**Compositional and physiological impacts of essential fatty acid deficient diet on zebrafish (*Danio rario)***

Trevor A. Gearhart1, Peter Euclide1, Jana Kraft2,and Jason D. Stockwell3  
  
1University of Vermont

Department of Biology  
Marsh Life Sciences Building   
Burlington, VT 05405

2University of Vermont

Department of Animal and Veterinary Sciences  
Terrill Building  
Burlington, VT 05405

3University of Vermont Rubenstein Ecosystem Science Laboratory

3 College Street  
Burlington, VT 05401

**Abstract**This study examines the consequences of potential decreases in diet quality for fish due to increases in cyanobacteria dominance in freshwater ecosystems. The timing and extent of decreases in essential fatty acids within fish fed a diet deficient in these compounds were tracked in zebrafish (*Danio rario*). Physiological parameters such as swim performance and respiration were collected in conjunction with fatty acid profiles to address the hypotheses that decreasing EFA levels will lead to lower physiological fitness. This study also evaluates the use of mead acid as an indicator for EFA limitation within fish.

**Keywords**

**Introduction**

Anthropogenic impacts on aquatic ecosystem health, such as climate change, nutrient loading, and habitat degradation create stress for ecological communities ([Ficke *et al.*, 2007](#_ENREF_7)). The cumulative impacts of anthropogenic stress manifest through intra- and inter-species competition, reducing habitat availability, altering community composition, and shifting available prey ([Buskey, 2008](#_ENREF_2); [Donnelly *et al.*, 2011](#_ENREF_6); [McMeans *et al.*, 2015](#_ENREF_9)). Changes in prey community can lead to shifts in food source quality for many organisms exacerbating competition and increasing stress to higher trophic levels ([Demott and Muller-Navarra, 1997](#_ENREF_5)).

Increased nutrient loading and phosphorous concentrations in freshwater systems is a major anthropogenic impact that has and will continue to increase cyanobacteria dominance in phytoplankton communities (REFS). However, Cyanobacteria are considered an inferior food source for zooplankton compared to other aquatic primary producers (REFS). Consequently, Cyanobacteria-based diets result in diminished growth and reproduction in zooplankton ([Brett, 2006 #77][Ravet, 2006 #731]). One issue with cyanobacteria based food webs is a lack of essential fatty acids. Specifically, cyanobacteria lacks three important bioactive fatty acids which cannot be synthesized by secondary consumers – docosahexaenoic acid (22:6 n-3, DHA), eicosapentaenoic acid (20:5 n-3, EPA) and arachidonic acid (20:4 n-6, ARA) ([Ahlgren, 1992 #292]). While having increased proportions of the essential fatty acids α-linolenic (18:3 n-3, ALA) and linoleic (18:2 n-2, LA) acid, with subsequent decreases in their downstream metabolites ([Perga, 2013 #773][Gearhart, 2016 #870]). Shifts in the availability and stoichiometry of these fatty acids, particularly the proportions of DHA, EPA and ARA, have many significant consequences for organism health including development and growth, hormone synthesis and composition, and immune response ([Sargent *et al.*, 1999](#_ENREF_12); [Tocher, 2003](#_ENREF_13); [2010](#_ENREF_14)).

The influence of diet on fatty acid composition and health is complex and remains uncertain. The fatty acid composition of fish is affected by dietary fatty acids found in diets (REFS), but metabolic processes can regulate composition (REFS), and thus may obscure the potential true cost of a diet shift and lead to unexpected outcomes. One process known to be influenced by diet is swimming performance and respiration. For example, arctic charr *Salvlinus alpinus* fed a diet based on fish oil had significantly higher swimming performance than charr fed a rapeseed and palm oil diet and showed significant changes in fatty acid composition over the treatment period (Pettersson, Pickova, & Brännäs, 2010). Alternatively, juvenile rainbow trout *Oncorhynchus*

*mykiss* fed diets of varying protein and lipid content showed no differences in metabolism over an eight week study indicating that the effects of diet are still not completely understood (Eliason, Higgs, & Farrell, 2007).

To better understand the impacts of altered fatty acid content on fish health and fitness, two key underlying uncertainties need to be addressed. First, how long must a fish experience limited essential fatty acid supply before it is unable to maintain essential fatty acid stoichiometry? Second, what are the potential consequences of essential fatty acid deficiency over time? These two questions are intertwined, and confounded in nature by variations in organism (*i.e*., local adaptation) and environmental (*i.e*., temperature). Additionally, to apply findings to the field, specific tracers of dietary stress must be identified to efficiently assess stress in natural communities. Controlling for these confounds by using experimental conditions and model organisms will help to accurately predict the impacts of low diet quality resulting from shifts in prey resources while allowing for the evaluation of potential tracers that correlate with dietary stress.

