1 **Uptake and selective partitioning of dietary lipids to ovarian and muscle tissue of**
2 **maturing female coho salmon, *Oncorhynchus kisutch*, during secondary oocyte**
3 **growth.**

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23
24 **Abstract**

25 Female coho salmon, *Oncorhynchus kisutch*, were fed one of two experimental
26 feeds containing lipids with markedly different stable ¹³C isotope signatures during the
27 late cortical alveolus, lipid droplet, and vitellogenesis stages of secondary oocyte growth.
28 Ovarian and muscle lipids fatty acid concentrations were significantly affected by
29 treatment during all three stages of development. Stable ¹³C isotope analyses confirmed
30 that dietary lipids were incorporated into both ovarian and muscle lipids during all three
31 stages and revealed that ovarian lipids were more affected than muscle lipids during

vitellogenesis. Arachidonic acid (ARA) was incorporated into ovarian lipids at the highest rate of all fatty acids examined with the greatest uptake observed during the cortical alveolus and lipid droplet stages of development. Docosahexaenoic acid (DHA) was incorporated into ovarian lipids at the next highest rate with the greatest uptake observed during the lipid droplet stage of development. The presence of an ovary specific, fatty acid transfer mechanism is proposed. Results from this study demonstrate the ability to greatly alter the fatty acid composition of ovarian lipids through a dietary change during secondary oocyte growth and may be of great interest to producers of farmed salmon and salmon broodstock programs.

Key words: secondary oogenesis, fatty acids, stable isotopes, lipid transport, broodstock nutrition

1. Introduction

Coho salmon *Oncorhynchus kisutch* are produced in fresh water and marine aquaculture systems, worldwide. Annual production typically exceeds 150,000 metric tons (FAO, 2016). In culture, coho salmon can reach weights over 4 kg and become sexually mature within two years. Like other Pacific salmon species, coho salmon are semelparous with highly synchronized oocyte growth. Gonadal somatic indices (GSI= gonadal weight/ whole body weight x 100) exceeding 20 are common among spawning females. The early maturation of females in some pedigrees enables the producer of coho salmon to harvest fish for meat and eggs, simultaneously.

Producers of farmed salmon are concerned with the human health benefits associated with their products as well as their sensory attributes (Sargent et al., 2001). Salmon are an excellent source of n-3 highly unsaturated fatty acids (HUFAs) and producers of farmed salmon strive to produce fish with n-3 HUFA levels that are similar to those of their wild counterparts. Until recently, this was easily achieved by selecting feeds rich in fish meal and fish oil, containing high amounts of n-3 HUFAs. Salmon receiving these marine based feeds throughout the production cycle consistently possessed muscle n-3 HUFA levels that were similar to that of wild fish or higher (Hamilton et al., 2005; Tocher 2015). This practice, however, has become costly in

63 recent years as increased global demand for the finite amounts of fish meal and fish oil
64 produced has resulted in dramatic increases in the costs of these ingredients (Sprague et
65 al., 2016; FAO, 2014). Many salmon producers have transitioned to lower cost,
66 terrestrial based feeds for the majority of the production period, and use higher cost,
67 marine based, “finishing” feeds shortly before harvest.

68 There is some debate as to the optimum timing and duration of a pre-harvest
69 “finishing” period for coho salmon, especially when harvesting maturing females. A
70 finishing period of 12 weeks is sufficient to restore muscle n-3 HUFAs in Atlantic
71 salmon (*Salmo salar*), previously fed plant oils, to levels similar to fish exclusively fed
72 fish oils (Bell et al., 2003). Robin et al. (2003) introduced a model to estimate muscle
73 lipid finishing periods that has been validated for a number of fish species (Jobling, 2004;
74 Lane et al., 2006; Turchini et al., 2006; Jobling et al., 2008; Benedito-Palos et al., 2009;
75 Woitel et al. 2014). The model, however, is not applicable to maturing fish and is
76 consequently of limited use to producers of coho salmon that harvest fish at late stages of
77 sexual maturity. Like many fish species, coho salmon undergo a period of depletion
78 during sexual maturation where nutrients are transported from adipose stores to the
79 gonads (Love, 1970). Weight gain is often minimal during this period which proves
80 problematic when applying the model proposed by Robin et al. (2003). The semelparous
81 nature of coho salmon further exasperates this issue as fish voluntarily cease feeding at
82 late stages of maturity.

83 To evaluate the efficiency of a finishing period, it is necessary to monitor the
84 amount of dietary lipid that is transported to target tissues. As in the aforementioned
85 studies, this is typically estimated from tissue weight gain and lipid composition. A more
86 direct approach is to incorporate a chemically identical, isotopic marker into the lipid
87 component of the finishing feed and monitor the emergence of this marker in the lipids of
88 target tissues. During periods of lipid accrual, this would differentiate what proportion of
89 the observed increase originated from dietary lipids rather than excess dietary protein or
90 carbohydrates. During periods of lipid depletion, this would allow for the detection of
91 minor dietary lipid contributions to target tissue when lipid mass balance calculations
92 suggest dietary lipids have no contribution.

93 Analogous to radiolabeled molecules that have been employed in metabolic
94 research for decades, biomolecules enriched with the stable isotopes of carbon, nitrogen,
95 and hydrogen have been successfully employed in metabolic studies (Wolfe and Chinkes,
96 2005; Makkar, 2008). Stable isotope markers have several advantages over their
97 radioactive counterparts. They are less hazardous to the health of both the animal subject
98 and the researcher and they eliminate the need for expensive specialized disposal.
99 Naturally occurring differences in ^{13}C and ^{15}N abundance exist in readily available feed
100 ingredients and allow for an economical way to monitor the assimilation of dietary
101 nutrients in large scale animal feeding studies. Natural differences in stable isotope
102 abundance exist between marine and terrestrial food webs, C4 and C3 terrestrial plants
103 (Kelly, 2000; West et al., 2006), and the lipid and non-lipid fractions of higher order
104 organisms (DeNiro and Epstein, 1977). Stable isotopes have been successfully used to
105 monitor the transport and bioconversion of nutrients in mammals (Wijendran et al., 2002;
106 Larque et al., 2003; Sarkadi-Nagy et al., 2004), and fish, including tilapia (Gay-
107 Siessegger et al. 2003), carp (Schlechtriem et al., 2004), and recently, sea cucumbers (Yu
108 et al., 2015).

109 In the following study, two feeds containing lipids with similar n-3 HUFA
110 content and markedly different stable ^{13}C isotope signatures were employed to investigate
111 the uptake of dietary lipids to ovarian and muscle lipids in coho salmon. The two feeds
112 had identical formulations, except for the lipid component, which either had a marine ^{13}C
113 signature or a terrestrial ^{13}C signature. As study fish were previously reared on marine
114 based feeds at our laboratory, the feed with the terrestrial ^{13}C signature was employed as
115 the “finishing” feed. However, in an actual commercial setting, the transition of salmon
116 from a terrestrial base feed to a marine based finishing feed would be more likely. The
117 three finishing periods examined coincided with the late cortical alveolus, lipid droplet,
118 and vitellogenesis stages of secondary oocyte development of female salmonids as
119 described by Nagahama (1983). At each interval, replicate tanks of fish were transitioned
120 to the finishing feed to monitor changes in stable ^{13}C isotope abundance of tissue lipids at
121 harvest and to assess the potential impact of dietary lipids on muscle and ovarian lipids
122 during that stage of oocyte development. A by-product of the research was the detection

123 of a selective partitioning of dietary fatty acids between muscle and ovarian lipids during
124 secondary oocyte growth.

