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

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Abstract

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

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

2. Methods

2. 1. Feeds

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

2.2. Fish culture

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

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

2.3. Finishing periods

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

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

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

2.4. Tissue sampling

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

2.5. Chemical analysis

2.5.1. Proximate analysis

White muscle tissue samples were sectioned from fillets of sacrificed fish above the lateral line and anterior to the dorsal fin. All skin and red muscle was trimmed from the muscle samples prior to chemical analysis. For previtellogenic fish, an ovarian tissue sample was a complete ovary. For vitellogenic fish, an ovarian tissue sample was a cross section of the anterior portion of one ovary. Muscle and ovarian tissue was lyophilized at -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, $fa_{(o/m)}$ = fatty acid concentration in ovarian lipids / fatty acid concentration in muscle lipids.

2.5.3. Stable ^{13}C isotope analysis

Stable ^{13}C 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.

Stable carbon isotope ratios were expressed in δ notation as per mil (‰) according to the following expression,

$$\delta^{13}C = [(R_{sample}/R_{standard})-1] \times 1000$$

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

2.6. Statistical analysis

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

Fatty acid partitioning coefficients, $fa_{(o/m)}$, were $\log_{10}(x+1)$ transformed prior to statistical analysis as described by Zar (1999) to minimize variability differences between the results from individual fatty acids. Box and whisker plots were constructed to visualize differences in $fa_{(o/m)}$ for each sampling. One-way ANOVA, main effect, 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 $\delta^{13}C$ 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 ± 120g at late vitellogenesis (Figure
293 2a). Eggs sampled at late vitellogenesis were 5.5 ± 0.1mm 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 ± 5 g kg^{-1}, at the MV sampling and
304 decreased to a minimum of 195 ± 5 g kg^{-1} by LV. Ovarian protein content continually
305 increased over the course of the study from a minimum of 195 ± 5 g kg^{-1} at the LD
306 sampling to a maximum of 282 ± 4 g kg^{-1} at LV.

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

3.3. Tissue Fatty Acids

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

3.3.1. Cortical Alveolus Feeding Interval.

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

Box and whisker plots revealed differences in fatty acid partitioning associated with fatty acid structure during the cortical alveolus feeding interval (Figure 4a). Fatty 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.

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 ($P=0.009$, $P=0.001$).

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

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

The transitioning of EARLY fish to a fish oil feed at early vitellogenesis had a greater effect on the fatty acid concentrations of ovarian lipids than muscle lipids at the MV sampling. While the EPA and LA levels in the muscle lipids of EARLY fish were similar to those of LONG fish at the MV sampling ($P=0.194$, $P=0.547$), significant differences were observed in the ovarian lipids ($P=0.002$, $P<0.001$). By the LV sampling, fatty acid levels in the ovarian lipids of EARLY fish were intermediate and often significantly different to levels observed in both control and LONG fish. In contrast, fatty acid levels in the ovarian lipids of EARLY and SHORT fish were very similar by the LV sampling, despite EARLY fish having received algal oil feeds for 21 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, $\delta^{13}C$, 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) $\delta^{13}C$ values than those of the control fish. Within a treatment, significant differences were observed

459 between the $\delta^{13}C$ values of ovarian and muscle lipids over the course of the study. In
460 control fish, ovarian lipid $\delta^{13}C$ values were lower (more negative) than that of muscle
461 lipids at every sampling (Figure 6a). EARLY fish, which initially were fed the algal oil
462 feed, had ovarian lipids with $\delta^{13}C$ values that were higher (less negative) than that of
463 muscle lipids after the cortical alveolus and lipid droplet feeding intervals, and then
464 ovarian lipid $\delta^{13}C$ values were lower than muscle lipid $\delta^{13}C$ values after fish transitioned
465 to the fish oil feed during vitellogenesis (Figure 6b). In LONG fish, ovarian lipid $\delta^{13}C$
466 values were higher than muscle lipid $\delta^{13}C$ values at the EV and MV samplings, but
467 similar at the LV sampling (Figure 6c). In SHORT fish, ovarian lipid $\delta^{13}C$ values were
468 higher than muscle lipid $\delta^{13}C$ values after fish were transitioned to the algal oil feed
469 during vitellogenesis (Figure 6d).
470
471 **4. Discussion**
472 Results from this study demonstrate the ability to significantly alter the fatty acid
473 composition of muscle and ovarian lipids in cultured coho salmon during secondary
474 oocyte growth through changes in dietary lipids. This is in agreement with previous
475 studies showing dietary lipids to directly influence the fatty acid profile of muscle lipids
476 (Polvi and Ackman, 1992; Hardy et al., 1987; Bell et al., 2001; Torstensen et al., 2005;
477 Higgs et al., 2006) and to a lesser extent, eggs lipids (Hardy et al., 1990; Rennie et al.,
478 2005) in salmonids. At all samplings, the stable ^{13}C isotope abundance in ovarian and
479 muscle lipids of study fish reflected the stable ^{13}C isotope abundance of the feed lipids
480 they had received. Control fish, which received only fish oil feeds during the study, had
481 ovarian and muscle lipids with a marine $\delta^{13}C$ signature, which was significantly lower
482 (more negative) than fish from other treatments. The $\delta^{13}C$ values of both ovarian and
483 muscle lipids of EARLY, LONG, and SHORT fish were significantly higher (less
484 negative) to those of control fish after the cortical alveolus, lipid droplet, and
485 vitellogenesis feeding intervals, respectively. This suggests that dietary lipids are
486 incorporated into both egg and muscle tissue of coho salmon at appreciable amounts
487 during all three stages of secondary oocyte development.
488 Regarding fatty acid composition, the DHA content of ovarian and muscle lipids
489 significantly increased when fish received a high DHA, algal oil feed. It was additionally

