 LC-PUFA does not increase or decrease with feed values but increases as a function of increased storage capacity associated with increasing fish size (i.e. larger fish can store more lipid and n-3 LC-PUFA per weight of fillet, Fig. 2a). Previous evidence for a threshold effect also exists. For example, Bell, Tocher *et al.* (2003) found that decreasing dietary values of n-3 LC-PUFA by replacing 33% of the marine oil component with vegetable oil resulted in appreciable declines in Atlantic Salmon EPA and DHA, but that providing dietary EPA beyond 1800 mg·100 g^{-1} (the lowest EPA of the experimental diets tested) did not produce a 1:1 increase in salmon muscle (Bell, Tocher *et al.* 2003). Instead, salmon appeared to preferentially catabolize EPA above this level (Bell, Tocher *et al.* 2003). Dietary n-3 LC-PUFA should therefore be provided in sufficient levels to promote maximum incorporation, which is a function of fish size based on our findings, but not at such high levels that exceeds storage capacity and will likely result in selective catabolism.

Lipid source, regional and farmed vs. wild differences

Two sources of data for harvest size, west coast (British Columbia) farmed Atlantic Salmon were available for comparison to our east coast results: (1) salmon sampled prior to 2003 that were reared on a more traditional marine oil diet (Ikonomou *et al.* 2007) and (2) salmon sampled after 2003 that were reared on Feed C, which, similar to feeds A and B, contains terrestrial sources of lipids (Friesen *et al.* 2008). The salmon reared on a marine fish oil diet had significantly higher flesh n-3 LC-PUFA levels than fish reared on Feed C on both the east (present study) and west coasts (Friesen *et al.* 2008). This agrees with data from Australian farmed Atlantic Salmon that exhibited a decline in n-3 LC-PUFA from 2002 to 2013 as terrestrial lipids were incorporated into feed to replace marine oil (Nichols, Glencross, Petrie & Singh 2014).

There was no difference between east and west coast salmon reared on Feed C (based on t-test, Fig. 3). A previous study reported higher n-3 LC-PUFA in west vs. east coast Canadian farmed Atlantic Salmon, but did not separate the salmon by those fed a marine (i.e. higher n-3 LC-PUFA) or terrestrially subsidized diets (i.e. lower n-3 LC-PUFA) (Hamilton *et al.* 2005), which could explain the discrepancy with our findings. Based on the present study, diet, not environmental differences, is the major driver of variability in Atlantic Salmon flesh quality both within and between North American east and west coasts.

Compared to farmed Atlantic Salmon, wild Pacific salmon had high individual variability and chum and coho had significantly lower mean n-3

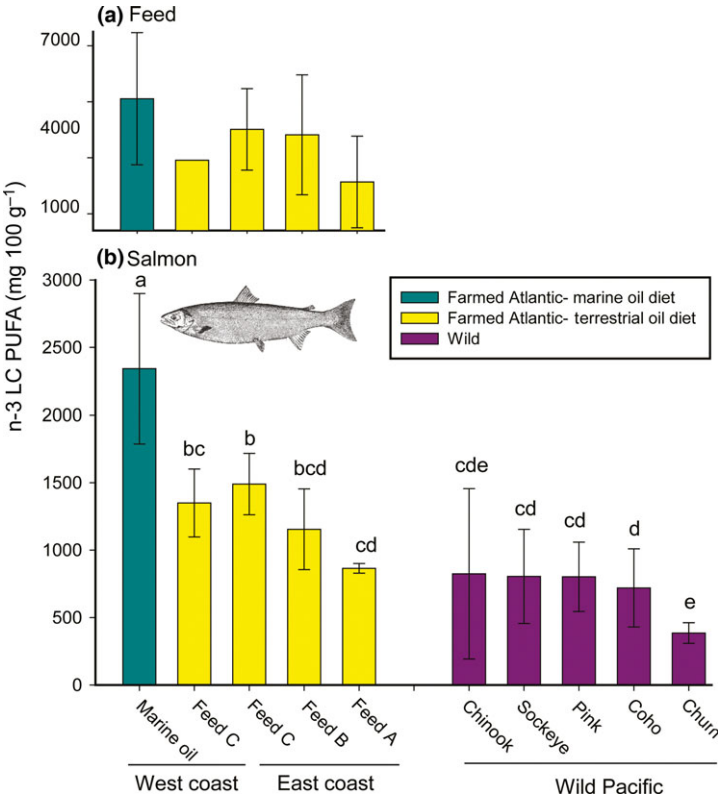

Figure 3 Feed (a) and flesh (b) n-3 LC-PUFA levels from harvest size farmed Atlantic Salmon reared on the east and west coasts of North America and from five wild Pacific salmonids. Farmed Atlantic Salmon were fed either marine (marine oil)- or terrestrial (A, B, C)-based diets. Significant differences among the salmon diet and geographical location groups based on pairwise t-tests (with non-pooled standard deviation due to unequal variances and with Bonferroni p adjustment) do not share the same letter.