125

126 **2. Methods**

127 **2. 1. Feeds**

128 Two experimental diets, a fish oil rich diet and an algal oil rich diet, were
129 prepared to contain similar lipid and n-3 HUFA content, but different ^{13}C stable isotope
130 signatures. Diets were identical except for the added lipid. The non-lipid portion of the
131 diets was produced and formulated by Nelson and Sons, Murray, UT, USA (now
132 Skretting) and ingredients are listed in order of abundance in Table 1. Fish oil feeds
133 contained 9% (w/w) sardine oil, produced in Mexico. Algal oil feeds contained 9%
134 (w/w) of a blend of algal and corn oils. The algal oil was DHASCO-T algal oil produced
135 by Martek Biosciences, Columbia, MD, USA (now DSM). Martek Biosciences cultured
136 *Schizochytrium spp* in land based, fermentation tanks to produce their DHASCO-T oil.
137 As a result of their process, the DHASCO-T oil possessed a stable isotope signature
138 similar to that of a C4 terrestrial plant, such as corn, elevated in ^{13}C and markedly
139 different than the marine lipids in the fish oil diet. The corn oil used in the algal oil diet
140 was a food grade cooking oil produced in the USA. As the intent of the study was to
141 formulate two experimental diets with similar lipid and n-3 HUFA content, but differing
142 stable isotope signatures, it was necessary to blend corn oil with algal oil to match the n-3
143 HUFA content of the fish oil. Corn oil was chosen over other plant oils due to its low n-3
144 HUFA content and similar stable ^{13}C isotope content to the algal oil. During preliminary
145 experiments, we determined the $\delta^{13}\text{C}$ values of sardine, corn, and DHASCO-T algal oils
146 to be approximately -24.5, -15.0 and -13.5‰, respectively (see explanation of $\delta^{13}\text{C}$
147 notation below). Proximate and fatty acid compositions of the feeds are listed in Table 1.

148

149 **2.2. Fish culture**

150 Coho salmon smolts, age 0, were purchased from Aquaseed Corporation,
151 Rochester, WA, USA (now Riverence LLC) in early September and gradually
152 transitioned from fresh to salt water in the indoor recirculating seawater system at our
153 laboratory. Average water temperature and salinity were 11°C and 29 g L⁻¹, respectively.

154 Lighting was programmed to mirror the natural photoperiod (47° 40' N). Fish were of
155 mixed sex. The typical sex ratio of males to females in this species is approximately 1:1.
156 Average fish weight was 27 g at time of purchase and fish were fed a commercial Pacific
157 salmon grower feed (EWOS Canada Ltd., Surrey, BC, Canada) until the beginning of the
158 feeding study, the following April. At the beginning of the study, 480 fish of mixed sex
159 were equally distributed among eight 3600 L tanks and four experimental treatments
160 were randomly assigned to two tanks each. Average fish weight (mixed sex) at the
161 beginning of the feeding study was 420 ± 57 g.

162

163 **2.3. Finishing periods**

164 A coho salmon producer who times the harvest of female fish for the end of
165 vitellogenesis is able to harvest both muscle for the meat market and eggs for the roe
166 market. This is the target harvest time we envisioned when naming our experimental
167 treatments. Treatments differed in when fish were transitioned to an algal oil finishing
168 feed (Figure 1). Finishing periods spanned the late cortical alveolus (experimental weeks
169 1-11), lipid droplet (experimental weeks 12-21), and vitellogenesis (experimental weeks
170 22-33) stages of secondary oocyte growth. Control fish were fed fish oil feeds
171 throughout the study. The LONG treatment corresponded to a 22 week finishing period,
172 timed late in the experiment. LONG fish were transitioned to algal oil feeds 22 weeks
173 before harvest, during the lipid droplet and vitellogenesis stages. The SHORT treatment
174 corresponded to a 12 week finishing period, timed late in the experiment. SHORT fish
175 were transitioned to algal oil feeds 12 weeks before harvest, during vitellogenesis. The
176 EARLY treatment corresponded to a 21 week finishing period, timed earlier in the
177 experiment. EARLY fish were transitioned to algal oil feeds 33 weeks before harvest,
178 during the cortical alveolus and lipid droplet stages, and then returned to fish oil feeds 12
179 weeks before harvest, during vitellogenesis.

180 Fish were fed 5 d/week during the experiment, but average daily feed rations were
181 calculated on a 7 d/week basis. The daily feed ration was initially set at 0.85% body
182 weight (bw)/d at the beginning of the feeding study and afterwards uniformly adjusted for
183 all tanks based on observed consumption and uneaten feed in the tanks. Fish were fed a
184 half ration by hand and a half ration using delay belt feeders. The feed ration remained at

185 0.85% bw/d until early vitellogenesis, when it was reduced to 0.7% bw/d, due to reduced
186 feeding response. The feed ration was further reduced to 0.4% bw/d after the mid
187 vitellogenesis sampling, and eventually reduced to 0.2% bw/d, two weeks later. The feed
188 ration was maintained at 0.2% bw/d until the end of the study.

189

190 **2.4. Tissue sampling**

191 Fish were sacrificed to obtain ovarian and muscle tissue at four sampling events
192 during the feeding study; LD-lipid droplet, EV-early vitellogenesis, MV-mid
193 vitellogenesis, and LV-late vitellogenesis as depicted on the study timeline (Figure 1).
194 Three of the sampling events (LD, EV, and LV) coincided with the end of a feeding
195 interval. The cortical alveolus interval ended with the LD sampling, the lipid droplet
196 interval ended with the EV sampling, and the vitellogenesis interval ended at the LV
197 sampling. Fish were additionally sampled mid-way through the vitellogenesis interval, at
198 week 28 (MV sampling). Whole body weights, lengths, hepatosomatic index (HSI) and
199 GSI were recorded for all sacrificed fish. Ovarian and muscle tissue were sampled and
200 saved at -80°C for subsequent proximate, fatty acid, and stable isotope analyses. Ovarian
201 tissue collected was also used for histological analysis to confirm stage of sexual
202 maturity. Average egg diameter was recorded for all female fish sampled at the end of
203 the study (LV). Study fish were cared for humanely in accordance with practices
204 developed by the National Research Council of the United States of America for aquatic
205 animals (NRC, 2011) and were euthanized in accordance with American Veterinary
206 Medical Association guidelines (AVMA, 2007).

207

208 **2.5. Chemical analysis**

209 **2.5.1. Proximate analysis**

210 White muscle tissue samples were sectioned from fillets of sacrificed fish above
211 the lateral line and anterior to the dorsal fin. All skin and red muscle was trimmed from
212 the muscle samples prior to chemical analysis. For previtellogenic fish, an ovarian tissue
213 sample was a complete ovary. For vitellogenic fish, an ovarian tissue sample was a cross
214 section of the anterior portion of one ovary. Muscle and ovarian tissue was lyophilized at
215 -20C prior to analyses. Protein and ash content of feeds and tissue samples were

performed in accordance with AOAC Official Methods 968.06 and 942.05, respectively (AOAC International, 2000). Lipid content of feed and tissue samples were determined via supercritical fluid extraction (SFE) as described by Johnson and Barnett (2003) and extracts saved for subsequent fatty acid and stable isotope analyses. Supercritical carbon dioxide with an ethanol modifier was employed as the eluent in the SFE procedure to ensure the complete recovery of both neutral and polar lipids.

2.5.2. Fatty acid analysis.

Fatty acid methyl esters were prepared from extracted feed and tissue lipids in accordance with AOAC Official Method 969.33 (AOAC International, 2000) and chromatographically quantified on a Hewlett Packard 5890 gas chromatograph employing a DB-225 polysiloxane column with a flame ionization detector, Agilent Technologies, Wilmington, DE, USA. Fatty acid methyl esters were solvated in decane rather than isooctane as listed in the official method. The separation method employed splitless injection and a carrier gas flow rate of 1.0mL/min. The thermal gradient profile began at 180°C for 2 min, increased by 2°C /min to 236°C, and then remained at 236°C for 15 min. The temperature of both the inlet and the detector was 275°C. The accuracy of the method was verified with an American Oil Chemistry Society (AOCS) reference standard (menhaden oil) from their laboratory proficiency program. Fatty acid concentrations in lipids were expressed on a percentage basis as mg fatty acid per 100 mg total fatty acids. Individual fatty acids were presented in tables employing the shorthand notation, X:Y, where X is the number of carbons in the aliphatic chain and Y is the number of double bonds. Fatty acid partitioning coefficients, $fa_{(o/m)}$, were computed for individual fish from paired samples of ovarian and muscle tissue by the equation, $fa_{(o/m)} = \text{fatty acid concentration in ovarian lipids} / \text{fatty acid concentration in muscle lipids}$.

2.5.3. Stable ¹³C isotope analysis

Stable ¹³C isotope ratios for feed, muscle, and ovarian lipid extracts were determined on a Costech ECS 4010 elemental analyzer, Valencia, CA, USA, coupled to a Thermo Electron Delta Plus stable isotope ratio mass spectrometer, Bremen, Germany.