Zebrafish, *Danio rario* (Hamilton, 1822), is a model organism increasingly being used in fatty acid research ([Hölttä-Vuori *et al.*, 2010](#_ENREF_8); [Miyares *et al.*, 2014](#_ENREF_10)). Using zebrafish as a model, the impact of a shift in baseline fatty acid on higher trophic levels was experimentally simulated to explore the potential organism-level effects on fatty acid composition and physiological performance. Fish were fed manufactured diets, which varied only in their fatty acid content, simulating conditions similar to cyanobacteria blooms. The study was designed to test the hypothesis that fish consuming diets deficient in DHA, EPA and ARA, but still containing LA and ALA, would experience decreased metabolic efficiency in terms of respiration and swimming performance. The study also tested the hypothesis that in addition to an altered EFA stoichiometry, fish consuming EFA-deficient diets would exhibit an increased concentration in mead acid (C20:3 n-9) where?, an known indicator of EFA deficiency ([Ahlgren *et al.*, 1992](#_ENREF_1)) **Tocher, 2010 #866**.

**Materials and Methods**

*Experimental Design*

AB strain wild-type zebrafish were housed in the Rubenstein Ecological Sciences Laboratory at the University of Vermont in accordance with protocols approved by the Institutional Animal Care and Use Committee.

Ninety-eight six-month-old zebrafish were housed at 27ºC on a 12-h-light/12-h-dark cycle in a 300-gallon circulating system consisting of 150-gallon cistern with 15 10-gallon fish tanks receiving water filtered through a Pentair Aquatic Eco CSK1 commercial bead filtration unit (Apopka, Florida). The tanks were stacked in three levels with each level having five tanks, however only 4 tanks were used on the top shelf. Each tank housed seven fish with one fish removed from each tank at each sampling time point. Dissolved oxygen, pH (value) and temperature were constantly monitored throughout the experiments and remained consistent among tanks.

Diets were distributed in a stratified random manner with an equal amount of both diets and a control allotted to each level of the flow through system. Each day, fish were fed an excess of food (0.20 mg for the first 4 weeks and then dropped 0.13 mg once the number of fish dropped to 4) in the morning and afternoon. Feed intake was measured throughout the experiment by comparing the pre- and post weight of food in each tank. Post weight determined by siphoning each tank 30 minutes after feeding separately onto filter paper which was then dried to determine the dry weight of matter left in each tank. One fish was sampled from each tank at zero (baseline), one, two, four, six, and eight weeks for swimming performance, respiration measurements, and fatty acid content.

*Diets.*

The cod oil (FO) and coconut oil (CO) containing diets consisted of identical ingredients in identical proportions (how much total fat?), differing only in lipid sources and thus, in their fatty acid profile (Tables 1 and S1). Say something here about the important differences between the two diets in terms of EFA.

*Swimming performance*

Prior to swimming performance experiments, individual fish were sexed, measured to the nearest mm, weighed to the nearest 0.1 g, and then allowed to acclimate in the swim tunnels at the lowest flow rate (approximately 1 body length/s) for 1.5 to 2.5 hours to reduce the effects of handling stress. Swim performance of zebrafish was measured using Blazka-type Loligo Systems Mini Swim Tunnel Respirometers, a DAQ-M control device and the AutoResp software version 2.2.0 (Loligo Systems, Denmark). At 0, 1, 2, 4, 6, and 8 weeks, critical swimming speed (Ucrit) was measured for six fish from each treatment however, Ucrit and respiration values from time 0 had to be removed from analysis due to complications with respirometers. Swim trial protocol was adapted from other studies (Goertzen, Driessnack, Janz, & Weber, 2011; Mager & Grosell, 2011). In brief, fish were subjected to stepwise swimming speed by increasing water velocity by 8.0 cm/s every 20 minutes until exhaustion (swimming stopped). Exhaust time was counted as the time when fish would not return to swimming after a 5 to 10 second period of reduced flow. Ucrit was then calculated using the following equation:

where Vi is the velocity increase per increment, Vp is the final velocity the fish swam for the entire 20 minutes, ti is the increment time length and tf is the duration of the last velocity increment until exhaustion. Water temperature was kept at 26°C for all trials and was recorded using a TEMP-4 temperature probe system (Loligo Systems, Denmark). Higher Ucrit values are generally associated with higher fish health (Hammer, 1995; Plaut, 2001). Therefore, we expected Ucrit values for fish fed the FO diet to be greater than values for those fed the CO diet.