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

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

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

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

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

552 phosphoinositol lipid class, has been positively correlated with larval fish survival in a
553 number of teleost species (Pickova et al., 1999; Cejas et al., 2003; Bell and Sargent,
554 2003; Salze et al., 2005). From mass balance calculations, more ARA was deposited into
555 the developing oocyte during vitellogenesis than any other stage of development, but as
556 the phosphoinositol lipid class represents only a minor component of fish egg lipids
557 (Tocher and Sargent, 1984; Navas et al., 1997; Salze et al., 2005; Murzina et al. 2009),
558 the uptake of ARA during previtellogenic growth may be physiologically essential for the
559 proper manufacture of this lipoprotein class. Correspondingly, it may prove extremely
560 important to supplement salmon broodstock diets with ARA during previtellogenic
561 growth when the preferential deposition of ARA to ovarian lipids is the highest.
562 While the experimental feeds in this study were balanced for n-3 fatty acids, they
563 were not balanced for n-6 fatty acids. This resulted in the algal oil feeds being higher in
564 polyunsaturated fatty acids and lower in saturated and monounsaturated fatty acids.
565 Elevated levels of LA, the dominant n-6 fatty acid, in the algal oil feed resulted in
566 elevated levels of n-6 fatty acids in the ovarian lipids of SHORT, LONG, and EARLY
567 fish at every sampling. Elevated levels of n-6 fatty acids were balanced with reduced
568 levels of monounsaturated fatty acids in ovarian lipids. Concentrations of saturated fatty
569 acids in ovarian lipids were not affected by treatment. While it is currently unknown
570 whether saturated fatty acids are physiological essential for embryonic growth, the
571 increased partitioning of stearic acid, 18:0, into ovarian lipids during vitellogenesis
572 suggests there may be some physiological requirement. Concomitant increases in 18:0 in
573 the liver and ovarian tissue during sexual maturation has previously been observed in
574 rainbow trout by Manor et al. (2012) and was speculated to derive from muscle lipid
575 stores and possibly, *de novo* synthesis in the liver. Both saturated and monounsaturated
576 fatty acids are highly utilized as energy stores by the developing fish embryo but
577 saturated fatty acids additionally provide the resulting larvae with higher functional
578 properties as they are less fluid. If saturated fatty acids prove to be physiologically
579 essential for embryonic growth, the anticipated transition of salmon broodstock feeds
580 from animal to plant based feed ingredients may prove to be problematic as
581 concentrations of stearic acid are typically lower in the latter.

582 For control fish, the $\delta^{13}C$ 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 (≤ 0.6 ‰) 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 $\delta^{13}C$. The $\delta^{13}C$ 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 $\delta^{13}C$ values of muscle and ovarian
596 lipids from control fish.
597 For fish in the other three treatments, ovarian lipid $\delta^{13}C$ 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 $\delta^{13}C$
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 $\delta^{13}C$ values in the study, it does not explain
608 the large differences (≥ 1.5‰) observed between egg and muscle lipid $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ values during