247 Stable carbon isotope ratios were expressed in δ notation as per mil (‰) according to the
 248 following expression,
 249
$$\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}})-1] \times 1000$$

 250 where R_{sample} is the ratio of $^{13}\text{C} : ^{12}\text{C}$ detected in the sample, and R_{standard} is the ratio of ^{13}C
 251 $: ^{12}\text{C}$ detected in the corresponding standard. Two isotopic standards for carbon were used
 252 to define the line used to convert the mass spectrometer signal to sample $\delta^{13}\text{C}$ values.
 253 Precision for isotope analysis was $\leq \pm 0.2\text{‰}$ for $\delta^{13}\text{C}$. All carbon values were referenced
 254 to Vienna Pee Dee Belemnite, a.k.a. NBS 19 [$\delta^{13}\text{C}$ of NBS 19 $\equiv 1.95\text{‰}$ (Coplen et al.,
 255 2006)].

257 **2.6. Statistical analysis**

258 All statistical analyses were performed with R version 2.13.0 statistical software
 259 (The R Foundation for Statistical Computing, Palo Alto, CA, USA). Differences were
 260 deemed significant when $P < 0.05$. Treatment means are reported as mean $\pm$ standard
 261 deviation. Student's t-test was performed to detect significant differences in tissue
 262 composition (proximate composition, fatty acid composition, and stable ^{13}C isotope
 263 abundance in tissue lipids) between the two treatments present at the LD sampling. One-
 264 way analysis of variance (ANOVA) was performed to detect significant differences in
 265 tissue composition attributable to treatment at the EV, MV, and LV samplings. When
 266 significant differences were detected by ANOVA, the Tukey HSD test was subsequently
 267 employed to assess the significance of differences between treatment means. Mean tissue
 268 proximate compositions of fish from all tanks were additionally grouped by sampling
 269 ($n=8$), and significant changes in these parameters over the course of the feeding study
 270 were assessed by one-way ANOVA, main effect, with Tukey HSD employed to detect
 271 significant difference between individual samplings.

272 Fatty acid partitioning coefficients, $fa_{(o/m)}$, were $\log_{10}(x + 1)$ transformed prior to
 273 statistical analysis as described by Zar (1999) to minimize variability differences between
 274 the results from individual fatty acids. Box and whisker plots were constructed to
 275 visualize differences in $fa_{(o/m)}$ for each sampling. One-way ANOVA, main effect,
 276 followed by Tukey HSD post hoc test was performed for the $fa_{(o/m)}$ for each fatty acid

277 over the course of the study to detect differences in fatty acid partitioning during the three
278 stages of secondary oocyte growth.

279 From examination of the effect of treatment on the stable ^{13}C isotope abundance
280 in tissue lipids, it became evident that differences existed between the $\delta^{13}\text{C}$ values of
281 muscle and ovarian lipids within a treatment. As a result, a series of paired Student's t-
282 tests were performed to determine the significance of these differences at each sampling.
283 Results were graphed by treatment and potential reasons for these differences discussed.

284

285 **3. Results**

286 *3.1. Fish Growth*

287 Fish growth and body indices have been presented previously (Johnson et al.
288 2014). Briefly, female fish grew well and sexually matured during the experiment and
289 there were no detectable differences in fish weight, fish length, condition factor, HSI, or
290 GSI attributable to feed treatment. Histological examination of ovarian tissue confirmed
291 the correct timing of the cortical alveolus, lipid droplet, and vitellogenesis feeding
292 intervals. Female fish reached a final weight of $1740 \pm 120\text{g}$ at late vitellogenesis (Figure
293 2a). Eggs sampled at late vitellogenesis were $5.5 \pm 0.1\text{mm}$ in diameter and a suitable size
294 for the manufacture of salmon caviar. Gonadal somatic index, GSI, increased from 0.3 at
295 the start of the experiment to 17.2 ± 0.5 at late vitellogenesis (Figure 2b).

296

297 *3.2. Tissue Proximate Composition*

298 At each sampling, fish from different treatments possessed ovarian and muscle
299 tissue with similar proximate compositions. Tissue proximate composition changed,
300 however, over the course of the study as fish sexually matured ($P < 0.05$). Muscle
301 protein content fluctuated slightly during the lipid droplet feeding interval through mid
302 vitellogenesis, and then significantly decreased between mid and late vitellogenesis
303 (Figure 3a). Muscle protein content was greatest, $215 \pm 5 \text{ g kg}^{-1}$, at the MV sampling and
304 decreased to a minimum of $195 \pm 5 \text{ g kg}^{-1}$ by LV. Ovarian protein content continually
305 increased over the course of the study from a minimum of $195 \pm 5 \text{ g kg}^{-1}$ at the LD
306 sampling to a maximum of $282 \pm 4 \text{ g kg}^{-1}$ at LV.

307 Muscle lipid content increased during the lipid droplet feeding interval, decreased
308 between early and mid vitellogenesis, and remained constant from mid through late
309 vitellogenesis (Figure 3b). Muscle lipid content was greatest, $53 \pm 5 \text{ g kg}^{-1}$, at the EV
310 sampling. Ovarian lipid content followed a similar pattern to that of muscle lipids.
311 Ovarian lipid content was greatest, $154 \pm 5 \text{ g kg}^{-1}$, at the EV sampling.

312

313 *3.3. Tissue Fatty Acids*

314 Dietary lipids strongly influenced the fatty acid composition of muscle and
315 ovarian lipids at every sampling. Additionally, there were differences in how dietary
316 fatty acids were partitioned between ovarian and muscle tissue over the course of the
317 study. Fatty acid results are presented below by feeding interval. Observed changes in
318 fatty acid partitioning between ovarian and muscle lipids over the course of the study are
319 then presented.

320

321 *3.3.1. Cortical Alveolus Feeding Interval.*

322 As expected, fish transitioned to the algal oil feed during the late cortical alveolus
323 stage of oocyte development (EARLY fish) possessed muscle and ovarian lipids with
324 significantly different fatty acid composition than control fish at the LD sampling (Tables
325 2 and 3). Reflective of the fatty acid composition of the feeds, EARLY fish muscle and
326 ovarian lipids contained elevated levels of DHA ($P=0.005$, $P<0.001$) and linoleic acid,
327 LA ($P<0.001$, $P<0.001$) and decreased levels of eicosapentaenoic acid, EPA ($P<0.001$,
328 $P<0.001$). Elevated levels of arachidonic acid, ARA, in the fish oil feed resulted in
329 elevated levels of ARA in the ovarian ($P=0.007$) and muscle ($P=0.013$) lipids of control
330 fish. Despite feed lipids being balanced for total n-3 fatty acid content (Table 1), muscle
331 lipids of control fish contained significantly more n-3 fatty acid than that of EARLY fish
332 ($P=0.016$). Ovarian lipid n-3 content was not significantly different ($P=0.153$) between
333 the two treatments. Reflective of the LA levels in the feeds, EARLY fish tissue lipids
334 were significantly elevated in total n-6 fatty acids ($P<0.001$).

335 Box and whisker plots revealed differences in fatty acid partitioning associated
336 with fatty acid structure during the cortical alveolus feeding interval (Figure 4a). Fatty
337 acids with at least 20 carbons in their aliphatic chain and 4 double bonds were elevated in

ovarian lipids over muscle lipids. With the exception of palmitic acid, c16:0, saturated and monounsaturated fatty acids were elevated in muscle lipids over ovarian lipids. Arachidonic acid, ARA, was selectively partitioned into ovarian lipids at the highest rate of any fatty acid examined. Docosahexaenoic acid, DHA, was selectively partitioned into ovarian lipids at the next highest rate, above that observed for EPA and DPA.

343

3.3.2. Lipid Droplet Feeding Interval.

Transitioning fish from the fish oil feed to the algal oil feed during the lipid droplet stage of oocyte development (LONG fish) resulted in ovarian and muscle lipid fatty acid profiles that were distinctly different to those of control fish and EARLY fish at the early vitellogenesis sampling (Tables 2 and 3). Concentrations of individual fatty acids in the muscle lipids of LONG fish were often intermediate, and occasionally significantly different, to that observed in control fish and EARLY fish. Significant differences were observed with palmitoleic acid, c16:1 ($P<0.001$), LA ($P<0.001$), EPA ($P<0.001$), and DPA ($P<0.001$). Levels of DHA in the muscle lipids of LONG fish were intermediate to the other two treatments, but were not significantly different to those of EARLY fish. Muscle lipid n-3 content was similar between treatments ($P=0.749$) and, as observed with LA, the total n-6 content of LONG muscle lipids was intermediate and significantly different to that observed in the other two treatments ($P<0.001$).