*Respiration (Oxygen consumption)*

Oxygen consumption (MO2) during swim performance trials was measured using intermittent respirometry at 26°C with Pt100 fiber optic probe connected to an Oxy-4 Mini device and AutoResp software version 2.2.0 (Loligo Systems, Denmark). Independent measurements of MO2 were recorded at 10-minute intervals. All chamber water was flushed with oxygenated water following each interval. Standard metabolic rate (SMR) was defined as the y intercept of the logarithm of oxygen consumption versus swimming speed. Active metabolic rate (AMR) was defined as the maximum three values of MO2 and aerobic scope was defined as AMR - SMR for each fish (Mager & Grosell, 2011). If an EFA-deficient diet affected fish metabolism, then we would expect AMR and aerobic scope to be higher in the higher quality FO based diet while SMR would remain stable or decrease in the FO based diet.

*Lipid extraction and fatty acid methylation of zebrafish*

Lipids from zebrafish were extracted using chloroform-methanol (2:1; *v*/*v*) according to Bligh and Dyer (1959). Total lipids were then transesterified into fatty acid methyl esters (FAME) using a 1-step procedure with toluene and 1% sulfuric acid in methanol ([Christie, 1989](#_ENREF_4)) at 50°C for 18 hours.

*Fatty acid analysis*

The resulting FAME (~0.01% solution) were analyzed by gas chromatography-mass spectrometry using a Shimadzu GCMS-QP2010plus (Shimadzu Co, Kyoto) equipped with a Rtx®-2330 column (100m x 0.25 mm x 0.15 µm; Restek) with the following temperature program: 45°C was maintained for 4 min, then the temperature was increased at 13°C min-1 to 150°C, and maintained for 27 min, and finally heated at 3°C min-1 to 215°C and held for 35 min. Helium was used as a carrier gas with an average velocity of 34 cm sec-1. The chromatograms were analyzed using GCMSsolution software (V. 2.70, Shimadzu, Kyoto). Individual fatty acids were identified as percent composition based standards (Supelco 37 component FAME mix, Nu-Chek Prep mix # 463 and mix # 674) and confirmed using mass spectral libraries (NIST, 2012; Wiley 10th edition).

*Statistical analyses*

To test how diet treatments impacted fatty acid composition of zebrafish, comparisons were made between CO and FO based diet treatments across the 6 sample dates using sex as a covariate. The analysis was limited to only biologically relevant fatty acids by choosing a suite of fatty acids based on previously observed correlations between fatty acid composition and cyanobacteria dominance in a freshwater lake (Gearhart et al, 2016) or fatty acids which were most likely to be impacted by EFA-deficient diets and known to have physiological consequences for the fish. The fatty acid categories included summed totals of: saturated fatty acids (∑saturated), monounsaturated fatty acids (∑monounsaturated), polyunsaturated fatty acids (∑polyunsaturated), essential fatty acids (∑EFA), n-3 fatty acids (∑n-3), and n-6 fatty acids (∑n-6). We also examined the specific fatty acids LA, ALA, ARA, EPA, DHA, and mead acid. [Somewhere in here you should also point out your test of the mead acid hypothesis – that you expected mead acid to be present in CO-fed fish but not FO-fed fish, and that you further tested correlations between mead acid and other fatty acids in fish from each diet group separately. You would expect specific relationships based on the production of mead acid in relation to availability/unavailability of certain FA, correct? You have some of this in the Results that you should transfer to here. At some point you will need to explain the mead acid “production line”, so to speak, but that might be better explain in the Discussion (i.e., here’s the mechanisms that explain the results).

Differences in swimming performance (Ucrit), respiration measurements (AMR, SMR, and aerobic scope), and fatty acids (individual and grouped) were evaluated using three-way analysis of variance (ANOVA) with treatment, time, and sex as principal factors. Significant differences (*P* < 0.05) were further evaluated with a post-hoc Tukey test to determine which treatments and time points differed. Resulting *P* values were then adjusted with a bonferonni correction to correct for the multiple two-way ANOVA tests. Prior to ANOVA, outliers were identified for each sampling period using using a modified Z-score (MOD) ([Iglewicz, 1993 #871]). Any value with a corresponding MOD of beyond ± 3.0 was removed from analysis. Respiration measurement metrics are not independent from one another. Thus, therefore if an individuala fish was an outlier in one respiration metric, it was removed from all subsequent respiration analyses. For example, because fish “ZF\_C\_5” was an outlier for aerobic scope at time 1, it was removed from analysis for aerobic scope, and for SMR, and AMR, but was included in Ucrit analysis.