613 the vitellogenesis feeding interval represent a steady state condition. In contrast, EARLY
614 fish received algal oil feed during the cortical alveolus and lipid droplet feeding intervals
615 and $\delta^{13}C$ values during the vitellogenesis feeding interval are in a state of transition due
616 to the incorporation of new marine derived lipids from the fish oil feed. The large
617 differences observed between the $\delta^{13}C$ values of egg and muscle lipids suggests that these
618 two tissues incorporate dietary lipids at different rates. As ovarian lipids $\delta^{13}C$ values in
619 EARLY fish were closer to the $\delta^{13}C$ value of feed lipids than muscle lipid $\delta^{13}C$ values,
620 our results suggest dietary lipids were incorporated into ovarian lipids at a higher rate
621 than muscle lipids during this stage of development.
622
623 **5. Conclusions**
624 Results from this study demonstrate the potential of finishing feeds to greatly
625 influence the fatty acid profiles of muscle and ovarian lipids in coho salmon during
626 secondary oogenesis. At the end of the study, muscle lipid DHA was directly related to
627 the duration fish received the high DHA algal oil feed, while ovarian lipid DHA was the
628 highest when fish received the algal feed during vitellogenesis. It was also observed that
629 feed lipids have a greater impact on ovarian lipids than muscle lipids during
630 vitellogenesis. It was additionally observed that physiologically essential n-3 and n-6
631 HUFAs, particularly ARA and DHA, were preferentially deposited into ovarian lipids at
632 all three stages of secondary oocyte development. Arachidonic acid was incorporated
633 into ovarian lipids at the highest rate of any fatty acid during all three stages of
634 development. Also, the partitioning of individual fatty acids into egg and muscle lipids
635 changed over the course of the study. Arachidonic acid, ARA, was incorporated into
636 ovarian lipids at the highest rate during the cortical alveolus and lipid droplet stages of
637 development and DHA was incorporated into ovarian lipids at the highest rate during the
638 lipid droplet stage of development.
639
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Figure Captions

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

Figure 2. Growth (A) and gonadal somatic index, GSI (B) of maturing female coho salmon (mean ± SD). Sample replicates (n=8) are tank means.

Figure 3. Protein (A) and lipid (B) content of muscle and ovarian tissue of maturing female coho salmon (mean ± SD). Sample replicates (n=8) are tank means.

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

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

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

968 Asterisks (*) donate when $\delta^{13}C$ 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 ± SD (*n*=3).

	Experimental Feeds [a]		
	Fish Oil	Algal Oil	P-value [b]
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			
$\delta^{13}C$ (‰)	-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.

	Feeding Interval (sampling)												
						Vitellogenesis							
	Cortical alveolus (LD)		Lipid droplet (EV)			Early Vitellogenesis (MV)				Late Vitellogenesis (LV)			
Treatment	Control *n*=6	EARLY *n*=2	Control *n*=4	EARLY *n*=2	LONG *n*=2	Control *n*=2	EARLY *n*=2	LONG *n*=2	SHORT *n*=2	Control *n*=2	EARLY *n*=2	LONG *n*=2	SHORT *n*=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													
$\delta^{13}C$ (‰)	-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.

	Feeding Interval (sampling)												
						Vitellogenesis							
	Cortical alveolus (LD)		Lipid droplet (EV)			Early Vitellogenesis (MV)				Late Vitellogenesis (LV)			
Treatment	Control *n*=6	EARLY *n*=2	Control *n*=4	EARLY *n*=2	LONG *n*=2	Control *n*=2	EARLY *n*=2	LONG *n*=2	SHORT *n*=2	Control *n*=2	EARLY *n*=2	LONG *n*=2	SHORT *n*=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													
$\delta^{13}C$ (‰)	-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}