As observed with muscle lipids, concentrations of individual fatty acids in the ovarian lipids of LONG fish were often intermediate to that observed in the other two treatments. There were fewer instances when the fatty acid concentrations of LONG fish ovarian lipids were significantly different to the other two treatments, but this was observed with c16:1 ($P<0.001$) and DHA ($P<0.001$). Levels of LA, EPA, and DPA in the ovarian lipids of LONG fish were not significantly different to those observed among EARLY fish. In contrast to that observed for muscle lipids, the total n-3 fatty acid content of ovarian lipids of LONG fish were significantly less than that observed in the other two treatments ($P=0.048$). Reflective of LA concentrations, the total n-6 fatty acid content of LONG fish ovarian lipids were significantly different to that of control fish ($P<0.001$) and were not significantly different to that of EARLY fish.

Box and whisker plots illustrate differences in fatty acid partitioning associated with fatty acid structure during the lipid droplet feeding interval (Figure 4b). As observed previously during the cortical alveolus feeding interval, fatty acids with at least 20 carbons and 4 double bonds were elevated in ovarian lipids. Saturated and monounsaturated fatty acids were elevated in muscle tissue. As observed previously, ARA was selectively partitioned into ovarian lipids at the highest rate of any fatty acid examined at this stage of development, followed by DHA.

3.3.3. Vitellogenesis Feeding Interval.

Fish tissue was sampled from female fish at the beginning, mid-way, and at the end of the vitellogenesis stage of oocyte development. Fish transitioned to the algal oil feed at the beginning of vitellogenesis (SHORT fish) possessed ovarian and muscle lipids with significantly different fatty acid profiles than control and LONG fish at the MV and LV samplings (Tables 2 and 3). EARLY fish, which had been fed the algal oil feed throughout the cortical alveolus and lipid droplet stages of oocyte development, were transitioned to the fish oil feed during this feeding interval. As a result, EARLY fish possessed ovarian and muscle lipids with distinctly different fatty acid profiles than the other three treatments by the LV sampling. EARLY fish muscle fatty acid profiles were similar to that of LONG fish at the MV sampling.

Fatty acid concentrations in the muscle lipids of SHORT fish were often intermediate, and occasionally significantly different, to that of control and LONG fish at the MV sampling. This was observed with eicosenoic acid, c20:1 ($P=0.006$), LA ($P=0.014$), EPA ($P=0.024$), and DPA ($P=0.015$). This trend continued to the LV sampling when c16:1 ($P=0.012$), c20:1 ($P=0.001$), LA ($P=0.001$), and EPA ($P<0.001$), levels in SHORT muscle lipids were intermediate and significantly different to that of control and LONG fish. In addition, levels of palmitic acid, c16:0 ($P=0.006$) and DHA ($P=0.011$) of SHORT fish muscle were significantly different to those of LONG fish by the LV sampling. The total n-3 fatty acid content of muscle lipids from control, SHORT, and LONG fish were similar at the MV and LV samplings ($P=0.676$, $P=0.214$) and total n-6 fatty acid levels were significantly different ($P=0.009$, $P=0.001$).

398 The fatty acid composition of muscle lipids from EARLY fish was similar to that
399 from LONG fish at the MV sampling. After transitioning EARLY fish to the fish oil feed
400 during early vitellogenesis, c16:1, EPA, and DPA levels in muscle lipids increased,
401 which resulted in similar levels to that of LONG fish at the MV sampling and higher EPA
402 levels than LONG fish at the LV sampling ($P=0.002$). The inverse trend was observed
403 with LA, which resulted in EARLY fish possessing significantly lower LA levels than
404 LONG fish at the LV sampling ($P=0.016$). Total levels of n-3 fatty acids were similar
405 among the muscle lipids of EARLY, LONG, and control fish at both the MV ($P=0.810$)
406 and LV ($P=0.103$) samplings and total n-6 fatty acids again mirrored the differences
407 observed with LA at both samplings.

408 Fatty acid levels in the ovarian lipids of SHORT fish were often intermediate to
409 that observed among control and LONG fish at both the MV and LV samplings. Only
410 LA levels were intermediate and significantly different to both the control and LONG
411 treatments at both samplings ($P=0.008$, $P=0.004$). Reflective of LA levels, total n-6 fatty
412 acids in the lipids of SHORT fish were also intermediate and significantly different from
413 control and LONG fish at both samplings ($P=0.002$, $P=0.006$). As observed at the EV
414 sampling, the levels of DHA in the ovarian lipids of LONG fish continued to be
415 significantly higher than that observed among control fish at the MV and LV samplings
416 ($P=0.006$, $P=0.024$). The total n-3 fatty acid content of ovarian lipids from control,
417 SHORT, and LONG fish were similar at MV and LV ($P=0.614$, $P=0.239$) samplings.

418 The transitioning of EARLY fish to a fish oil feed at early vitellogenesis had a
419 greater effect on the fatty acid concentrations of ovarian lipids than muscle lipids at the
420 MV sampling. While the EPA and LA levels in the muscle lipids of EARLY fish were
421 similar to those of LONG fish at the MV sampling ($P=0.194$, $P=0.547$), significant
422 differences were observed in the ovarian lipids ($P=0.002$, $P<0.001$). By the LV
423 sampling, fatty acid levels in the ovarian lipids of EARLY fish were intermediate and
424 often significantly different to levels observed in both control and LONG fish. In
425 contrast, fatty acid levels in the ovarian lipids of EARLY and SHORT fish were very
426 similar by the LV sampling, despite EARLY fish having received algal oil feeds for 21
427 weeks and SHORT fish having received algal oil feeds for only 12 weeks.

Box and whisker plots revealed differences in fatty acid partitioning associated with fatty acid structure during the vitellogenesis feeding interval (Figure 4c). As observed previously during the cortical alveolus and lipid droplet feeding intervals, fatty acids with at least 20 carbons and 4 double bonds were elevated in ovarian lipids. With the exception of stearic acid, c18:0, saturated and monounsaturated fatty acids were elevated in muscle tissue. Again, ARA was selectively partitioned into ovarian lipids at the highest rate of any fatty acid examined at this stage of development. Docosahexaenoic acid, EPA, and DPA were deposited at similar rates.

3.3.4. Changes in Fatty Acid Partitioning.

The partitioning of individual fatty acids between ovarian and muscle tissue significantly changed over the course of the study. Changes in fatty acid partitioning coefficients, $fa_{(o/m)}$, were observed over time for all fatty acids examined except LA. Stearic acid, c18:0, $fa_{(o/m)}$ changed the most of any fatty acid during the study. Stearic acid was selectively deposited into muscle tissue over ovarian tissue during the cortical alveolus and lipid droplet feeding intervals, but partitioning between the two tissues appeared equal after the vitellogenesis feeding interval (Figure 5a). Linoleic acid, LA, was selectively deposited into muscle tissue throughout the study and $fa_{(o/m)}$ was similar at all samplings (Figure 5b). Arachidonic acid, ARA, was selectively partitioned into ovarian lipids throughout the study and $fa_{(o/m)}$ was significantly higher during the cortical alveolus and lipid droplet feeding intervals than the vitellogenesis feeding interval (Figure 5c). Docosahexaenoic acid, DHA, was also selectively partitioned into ovarian lipids throughout the study and $fa_{(o/m)}$ was the highest during the lipid droplet feeding interval (Figure 5d).

3.4. Tissue Lipids Stable ^{13}C Isotope Signatures

The stable ^{13}C isotope signatures, $\delta^{13}\text{C}$, of ovarian and muscle lipids were significantly affected by treatment at every sampling (Tables 2 and 3, $P < 0.001$). Fish that received the algal oil feed (SHORT, LONG, and EARLY treatments) during the study possessed muscle and ovarian lipids with elevated (less negative) $\delta^{13}\text{C}$ values than those of the control fish. Within a treatment, significant differences were observed

between the $\delta^{13}\text{C}$ values of ovarian and muscle lipids over the course of the study. In control fish, ovarian lipid $\delta^{13}\text{C}$ values were lower (more negative) than that of muscle lipids at every sampling (Figure 6a). EARLY fish, which initially were fed the algal oil feed, had ovarian lipids with $\delta^{13}\text{C}$ values that were higher (less negative) than that of muscle lipids after the cortical alveolus and lipid droplet feeding intervals, and then ovarian lipid $\delta^{13}\text{C}$ values were lower than muscle lipid $\delta^{13}\text{C}$ values after fish transitioned to the fish oil feed during vitellogenesis (Figure 6b). In LONG fish, ovarian lipid $\delta^{13}\text{C}$ values were higher than muscle lipid $\delta^{13}\text{C}$ values at the EV and MV samplings, but similar at the LV sampling (Figure 6c). In SHORT fish, ovarian lipid $\delta^{13}\text{C}$ values were higher than muscle lipid $\delta^{13}\text{C}$ values after fish were transitioned to the algal oil feed during vitellogenesis (Figure 6d).