We used Pearson’s correlation to evaluate relationships between select fatty acids and metabolic measurements. Correlations were corrected using Benjamini-Hochberg false discovery rate and were considered significant at *P* < 0.05. Correlations were conducted on all fish within the study as a pooled sample study and were run on fish within each diet individually.

**Results**

*Experimental conditions*

Environmental conditions were consistent among tanks and throughout the experiment (temperature = 27 ± 1 °C; pH = 7.9; dissolved oxygen = 100 %). Consumption per tank per feeding did not differ between the two diet treatments (CO = 0.13 ± 0.003 g and FO = 0.11 ± 0.05 g; stats report here, t value and P value?, Table X) and all fish were seen actively feeding when food was introduced to each tank. No evidence of fungal growth or illness was observed in any of the tanks. The only evidence of stress was the death of a single fish (Tank 5, week 8) and which was not used for any analysis. The sex distribution, determined prior respirometry, was 14 males and 16 females for the CO diet treatment, and 20 males and 14 females for the FO diet.

[Suggest to follow order of statistical methods starting here. First, present FA composition of fish as function of experiments, and also relate to diet compositions. Also include the mead acid results here too. Then, do the experimental results of the Ucrit and then respirometry stuff. Next, correlations between FA and Ucrit and respirometry metrics.]

*Changes in fatty acid composition*

Average (SD) percent composition of all fatty acids analyzed, by diet and sex, are presented in Table 2. All the select fatty acids and categories, except for ∑polyunsaturated, varied by diets (P < 0.01; Tables 3 and S2). [statement on how they compared to their diets – what was gained or lost?]. Which ones changed through time and what were the patterns, and how quick did they change? Report on the interactions. Seems like the categories with PUFAs all had diet x sex interactions – seems to be of note. The select fatty acids and categories evaluated in this study to determine if and how quickly fatty acid compositions would change showed multiple statistically significant interactions within the ANOVA analyses performed.

*Swimming Performance and respiration*

Measurements of Ucrit and respiration were successfully taken from 75 fish. Five in had a MOD greater than 3.0 xxx (dimension?) in at least one or more metabolic measurement and were removed from analysis. Consequently, three individuals were removed from Ucrit analysis and two individuals were removed from respiration analyses. Fish fed the FO diet had higher Ucrit values than those fed the CO diet (F = 7.34, *P* < 0.01, Table S3), consistent with expectations of decreased/increased swimming performance with an EFA-deficient diet. Standard metabolic rate (SMR) decreased over time (F = 3.22 P < 0.02, Table S3) but was not affected by diet, sex, or interactions. Aerobic scope and AMR were not affected by any of the main factors or their interactions (Table S3).

[You only found differences in Ucrit (by diet) and SMR (by time) in your ANOVAs so I don’t think you should look for correlations between AMR/aerobic scope and fatty acids. If there was no change in AMR or aerobic scope, it does not make sense to look for significant relationships – perhaps I am thinking about this incorrectly? If I am correct in my thinking, then next two paragraph should be about the correlations of fatty acids with Ucrit (first paragraph) and SMR (second paragraph). All FA you looked at had significant diet effect (even PUFA had the diet x time interaction) so compare correlations of each of them with Ucrit, but do it separately for each diet treatment group (don’t pool all fish as you did below – it does not make sense to do this when there is a diet difference (to me anyway)). Only ALA, ARA, EPA, DHA and Sum(saturated) had time effects (or interactions that involved time) so these are probably the only ones to look at with SMR, I think. Because these 5 also had diet effects, you may want to look at correlations of these FA and SMR by diet groups. Make sense?]

*Correlations between lipids and physiology*

The evaluation of our hypothesis that a limited supply of essential fatty acids would lead to changes in physiological performance was also supported by significant correlations. When considering all fish, ∑monounsaturated fatty acids were positively correlated with Ucrit (r= 0.32, P = 0.006) and aerobic scope (r = 0.26; P = 0.033). ∑EFA and LA showed a positive relationship with SMR (r = 0.25, 0.25; P = 0.04, 0.03). ∑Saturated Fatty acids and ARA were negatively correlated with Ucrit (r = -0.28, -0.26; P = 0.01, 0.02).