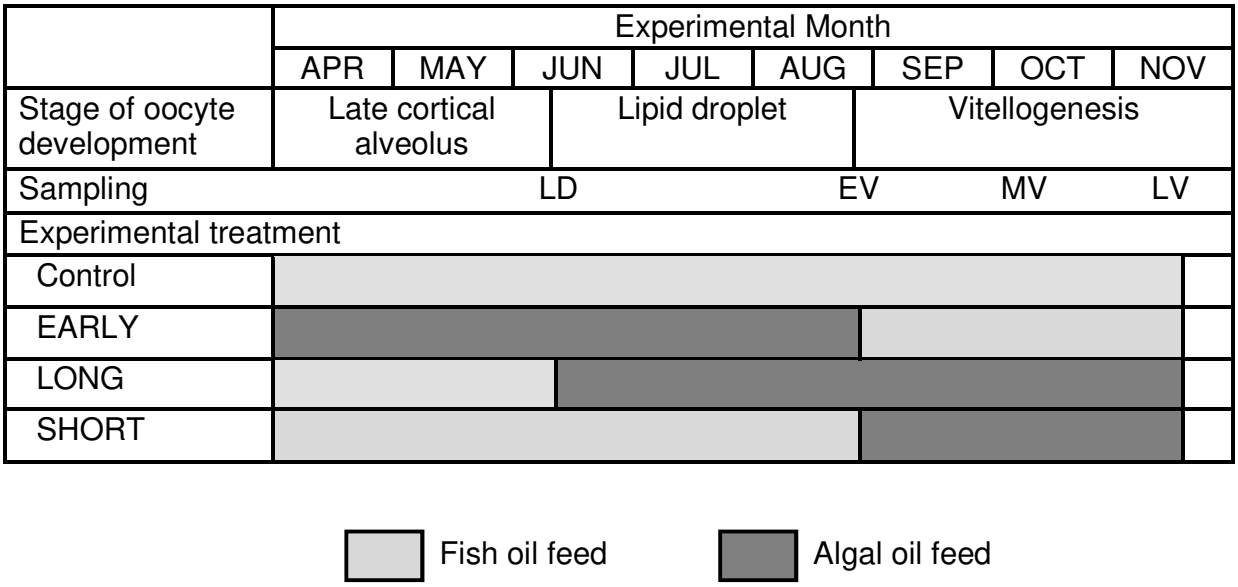
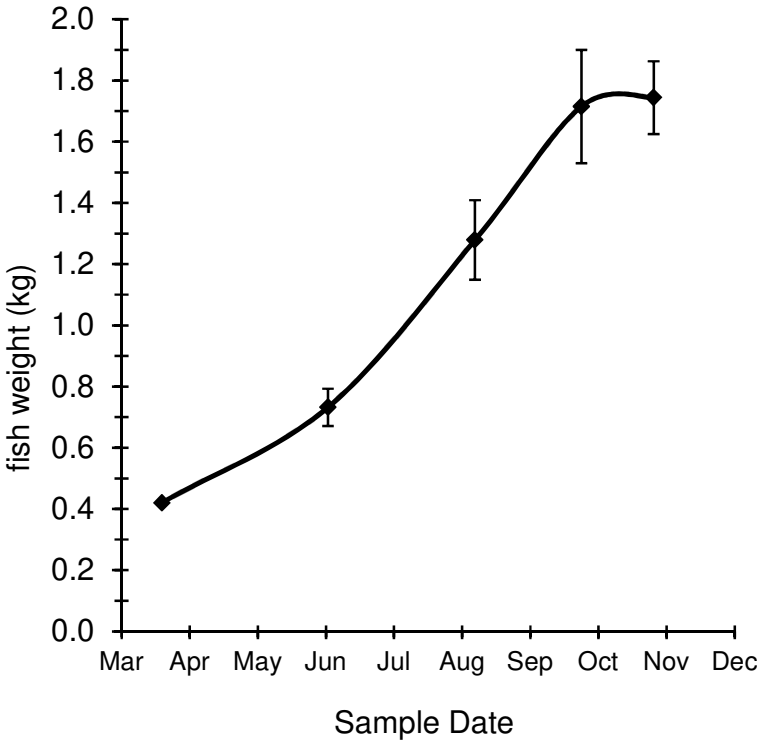

Experimental Month
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
Stage of oocyte development
Late cortical alveolus
Lipid droplet
Vitellogenesis
Sampling
LD
EV
MV
LV
Experimental treatment
Control
EARLY
LONG
SHORT
Fish oil feed
Algal oil feed