4. Discussion

Results from this study demonstrate the ability to significantly alter the fatty acid composition of muscle and ovarian lipids in cultured coho salmon during secondary oocyte growth through changes in dietary lipids. This is in agreement with previous studies showing dietary lipids to directly influence the fatty acid profile of muscle lipids (Polvi and Ackman, 1992; Hardy et al., 1987; Bell et al., 2001; Torstensen et al., 2005; Higgs et al., 2006) and to a lesser extent, eggs lipids (Hardy et al., 1990; Rennie et al., 2005) in salmonids. At all samplings, the stable ^{13}C isotope abundance in ovarian and muscle lipids of study fish reflected the stable ^{13}C isotope abundance of the feed lipids they had received. Control fish, which received only fish oil feeds during the study, had ovarian and muscle lipids with a marine $\delta^{13}\text{C}$ signature, which was significantly lower (more negative) than fish from other treatments. The $\delta^{13}\text{C}$ values of both ovarian and muscle lipids of EARLY, LONG, and SHORT fish were significantly higher (less negative) to those of control fish after the cortical alveolus, lipid droplet, and vitellogenesis feeding intervals, respectively. This suggests that dietary lipids are incorporated into both egg and muscle tissue of coho salmon at appreciable amounts during all three stages of secondary oocyte development.

Regarding fatty acid composition, the DHA content of ovarian and muscle lipids significantly increased when fish received a high DHA, algal oil feed. It was additionally

490 observed that DHA was selectively partitioned into ovarian lipids over muscle lipids at a
491 rate higher than any other fatty acid examined except for ARA, which reaffirms the
492 physiological importance of these fatty acids to egg viability and larval survival in fish
493 (Watanabe et al., 1984; Sargent et al., 1999; Izquierdo et al., 2001; Tocher, 2010, Luo et
494 al., 2015). For all three developmental stages (cortical alveolus, lipid droplet, and
495 vitellogenesis), increases in DHA were greater in ovarian lipids than muscle lipids when
496 fish were transitioned to the algal oil feed. This illustrates the significant contribution of
497 dietary lipids to ovarian lipids in maturing coho salmon and demonstrates the potential of
498 dietary DHA enrichments to more effectively increase the DHA content of ovarian lipids
499 than muscle lipids prior to harvest. This finding complements prior broodstock feeding
500 studies with cultured fish, where dietary enrichments immediately prior or during a
501 spawning period were shown to significantly alter the n-3 and n-6 HUFA content of egg
502 lipids (Bruce et al., 1999; Furuita et al., 2003; Fuiman and Faulk, 2014; Rodríguez-
503 Barreto et al., 2014; Røjbek et al., 2014; Xu et al., 2017).

504 It was additionally observed that HUFAs, with 20 carbons or more in their
505 aliphatic chain and 4 or more double bonds, were more concentrated in ovarian than
506 muscle lipids during all stages of secondary oocyte growth. Elevated levels of HUFAs in
507 the lipids of ripe eggs have been observed in a number of fish species (Wiegand, 1996;
508 Izquierdo et al., 2001, Rodríguez-Barreto et al. 2012) including coho salmon (Hardy et
509 al., 1990), and is suspected to be a result of the preferential catabolism of more easily
510 oxidized fatty acids in the liver during the mobilization of body lipids to gonadal lipids
511 (Henderson et al., 1984; Henderson and Almatar, 1989). In rainbow trout, Henderson
512 and Sargent (1985) observed higher liver mitochondrial β -oxidation rates for
513 monounsaturated and short-chain saturated fatty acids than for HUFAs. Docosahexaenoic
514 acid, in particular, is believed to be a poor substrate for mitochondrial β -oxidation in fish
515 (Tocher, 2003) and accumulates in the tissues of fish with high energy requirements such
516 as yellowfin tuna (Saito et al., 1996). While this partially explains the observed elevated
517 concentrations of HUFAs in ovarian lipids during and after vitellogenesis, it does not
518 explain the elevated concentrations of HUFAs observed in ovarian lipids when muscle
519 lipids were increasing, during the cortical alveolus and lipid droplet stages. As the VLDL
520 lipoprotein class is suspected of transporting the majority of lipids to all peripheral tissues

521 in fish (Sheridan, 1988), the higher partitioning of HUFAs into the ovary during these
522 earlier stages cannot be explained by the preferential catabolism of saturated and
523 monounsaturated fatty acids in the liver. Lipid catabolism within the ovary would
524 explain the elevated concentration of HUFAs, but this is unlikely to be the case as
525 ovarian lipid concentrations increased during these stages of development and reached
526 their highest levels after the lipid droplet feeding interval. Combined, our observations
527 suggest the presence of a currently unknown, selective fatty acid transport mechanism in
528 ovarian tissue that results in a concentration of physiologically essential n-3 and n-6
529 HUFAs in ovarian lipids.

530 The selective uptake of DHA has been observed by the brain of fish and other
531 animals (Xu et al., 1996; Haunerland and Spener, 2004; Liu et al., 2003). In mammals, a
532 tissue specific fatty acid binding protein, FABP7, has been detected in the brain that is
533 responsible for the preferential uptake of DHA from circulating lipoproteins. FABP7 has
534 a 20+ fold greater affinity for DHA than oleic acid or ARA (Xu et al., 1996). While
535 undiscovered in the majority of fish species, FABP7 mRNA has been isolated from brain
536 tissue of zebrafish (Liu et al., 2003) and Atlantic salmon (Lai et al. 2012). The
537 expression of FABP7 in ovarian tissue in the current study would explain the
538 accumulation of DHA in ovarian tissue during secondary oocyte growth. However, the
539 presence of FABP7 does not explain the preferential uptake of ARA and other HUFAs
540 during this period and the authors speculate there may be another, yet to be discovered,
541 ovary specific FABP that may be responsible for the selective uptake of ARA to ovarian
542 tissue.

543 In this study, ARA was preferentially deposited into ovarian lipids during all three
544 stages of secondary oocyte development at the highest rate of any fatty acid examined.
545 This preferential partitioning of ARA into ovarian lipids has been observed previously in
546 fish and dietary enrichments have been shown to effectively increase the ARA content of
547 egg lipids in European sea bass (Bruce et al. 1999), Japanese flounder (Furuita, 2003),
548 red drum (Fuiman and Faulk, 2013), and tongue sole (Xu et al., 2017). In the current
549 study, it was additionally observed that the partitioning of ARA into ovarian lipids was
550 greatest during the two previtellogenic stages of secondary oocyte growth. These
551 observations are particularly interesting as the ARA content of egg lipids, particularly the

phosphoinositol lipid class, has been positively correlated with larval fish survival in a number of teleost species (Pickova et al., 1999; Cejas et al., 2003; Bell and Sargent, 2003; Salze et al., 2005). From mass balance calculations, more ARA was deposited into the developing oocyte during vitellogenesis than any other stage of development, but as the phosphoinositol lipid class represents only a minor component of fish egg lipids (Tocher and Sargent, 1984; Navas et al., 1997; Salze et al., 2005; Murzina et al. 2009), the uptake of ARA during previtellogenic growth may be physiologically essential for the proper manufacture of this lipoprotein class. Correspondingly, it may prove extremely important to supplement salmon broodstock diets with ARA during previtellogenic growth when the preferential deposition of ARA to ovarian lipids is the highest.