Within the fish on the CO diet, ∑polyunsaturated fatty acids were negatively correlated with AMR and aerobic scope (r = -0.42, -0.43; P = 0.02, 0.02). ALA was negatively correlated with SMR and AMR (r = -0.46, -0.37; P= 0.02, 0.02). Within the FO diet, ∑n-3 fatty acids were negatively correlated with Ucrit (r = -0.46; P = 0.005), DHA was also found to negatively correlate with Ucrit (r = -0.41, P = 0.01), AMR (r = -0.37 ; P = 0.03 ) and aerobic scope (r = -0.38 ; P = 0.03).

~~Data concerning the hypothesis that Mead acid can be an indicator of decreased availability of important bioactive fatty acids without essential fatty acid deficiency are presented.~~ Correlations between mead acid and other fatty acids were considered for each individual diet because…. When looking at the fish fed the CO diet, mead acid was found to negatively correlate with ALA, EPA, ∑monounsaturated fatty acids (r = -0.47, -0.52, -0.65; P = >0.001, 0.005, 0.002). Mead acid in CO-fed fish was positively correlated with ARA, ∑polyunsaturated fatty acids, ∑n-6 fatty acids and ∑saturated fatty acids (r = 0.80, 0.49, 0.53, 0.50; P = <0.001, 0.009, 0.004, 0.088). Fish fed the FO diet also had multiple significant correlations between mead acid and other select fatty acids. Mead acid was positively correlated with ARA, and ∑saturated fatty acids (r = 0.83, 0.85; P >0.001). Mead acid was negatively correlated with EPA, ∑n-3, ∑monounsaturated fatty acids (r = -0.81, -0.63, -0.83; P > 0.001). Mead Acid was negatively correlated with Ucrit (r = -0.43; P = 0.01). Mead acid was also negatively correlated with AMR, and aerobic scope (r = -0.24, -0.24; P = 0.006, 0.04, 0.04).

None of the figures are referenced in the Results. Why?

**Discussion**

The results of this study indicate that diets deficient in Long Chain-EFA have immediate impacts on FA composition of consumers within one to two weeks and negatively influence organism metabolism and physical performance. We highlight four major findings, first, zebrafish fed a diet rich in LC-EFA (FO) had a higher Ucrit than fish fed a diet limited in LC-FA over an 8-week period. Second, we identified a suite fatty acids which show correlative relationships with swimming performance. Third, this experiment shows that competitive interactions between LC-EFA happen quickly and are dependent on both metabolic need and dietary source. Four, we establish that mead acid can be used as a biomarker to…… diets that are highly unbalanced in the n-3/n-6 ratios of EFA.

Physical performance (Ucrit) of zebrafish was correlated with specific LC-EFA. This is consistent with previous studies **(some human/mammal findings and fish stuff)**. In fish, PUFA from dietary sources can lead to decreased swimming performance and/or increased resting metabolic rate [Chatelier, 2006 #852;McKenzie, 2001 #862]. However, other research into the effects of fatty acids have found that increases in n-3 fatty acids lead to higher swimming performance ([Wagner *et al.*, 2004](#_ENREF_15); [Pettersson](#_ENREF_11" \o "Pettersson, 2010 #860) *[et al.](#_ENREF_11" \o "Pettersson, 2010 #860)*[, 2010](#_ENREF_11" \o "Pettersson, 2010 #860)). Interestingly, in the treatments where the FO has substantially higher PUFA, we see agreement with both Chatelier *et al.* and Wagners findings of higher overall swim performance but also a negative correlation between PUFA and metabolic measurements. Specifically, we found a positive correlation between LA and SMR, but no correlation with Ucrit, which has been previously observed [McKenzie, 2001 #862]. this is likely because all fish had ample LA supply and so there was not a large enough gradient of LA composition to pick up trends. This could be indicative of both lower and upper thresholds for PUFA in respect to swimming performance, or a result of specific ratios of fatty acids within the diets.