Figure 1

a) Fish growth



b) GSI

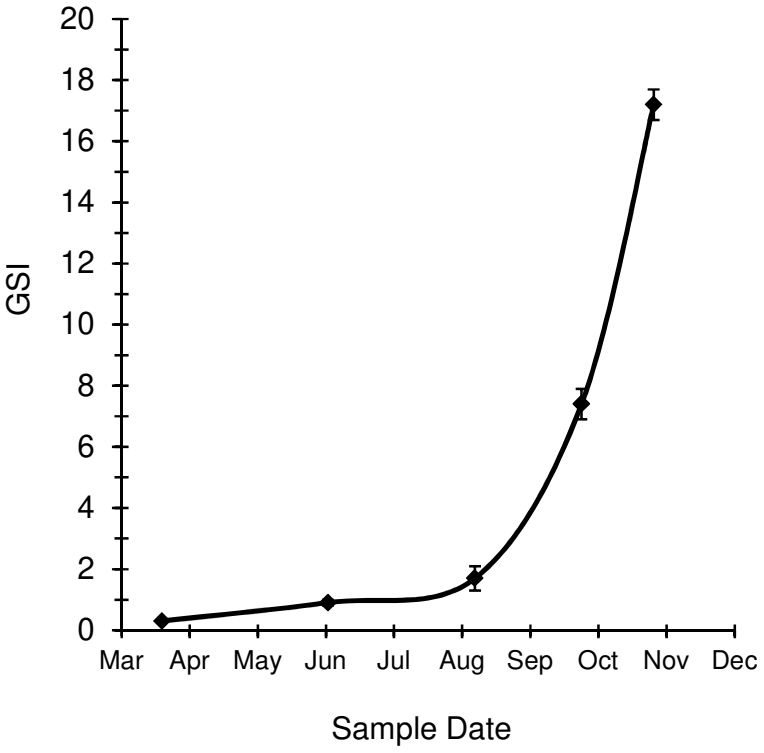
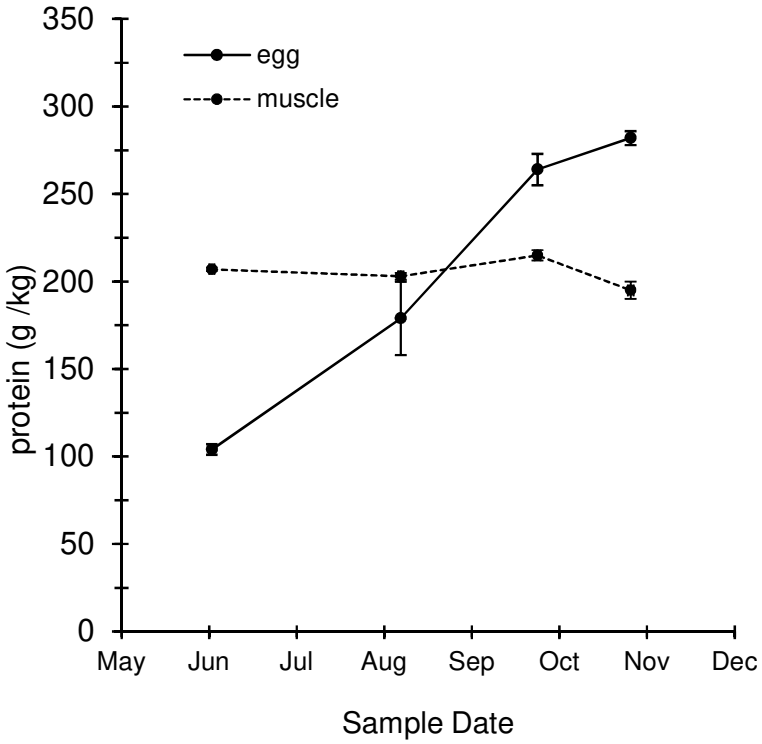