While the experimental feeds in this study were balanced for n-3 fatty acids, they were not balanced for n-6 fatty acids. This resulted in the algal oil feeds being higher in polyunsaturated fatty acids and lower in saturated and monounsaturated fatty acids. Elevated levels of LA, the dominant n-6 fatty acid, in the algal oil feed resulted in elevated levels of n-6 fatty acids in the ovarian lipids of SHORT, LONG, and EARLY fish at every sampling. Elevated levels of n-6 fatty acids were balanced with reduced levels of monounsaturated fatty acids in ovarian lipids. Concentrations of saturated fatty acids in ovarian lipids were not affected by treatment. While it is currently unknown whether saturated fatty acids are physiological essential for embryonic growth, the increased partitioning of stearic acid, 18:0, into ovarian lipids during vitellogenesis suggests there may be some physiological requirement. Concomitant increases in 18:0 in the liver and ovarian tissue during sexual maturation has previously been observed in rainbow trout by Manor et al. (2012) and was speculated to derive from muscle lipid stores and possibly, *de novo* synthesis in the liver. Both saturated and monounsaturated fatty acids are highly utilized as energy stores by the developing fish embryo but saturated fatty acids additionally provide the resulting larvae with higher functional properties as they are less fluid. If saturated fatty acids prove to be physiologically essential for embryonic growth, the anticipated transition of salmon broodstock feeds from animal to plant based feed ingredients may prove to be problematic as concentrations of stearic acid are typically lower in the latter.

582 For control fish, the $\delta^{13}\text{C}$ values of ovarian lipids were slightly less (more
583 negative) than that observed in the corresponding muscle lipids at every sampling. These
584 differences were small ($\leq 0.6\text{‰}$) and possibly linked to the higher partitioning of marine
585 derived n-3 HUFAs into ovarian lipids over muscle lipids. As illustrated by Yu et al.
586 (2015), fish feeds are complex blends of marine and terrestrial ingredients, each with a
587 different $\delta^{13}\text{C}$. The $\delta^{13}\text{C}$ values of feed lipids presented in Table 1 are averages, derived
588 from a mix of marine and terrestrial sources. In the fish oil feeds, n-3 HUFAs were
589 almost entirely marine derived, originating from the fish oil and fish meal feed
590 ingredients. In contrast, some of the medium chain fatty acids in the fish oil feeds
591 originated from minor, terrestrial based, feed ingredients such as poultry-by product
592 meal, corn gluten, and soybean meal. As discussed previously, these medium chain fatty
593 acids were observed to be more partitioned into muscle lipids than ovarian lipids. The
594 selective partitioning of certain fatty acids, originating from different feed ingredients,
595 most likely explains the small differences observed in $\delta^{13}\text{C}$ values of muscle and ovarian
596 lipids from control fish.

597 For fish in the other three treatments, ovarian lipid $\delta^{13}\text{C}$ values were similar or
598 slightly higher (less negative) than that of the corresponding muscle after fish consumed
599 the algal oil feed. While this trend is in contrast to what was observed among control
600 fish, it can be explained in a similar manner. Algal oil feed lipids contained high levels
601 of DHA, which was selectively partitioned into ovarian lipids over muscle lipids. The
602 majority of DHA in the algal oil feeds originated from the algal oil component, which
603 possessed elevated levels of the ^{13}C isotope due to its terrestrial origin. The higher $\delta^{13}\text{C}$
604 values of ovarian lipids from these fish can then be partially explained by the preferential
605 incorporation of dietary DHA into ovarian lipids over muscle lipids.

606 While the selective partitioning of n-3 HUFAs likely explains the small
607 differences observed between egg and muscle $\delta^{13}\text{C}$ values in the study, it does not explain
608 the large differences ($\geq 1.5\text{‰}$) observed between egg and muscle lipid $\delta^{13}\text{C}$ values of
609 EARLY fish during vitellogenesis. While both control and EARLY fish received the fish
610 oil feed during this period, differences between egg and muscle lipid $\delta^{13}\text{C}$ values of
611 EARLY fish greatly exceed what was observed with control fish. Control fish received
612 fish oil feeds throughout the study and differences in egg and muscle $\delta^{13}\text{C}$ values during

the vitellogenesis feeding interval represent a steady state condition. In contrast, EARLY fish received algal oil feed during the cortical alveolus and lipid droplet feeding intervals and $\delta^{13}\text{C}$ values during the vitellogenesis feeding interval are in a state of transition due to the incorporation of new marine derived lipids from the fish oil feed. The large differences observed between the $\delta^{13}\text{C}$ values of egg and muscle lipids suggests that these two tissues incorporate dietary lipids at different rates. As ovarian lipids $\delta^{13}\text{C}$ values in EARLY fish were closer to the $\delta^{13}\text{C}$ value of feed lipids than muscle lipid $\delta^{13}\text{C}$ values, our results suggest dietary lipids were incorporated into ovarian lipids at a higher rate than muscle lipids during this stage of development.

5. Conclusions

Results from this study demonstrate the potential of finishing feeds to greatly influence the fatty acid profiles of muscle and ovarian lipids in coho salmon during secondary oogenesis. At the end of the study, muscle lipid DHA was directly related to the duration fish received the high DHA algal oil feed, while ovarian lipid DHA was the highest when fish received the algal feed during vitellogenesis. It was also observed that feed lipids have a greater impact on ovarian lipids than muscle lipids during vitellogenesis. It was additionally observed that physiologically essential n-3 and n-6 HUFAs, particularly ARA and DHA, were preferentially deposited into ovarian lipids at all three stages of secondary oocyte development. Arachidonic acid was incorporated into ovarian lipids at the highest rate of any fatty acid during all three stages of development. Also, the partitioning of individual fatty acids into egg and muscle lipids changed over the course of the study. Arachidonic acid, ARA, was incorporated into ovarian lipids at the highest rate during the cortical alveolus and lipid droplet stages of development and DHA was incorporated into ovarian lipids at the highest rate during the lipid droplet stage of development.

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937 **Figure Captions**

938

939 **Figure 1.** Timing of experimental feeds and stages of secondary oocyte growth in
940 experimental fish. Experimental treatments differed in the interval that female coho
941 salmon were fed a high ^{13}C algal oil feed during secondary oocyte growth. Samplings:
942 LD-lipid droplet, EV-early vitellogenesis, MV-mid vitellogenesis, LV-late vitellogenesis.
943

944 **Figure 2.** Growth (A) and gonadal somatic index, GSI (B) of maturing female coho
945 salmon (mean $\pm$ SD). Sample replicates ($n=8$) are tank means.
946

947 **Figure 3.** Protein (A) and lipid (B) content of muscle and ovarian tissue of maturing
948 female coho salmon (mean $\pm$ SD). Sample replicates ($n=8$) are tank means.
949

950 **Figure 4.** Box and whisker plots of fatty acid partitioning ratios, $fa_{(o/m)}$, after the cortical
951 alveolus (A), lipid droplet (B), and vitellogenesis (C) stages of secondary oocyte growth.
952 Ratios are computed from fatty acid concentrations of ovarian and muscle lipids from
953 individual fish ($n=24$). Dashed horizontal line denotes equal partitioning of a fatty acid
954 between ovarian and muscle lipids.
955

956 **Figure 5.** Fatty acid partitioning ratios, $fa_{(o/m)}$, for select fatty acids during secondary
957 oocyte growth (mean $\pm$ SD, $n=24$). Stearic acid, c18:0 (A), Linoleic acid, c18:2 (B),
958 Arachidonic acid, c20:4 (C), and Docosahexaenoic acid, c22:6 (D). Replicates are ratios
959 computed from fatty acid concentrations of ovarian and muscle lipids of maturing female
960 fish. Samplings: LD-lipid droplet, EV-early vitellogenesis, MV-mid vitellogenesis, LV-
961 late vitellogenesis. Columns in the same graph with different letters are significantly
962 different ($P<0.05$).
963

964 **Figure 6.** Stable ^{13}C isotope abundance (mean $\pm$ SD, $n=6$) in ovarian and muscle lipids of
965 individual female fish during secondary oocyte development, separated by treatment:
966 Control fish (A), EARLY fish (B), LONG fish (C), and SHORT fish (D). Samplings:
967 LD-lipid droplet, EV-early vitellogenesis, MV-mid vitellogenesis, LV-late vitellogenesis.

968 Asterisks (*) donate when $\delta^{13}\text{C}$ values of egg and muscle lipids are significantly different
969 (P<0.05).
970

Table 1. Proximate composition of experimental feeds, and stable ^{13}C isotope and fatty acid content of feed lipids. Values are mean $\pm$ SD ($n=3$).