LC-PUFA values than farmed Atlantic Salmon (Fig. 3), which agrees with previous findings (Hamilton *et al.* 2005; Ikonomou *et al.* 2007). While we do not have wild Atlantic Salmon data for an explicit comparison, previous studies reported similar (Blanchet, Lucas, Julien, Morin, Gingras & Dewailly 2005) and higher concentrations of n-3 LC-PUFA in farmed vs. wild Atlantic Salmon (Henriques *et al.* 2014; Nichols *et al.* 2014). This could suggest that wild Atlantic Salmon provide more EPA and DHA for human consumption than wild Pacific salmon, which warrants further study.

Based on the above comparisons, farmed Atlantic Salmon reared on terrestrially subsidized feeds on both east and west coasts provide on average 1730 mg (Feed A fish) to 2980 mg (east coast Feed C fish) n-3 LC-PUFA in one 200 g serving. Thus, one weekly 200 g serving of any of the farmed fish sampled in the present study is greater than the FAO's 1500–200 mg per day recommendation for children between 4 to 6 years old and within the 200–250 mg per day recommended for children 6–10 (FAO, 2010). For adults, two weekly 200 g servings also surpass, although is slightly below in the Feed A fish, the recommendation of the National Heart Foundation of Australia of 500 mg per day to reduce the risk of coronary heart disease (CHD) and contribute to the 1000 mg per day suggested for adults with documented CHD (National Heart Foundation of Australia 2008). Thus, even given the observed decline in n-3 LC-PUFA levels following replacement of marine with terrestrially source oils (Bell, McGhee *et al.* 2003; Bell, Tocher *et al.* 2003; Friesen *et al.* 2008; Nichols *et al.* 2014), farmed Atlantic Salmon remains an excellent source of essential lipids.

Recommendations for feed manufactures

There is an increasing demand for feed manufacturers and farm managers to consider the sustainability of feed lipid source, which has motivated a rich area of study into the extent that marine oils can be replaced with terrestrial sourced oils with the smallest impact on fish growth and health (Bell, McGhee *et al.* 2003; Bell, Tocher *et al.* 2003; Bransden, Carter & Nichols 2003; Henriques *et al.* 2014; Higgs, Balfry, Oakes, Rowshandeli, Skura &

Deacon 2006). Our study contributes to this body of research by tracking the effect of diet on n-3 LC-PUFA levels over 8–12 months at 7 different farm locations and throughout ambient temperature fluctuations. Although the nuances between temperature and dietary lipid source on FA composition in Atlantic Salmon fillets are not completely clear (Bowyer *et al.* 2013), fat digestibility has been shown to improve by feeding a higher fat diet, whereas protein digestibility was improved by feeding terrestrial (vs. fish) oils to Atlantic Salmon parr at lower temperatures (2 vs. 8°C) (Bendiksen *et al.* 2003). Our findings, as they relate to feed manufacturing, indicate that feeding a diet with n-3 LC-PUFA above the apparent threshold level of 3000 mg·100 g^{-1} or 10% of total FA is important at both smaller (1 kg) and larger weights (3 and 5 kg). Further, regardless of the temperature variability across salmon farms and sampling months at each target weight class of 9.7 to 14.1°C at 1 kg (June to July), 1.5 to 4.2°C (February to January) at 3 kg and 7.5 to 17.6°C (May to August) at 5 kg (Table 2), diet appeared to be the primary driver of observed variability. Based on these findings, feed manufacturers can avoid costly diets drastically above the n-3 LC-PUFA feed threshold, which will not result in proportional increases in salmon flesh. Maintaining dietary levels above the feed threshold in all life cycle stages (important for the n-3 LC-PUFA levels in market-sized fish) and a higher degree of consistency among the different feed formulae (as long as all are above the feed threshold) is also important based on our findings. Alternative sources of lipids should be explored for their capacity to meet these requirements to prevent increasing harvest pressure on overburdened marine resources.

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