Figure 2

a) Tissue protein content



b) Tissue lipid content

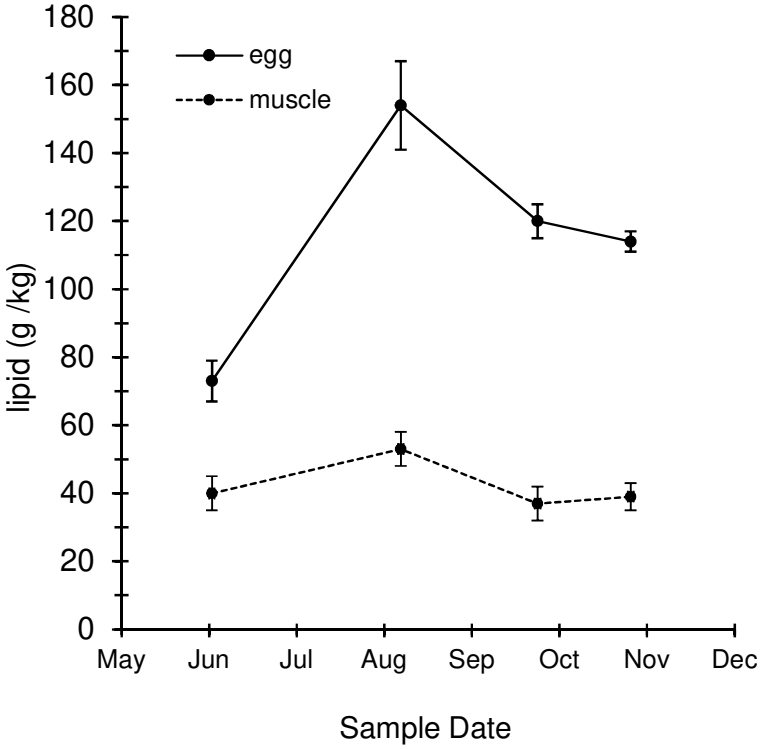
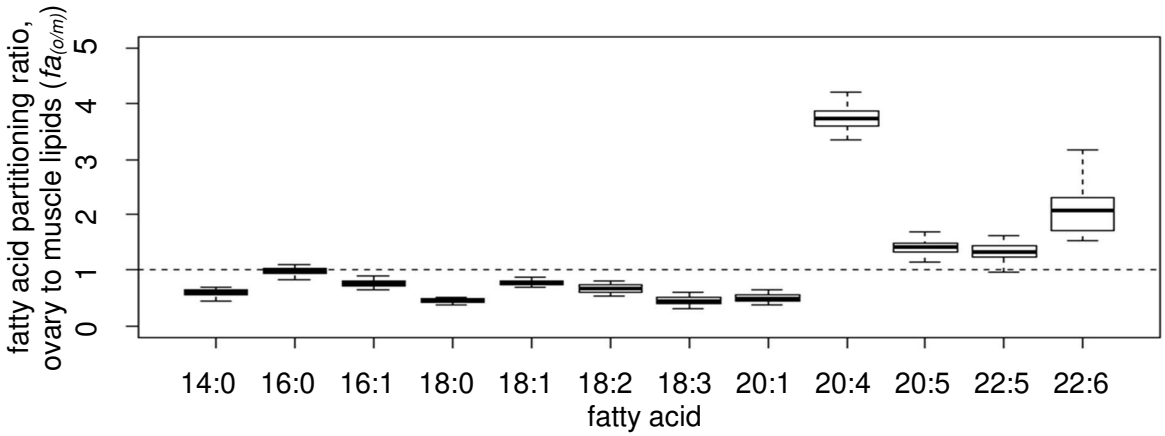