	Experimental Feeds ^a		P-value ^b
	Fish Oil	Algal Oil	
Proximate composition (g kg ⁻¹)			
Protein	409 ± 9	412 ± 8	
Lipid	237 ± 6	236 ± 6	
Ash	83 ± 6	84 ± 4	
Moisture	79 ± 9	77 ± 9	
Stable ¹³ carbon isotope ratio, feed lipids			
δ ¹³ C (‰)	-23.9 ± 0.4	-19.4 ± 0.2	***
Simplified fatty acid composition (g [100g total fatty acids] ⁻¹) ^c			
14:0	4.0 ± 0.5	4.8 ± 0.5	
16:0	17.9 ± 1.4	16.6 ± 0.5	
18:0	4.4 ± 0.3	3.5 ± 0.3	*
Σ SFA	26.3 ± 2.2	25.1 ± 0.9	
16:1	6.1 ± 0.4	3.4 ± 0.3	***
18:1 ^d	25.8 ± 2.6	28.2 ± 0.3	
20:1 ^d	4.3 ± 1.8	1.0 ± 0.2	*
Σ MUFA	39.8 ± 6.3	33.1 ± 0.2	
18:2 n-6 (LA)	6.7 ± 0.4	17.9 ± 1.9	***
20:4 n-6 (ARA)	0.8 ± 0.2	0.6 ± 0.1	
Σ n-6	8.0 ± 0.8	18.6 ± 2.0	***
18:3 n-3	0.9 ± 0.1	0.8 ± 0.0	
20:5 n-3	10.9 ± 0.5	4.8 ± 0.3	***
22:5 n-3	1.3 ± 0.5	0.9 ± 0.1	
22:6 n-3	7.2 ± 1.7	15.4 ± 2.0	**
Σ n-3	22.6 ± 2.5	22.4 ± 1.7	
Σ PUFA	32.4 ± 0.5	41.4 ± 0.8	*
DHA/EPA	0.7 ± 0.1	3.3 ± 0.6	**
EPA/ARA	13.3 ± 2.0	7.3 ± 0.2	**

^a Fish oil feed contained whole fish meal, wheat flour, stabilized sardine oil, poultry by-product meal, krill meal, corn gluten, soybean meal, astaxanthin, vitamin premix, and mineral premix. Algal oil feed contained whole fish meal, wheat flour, stabilized algal-corn oil blend, poultry by-product meal, krill meal, corn gluten, soybean meal, astaxanthin, vitamin premix, and mineral premix.

^b P-value key: *P < 0.05, **P < 0.01, ***P < 0.001.

^c Saturated fatty acids (SFA): 14:0, 16:0, 18:0, 20:0, 22:0, 24:0. Monounsaturated fatty acids (MUFA): 14:1, 16:1, 18:1, 20:1, 22:1, 24:1. N-6 fatty acids (n-6): 18:2 n-6, 20:2 n-6, 20:3 n-6, 20:4 n-6. N-3 fatty acids (n-3): 18:3 n-3, 18:4 n-3, 20:3 n-3, 20:4 n-3, 20:5 n-3, 21:5 n-3, 22:5 n-3, 22:6 n-3. Polyunsaturated fatty acids (PUFA): 16:2, 16:3, 16:4, 18:2, 18:3, 18:4, 20:2, 20:3 (n-6 and n-3), 20:4 (n-6 and n-3), 20:5, 21:5, 22:5, 22:6.

^d Sum of isomers.

Table 2. Proximate composition analysis of muscle tissue, and stable ^{13}C isotope and fatty acid content of muscle lipids from maturing female coho salmon, sampled four times during secondary oocyte growth. Values are means of replicate tank means, derived from 3 fish each. Within a row and sampling, values with different superscript letters are significantly different at $P < 0.05$. Samplings: LD-lipid droplet, EV-early vitellogenesis, MV-mid vitellogenesis, LV-late vitellogenesis. See Table 1 notes for fatty acids included in groupings.

Treatment	Feeding Interval (sampling)					Vitellogenesis							
	Cortical alveolus (LD)		Lipid droplet (EV)			Early Vitellogenesis (MV)				Late Vitellogenesis (LV)			
	Control <i>n</i> =6	EARLY <i>n</i> =2	Control <i>n</i> =4	EARLY <i>n</i> =2	LONG <i>n</i> =2	Control <i>n</i> =2	EARLY <i>n</i> =2	LONG <i>n</i> =2	SHORT <i>n</i> =2	Control <i>n</i> =2	EARLY <i>n</i> =2	LONG <i>n</i> =2	SHORT <i>n</i> =2
Proximate composition (g kg ⁻¹)													
Protein	207	208	204	202	202	215	212	217	215	191	196	196	200
Lipid	40	39	52	52	56	35	35	42	37	38	37	44	38
Moisture	744	745	737	738	734	742	746	736	742	762	763	755	756
Stable carbon isotope ratio of lipids $\delta^{13}\text{C}$ (‰)	-24.2 ^a	-21.2 ^b	-24.3 ^a	-20.5 ^b	-21.6 ^c	-24.1 ^a	-21.1 ^c	-20.9 ^c	-23.0 ^b	-24.1 ^a	-21.3 ^c	-20.7 ^d	-22.7 ^b
Simplified fatty acid composition (g [100g total fatty acids] ⁻¹)													
14:0	3.8	4.1	4.5 ^a	4.9 ^b	4.8 ^b	4.2	5.0	4.8	4.8	4.2	3.9	4.1	4.5
16:0	18.3 ^b	17.6 ^a	19.2 ^b	18.1 ^a	18.5 ^a	19.9 ^{ab}	20.7 ^{ab}	18.9 ^a	20.9 ^b	18.7 ^b	17.2 ^a	16.5 ^a	18.9 ^b
18:0	4.1 ^b	3.8 ^a	4.1 ^b	3.8 ^a	3.8 ^a	4.0	3.7	3.7	3.9	3.8	3.7	3.7	3.8
Σ SFA	26.2	25.5	27.7 ^b	26.8 ^a	27.2 ^{ab}	28.1	29.4	27.4	29.6	26.7 ^b	24.8 ^a	24.2 ^a	27.2 ^b
16:1	6.2 ^b	4.3 ^a	7.2 ^c	4.5 ^a	5.5 ^b	7.4 ^b	5.4 ^a	5.0 ^a	6.8 ^b	7.9 ^c	5.3 ^a	5.0 ^a	6.8 ^b
18:1	26.9	26.4	28.7	28.2	28.9	28.9	27.9	29.1	28.6	26.1	28.4	29.4	29.5
20:1	1.5 ^b	0.7 ^a	3.8 ^c	1.2 ^a	2.1 ^b	3.6 ^c	1.5 ^a	1.7 ^a	2.8 ^b	3.6 ^c	1.9 ^a	1.9 ^a	3.1 ^b
Σ MUFA	38.5 ^b	32.3 ^a	42.1 ^c	34.5 ^a	37.3 ^b	41.6 ^b	35.4 ^a	36.3 ^a	39.3 ^{ab}	42.2 ^b	36.3 ^a	36.8 ^a	40.6 ^{ab}
18:2 n-6 (LA)	6.5 ^a	13.5 ^b	6.2 ^a	14.8 ^c	12.0 ^b	6.2 ^a	12.7 ^c	13.8 ^c	8.8 ^b	6.9 ^a	12.9 ^c	14.3 ^c	9.6 ^b
20:4 n-6 (ARA)	0.8 ^b	0.7 ^a	0.6 ^b	0.6 ^a	0.6 ^{ab}	0.6	0.6	0.5	0.6	0.6 ^b	0.6 ^{ab}	0.6 ^a	0.6 ^{ab}
Σ n-6	7.9 ^a	14.9 ^b	7.4 ^a	16.3 ^c	13.4 ^b	7.4 ^a	14.1 ^c	15.3 ^c	10.1 ^b	8.2 ^a	14.5 ^c	15.9 ^c	11.0 ^b
18:3 n-3	1.3	1.3	1.0	1.0	1.0	0.9	0.8	0.8	0.8	1.0	0.9	0.9	0.9
20:5 n-3	6.1 ^b	3.8 ^a	5.8 ^c	3.1 ^a	3.9 ^b	5.3 ^c	3.2 ^a	2.9 ^a	4.3 ^b	5.1 ^d	3.6 ^b	2.9 ^a	4.0 ^c
22:5 n-3	2.4 ^b	1.8 ^a	2.2 ^c	1.5 ^a	1.7 ^b	2.5 ^c	1.6 ^a	1.6 ^a	2.0 ^b	2.8 ^b	2.4 ^a	2.2 ^a	2.5 ^a
22:6 n-3	12.2 ^a	16.5 ^b	9.0 ^a	13.6 ^b	11.8 ^b	9.9	12.9	13.0	10.3	9.8 ^a	14.8 ^b	14.8 ^b	11.3 ^a
Σ n-3	30.5 ^b	27.2 ^a	19.8	19.9	19.3	20.0	19.1	18.8	18.5	20.2	22.5	21.3	19.6
Σ PUFA	39.7 ^a	43.1 ^b	28.6 ^a	37.0 ^b	33.8 ^b	28.7 ^a	33.9 ^{bc}	34.9 ^c	29.7 ^{ab}	29.5 ^a	37.5 ^b	37.6 ^b	31.4 ^a
DHA/EPA	2.0 ^a	4.4 ^b	1.6 ^a	4.5 ^c	3.1 ^b	1.9 ^a	4.1 ^b	4.5 ^b	2.4 ^a	1.9 ^a	4.2 ^c	5.0 ^d	2.8 ^b
EPA/ARA	8.1 ^b	5.6 ^a	9.0 ^c	5.4 ^a	6.3 ^b	8.6 ^c	5.5 ^a	5.3 ^a	7.3 ^b	8.1 ^c	5.9 ^a	5.3 ^a	7.1 ^b