The n-3/n-6 ratios found in our diets were significantly altered likely due to an increase in LA found within both diets. Although this increase in LA means that the diets deviate from levels of LA found within cyanobacteria, this increase in LA enabled the examination of the potential impacts of imbalanced n-3/n-6 ratios on the incorporation and synthesis of EFA (Figure 1). Imbalances in the n-3/n-6 ratio have been implicated in leading to decreased immune efficiency [Oliva‐Teles, 2012 #863] and cardiovascular health [McKenzie, 2001 #865]. The rates of LA uptake in fish fed CO compared to FO diets are specifically exciting. There is evidence that while CO fed diets are incorporating large amounts of LA, and continuously increasing their levels, the levels of LA in fish fed the FO diet are actually decreasing even as their diet holds higher amounts of LA (Figure 2). These results are exciting as they show that the fish fed FO are likely modifying LA into another source of energy such as monounsaturated fatty acids for energy storage [Maillet, 2006 #864]. In CO-fed fish, levels of LA were higher, while levels of ALA, which was also available in the diet, were lower. One potential explanation for this is that it is being synthesized into EPA and then DHA, both of which also decreased throughout the experiment suggesting physiological demand for these fatty acids. From this we can deduce that metabolic processes were requiring the utilization of EPA and/or DHA and this need is possible drawing down ALA along with those two FA. Another potential explanation could be that LA and ARA are outcompeting their n-3 counterpoints at bonding points [where?] along the synthesis pathway and limiting the fish’s capability to increase downstream synthesis. This hypothesis is also supported by the minor but significant increase in ARA within the CO-fed fish, even though their food source did not contain any ARA.

We observed a drawdown of downstream EFA in our fish, which supports our hypothesis that mead acid can act biomarker of EFA deficiency even when C18 EFA are present. This study is unique as far as we know in that it looks not at a total EFA deficit, but an imbalance between n-6 and n-3 fatty acids and an enrichment of LA. Mead acid levels were shown to increased significantly in a diet that was highly imbalanced but not technically EFA deficient (Figure 3). Mead acid had consistent negative correlations with swim performance (Ucrit) within all fish considered and specifically within the CO fed treatments. Mead acids did not correlate with fish fed the FO diet, which is not surprising and suggests that although mead acid was detected in those fish it is not above a required threshold for physiological impacts.

These findings that dietary decreases in available long-chain EFA have detrimental impacts on the swimming capabilities of zebrafish build on a body of literature showing that decreases in availability of quality prey sources will have detrimental impacts on fish (figure 4). The fact that the changes within the fatty acid composition of these fish were significant within two weeks of a diet shift indicates that even short term, seasonal alterations in prey availability, such as mistiming in phenology or cyanobacteria blooms could have consequences of survival or foraging success in the near future. As climate change and habitat degradation continues and there are shifts in aquatic primary producer community as well as altered environmental stressors on those producers we can expect to see shifts in the fatty acid profile of available food. One such example is increases in lake temperature, which can lead to less desirable species such as cyanobacteria also decreases the physiological need for PUFA in primary producers as well as consumers. These compounding impacts will lead to energetic and nutritional constraints that are currently not thoroughly considered in the context of the potential impacts of climate change on organism health and community interactions.

The use of zebrafish as a means to test ecological hypotheses allows for us to both better understand the potential impacts of fatty acid shifts and to also evaluate the potential implications for climate change and eutrophication on wild fish populations which face these stresses as a result of climate change and land use shifts.

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Table I. Percent fatty acid composition of diets used in experiment.

Table II. Fatty acid % composition of all fish by diet (coconut oil (CO) or cod oil (FO)) and sex.

Table 3.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Diet | Time | Sex | Diet x Time | Diet x Sex |
| ΣSaturated | X |  |  | X |  |
| ΣMonounsaturated | X |  |  |  |  |
| ΣPolyunsaturated |  |  |  |  | X |
| ΣEFA | X |  |  |  | X |
| Σn\_3 | X |  |  |  | X |
| Σn\_6 | X |  |  |  | X |
| LA | X |  |  |  |  |
| ALA | X | X |  | X |  |
| ARA | X | X | X |  |  |
| EPA | X |  |  | X |  |
| DHA | X |  |  | X |  |
| Mead Acid | X |  | X |  |  |

Figure 1. The ratio of n-3 to n-6 fatty acids in zebrafish fed the coconut oil (CO), fish oil (FO) and the average of fish fed their growth diet prior to initiation of the experiment.

Figure 2. Percent composition of LA, ALA, ARA, and DHA in zebrafish fed fed the coconut oil (CO), fish oil (FO) over the course of the experiment. These fatty acids represent the initial and end products of the metabolic pathways concerning the creation of bioactive fatty acids.

Figure 3. Percent composition of Mead acid in zebrafish fed fed the coconut oil (CO), fish oil (FO) over the course of the experiment.

Figure 4. Ucrit values for zebra fish fed fed the coconut oil (CO), fish oil (FO) over the course of the experiment.