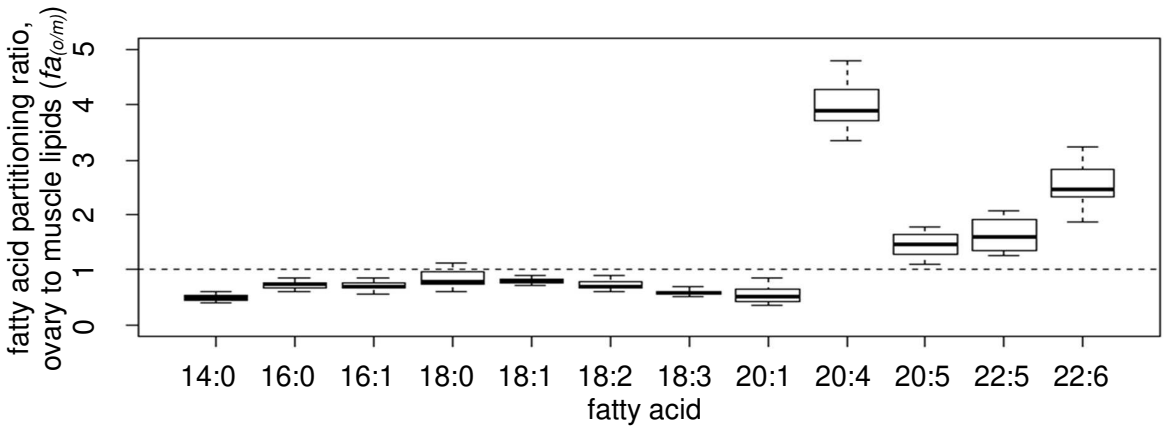
Figure 3

a) Cortical alveolus stage

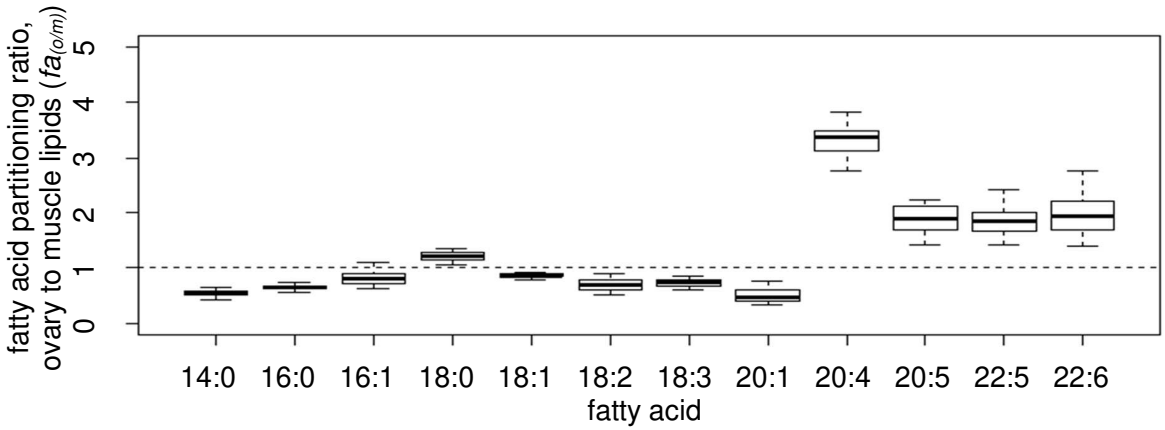
Figure 4



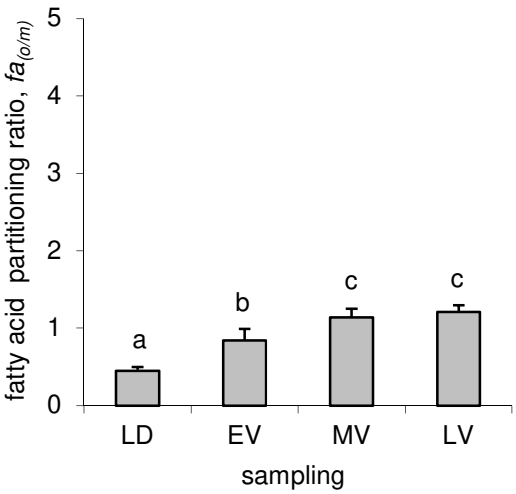
b) Lipid droplet stage



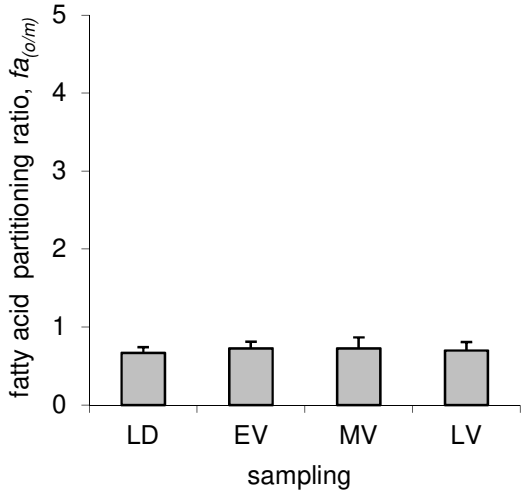
c) Vitellogenesis



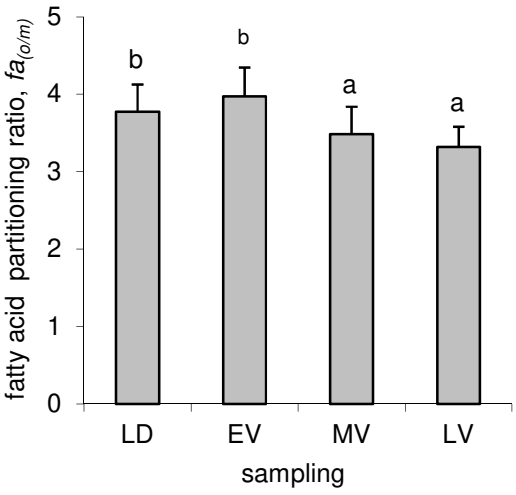
a) Stearic acid, c18:0