Table 3. Proximate composition analysis of ovarian tissue, and stable ^{13}C isotope and fatty acid content of ovarian lipids from maturing female coho salmon, sampled four times during secondary oocyte growth. Values are means of replicate tank means, derived from 3 fish each. Within a row and sampling, values with different superscript letters are significantly different at $P < 0.05$. Samplings: LD-lipid droplet, EV-early vitellogenesis, MV-mid vitellogenesis, LV-late vitellogenesis. See Table 1 notes for fatty acids included in groupings.

Treatment	Feeding Interval (sampling)					Vitellogenesis							
	Cortical alveolus (LD)		Lipid droplet (EV)			Early Vitellogenesis (MV)				Late Vitellogenesis (LV)			
	Control <i>n</i> =6	EARLY <i>n</i> =2	Control <i>n</i> =4	EARLY <i>n</i> =2	LONG <i>n</i> =2	Control <i>n</i> =2	EARLY <i>n</i> =2	LONG <i>n</i> =2	SHORT <i>n</i> =2	Control <i>n</i> =2	EARLY <i>n</i> =2	LONG <i>n</i> =2	SHORT <i>n</i> =2
Proximate composition (g kg ⁻¹)													
Protein	104	104	184	171	176	267	269	261	261	281	282	282	282
Lipid	74	70	152	151	160	119	117	123	120	114	112	115	115
Moisture	780	786	635	649	636	586	586	589	592	581	576	576	572
Stable carbon isotope ratio $\delta^{13}\text{C}$ (‰)	-24.4 ^a	-20.4 ^b	-24.6 ^a	-20.0 ^b	-20.8 ^c	-24.7 ^a	-22.7 ^b	-20.4 ^c	-22.1 ^b	-24.6 ^a	-22.8 ^b	-20.9 ^d	-22.0 ^c
Simplified fatty acid composition (g [100g total fatty acids] ⁻¹)													
14:0	2.3 ^a	2.5 ^b	2.2 ^a	2.4 ^{ab}	2.6 ^b	2.3	2.7	2.6	2.7	2.1	2.4	2.3	2.4
16:0	17.9	16.6	13.7	13.2	14.4	11.6	12.3	11.7	12.1	11.8	11.4	11.5	11.7
18:0	1.9	1.7	3.4	3.3	3.3	4.5	4.5	4.2	4.3	4.7	4.5	4.4	4.5
Σ SFA	22.0	20.7	19.3 ^{ab}	18.8 ^a	20.3 ^b	18.5	19.5	18.5	19.1	18.6	18.0	18.3	18.6
16:1	4.8 ^b	3.1 ^a	5.2 ^c	3.1 ^a	3.8 ^b	5.7 ^c	5.1 ^{bc}	3.4 ^a	4.3 ^{ab}	5.8 ^b	4.9 ^{ab}	4.3 ^a	4.7 ^{ab}
18:1	21.0	20.5	23.2	21.4	23.1	24.5	23.9	22.7	23.2	24.2	23.7	23.7	24.4
20:1	3.2 ^b	1.1 ^a	1.7 ^b	1.0 ^a	1.0 ^a	1.9 ^b	1.7 ^{ab}	1.4 ^a	1.5 ^a	1.5 ^b	1.2 ^a	1.0 ^a	1.2 ^a
Σ MUFA	27.3 ^b	24.3 ^a	30.2 ^b	25.6 ^a	28.0 ^{ab}	32.1 ^c	30.7 ^{bc}	27.6 ^a	29.0 ^{ab}	31.6	29.8	29.0	30.3
18:2 n-6 (LA)	4.2 ^a	9.9 ^b	4.3 ^a	10.6 ^b	9.7 ^b	4.3 ^a	7.1 ^b	10.1 ^c	8.0 ^b	4.5 ^a	7.5 ^b	10.4 ^c	8.0 ^b
20:4 n-6 (ARA)	2.8 ^b	2.5 ^a	2.5	2.4	2.3	2.0	2.0	2.1	2.0	2.0 ^b	1.9 ^{ab}	1.9 ^a	1.9 ^{ab}
Σ n-6	7.2 ^a	13.7 ^b	7.8 ^a	14.9 ^b	13.7 ^b	7.6 ^a	10.7 ^b	14.6 ^d	12.0 ^c	7.7 ^a	11.2 ^b	14.4 ^c	11.9 ^b
18:3 n-3	0.6	0.6	0.6	0.6	0.5	0.6	0.6	0.5	0.5	0.7	0.7	0.7	0.6
20:5 n-3	9.0 ^b	4.6 ^a	9.3 ^c	4.2 ^a	4.5 ^a	9.0 ^d	7.1 ^c	4.4 ^a	5.9 ^b	9.6 ^c	7.5 ^b	5.5 ^a	6.3 ^a
22:5 n-3	3.4 ^b	1.8 ^a	4.2 ^b	2.1 ^a	2.2 ^a	5.4 ^c	4.1 ^a	2.9 ^b	3.8 ^{ab}	5.9 ^c	4.8 ^a	3.5 ^a	4.4 ^{ab}
22:6 n-3	25.2 ^a	30.4 ^b	24.6 ^a	32.0 ^c	28.3 ^b	22.2 ^a	24.0 ^a	29.2 ^b	26.1 ^{ab}	21.7 ^a	24.3 ^{ab}	26.4 ^b	25.5 ^{ab}
Σ n-3	39.3	37.7	40.7 ^b	39.4 ^b	36.3 ^a	38.6	36.7	37.3	36.9	39.7	38.6	36.5	37.4
Σ PUFA	46.6 ^a	51.4 ^b	48.8 ^a	54.2 ^a	50.1 ^b	46.8 ^a	47.5 ^{ab}	52.0 ^b	49.0 ^{ab}	47.7	50.0	51.1	49.4
DHA/EPA	2.8 ^a	6.6 ^b	2.6 ^a	7.6 ^c	6.3 ^b	2.5 ^a	3.4 ^b	6.6 ^d	4.6 ^c	2.3 ^a	3.2 ^b	4.8 ^d	4.0 ^c
EPA/ARA	3.2 ^b	1.8 ^a	3.7 ^b	1.8 ^a	1.9 ^a	4.5 ^d	3.6 ^c	2.2 ^a	2.9 ^b	4.7 ^c	3.9 ^b	3.0 ^a	3.3 ^a

Fish oil feed

Algal oil feed

Figure 1

a) Fish growth

b) GSI

Figure 2

a) Tissue protein content

b) Tissue lipid content

Figure 3

a) Cortical alveolus stage

Figure 4

b) Lipid droplet stage

c) Vitellogenesis

a) Stearic acid, c18:0

b) Linoleic acid, c18:2 n-6

c) ARA, c20:4 n-6

d) DHA, c22:6 n-3

Figure 5

a) Control fish

b) EARLY fish

c) LONG fish

d) SHORT fish

Figure 6