b) Linoleic acid, c18:2 n-6



c) ARA, c20:4 n-6



d) DHA, c22:6 n-3

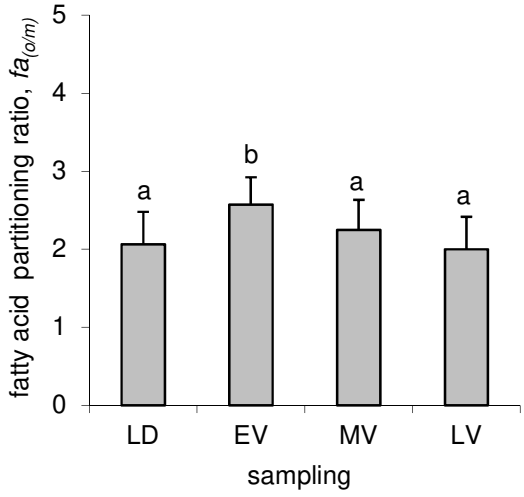


Figure 5

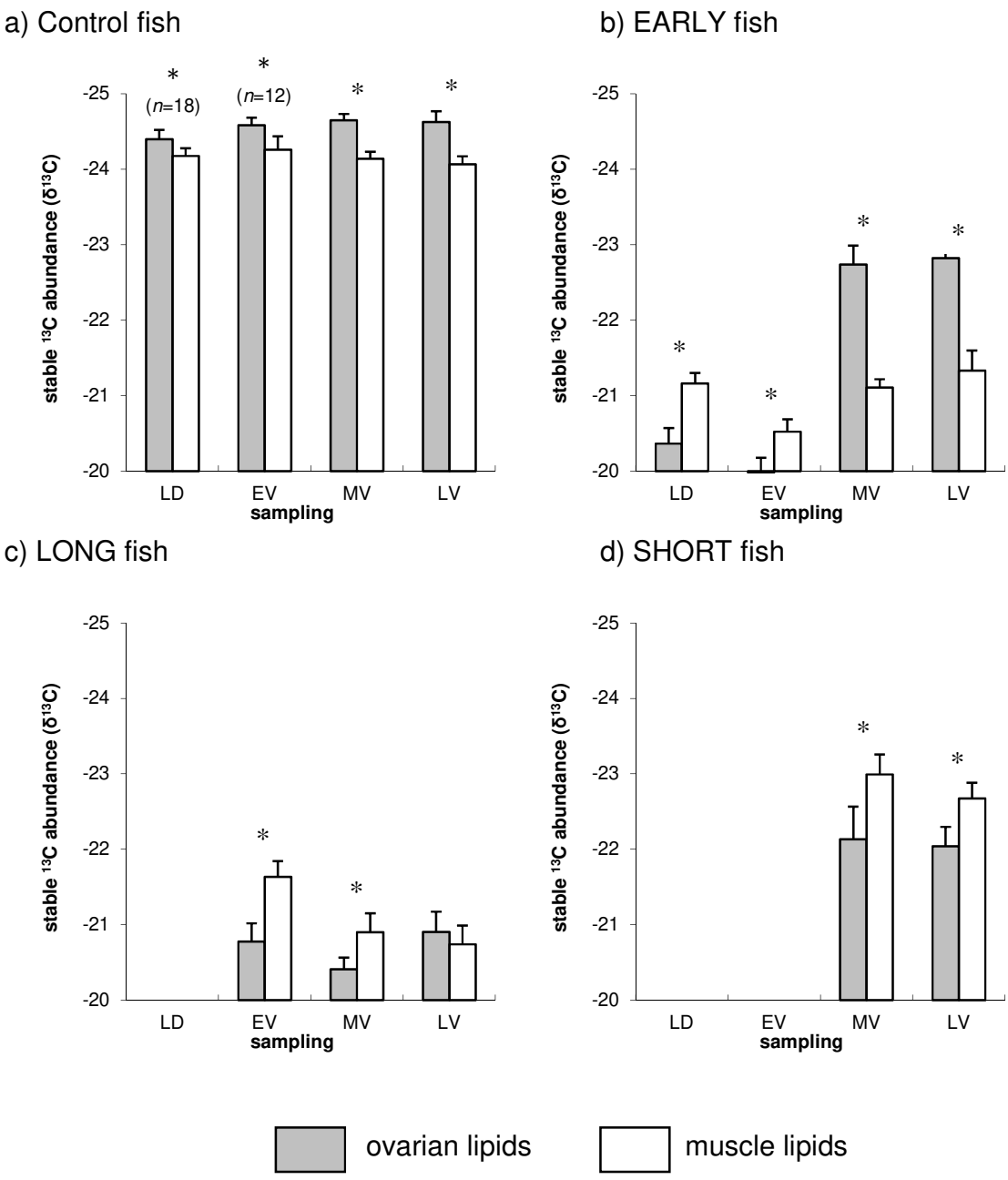

a) Control fish
b) EARLY fish
c) LONG fish
d) SHORT fish
stable ¹³C abundance (δ¹³C)
-25
-24
-23
-22
-21
-20
(n=18)
(n=12)
*
LD
EV
MV
LV
sampling
ovarian lipids
muscle lipids

Figure 6