INSECTS AS SOURCES OF PROTEIN AND LONG-CHAIN FATTY ACIDS FOR ENTOMOPHAGY

BY

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ABSTRACT OF THE THESIS

Insects as Sources of Protein and Long-Chain Fatty Acids for Entomophagy

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Current sources of protein and omega-3 fatty acids have become unsustainable. Livestock and farmed fish are fed unnatural diets in order to increase productivity and cut costs. This causes health problems for the animals and decreases the nutritional value of their meat. Meat from factory farms contains high concentrations of the omega-6, linoleic acid (LA) while lacking the omega-3, α-linolenic (ALA) acid. Aquaculture fish have less protein than wild-caught fish. Eating a diet with a high ratio of LA to ALA contributes to obesity and cardiovascular disease. Farming insects for entomophagy can be more cost effective than farming livestock or fish because insects require less water, feed, and space, have a much smaller carbon footprint and produce far less waste.

The objective of this study is to determine which local insect species have the highest concentrations of protein and beneficial long-chain fatty acids (LCFAs) along with the most balanced ratios of LCFAs. I compared the protein and long-chain fatty acid concentrations of six terrestrial insect species; *Acheta domesticus* (L.), *Tenebrio mollitor* (L.), *Hermetia illucens* (L.)., *Reticulitermes flavipes* (L.), *Dissosteira carolina* (L.), and *Diestrammena japanica* (Blatchley), and 4 aquatic insect species; *Acroneuria abnormis* (Newman), *Rhyacophila carolina* (Banks), *Hydropsyche betteni* (Ross), *Brachycentrus*

numerous (Say) Say, to those of three ground beef samples; grain-fed, grass-fed, and grass-finished beef and four commercial fish samples; farm-raised and wild-caught Atlantic salmon, *Salmo salar* (L.) and wild-caught European anchovy, *Engraulis encrasicolus* (L.), and Pacific sardine, *Sardinops sagax* (Jenyns). The 10 insect species evaluated represented insects of different natural diet, habitat, and insect phylogenetic positions.

While this research is exploratory in nature, working hypotheses based on existing research are as follows: First; Termites eaten in Africa have very high protein concentrations; therefore the local species of termites, *Reticulitermes flavipes* is likely to also be high in protein. Second, Camel crickets can jump much higher than *A. domesticus;* therefore, the Japanese camel cricket, *D. japanica* is likely to have a higher protein concentration than *A. domesticus* due to larger and stronger muscles. Third; Graminivore insects such as the Carolina grasshopper, *D. carolina* are likely to have high concentrations of alpha-linolenic acid and a balanced ratio of omega 6 to omega 3 fatty acids based on the comparison of beef raised on grass-only diets compared to beef rose on grain and soy diets. Fourth; Grasshoppers consume far more relative to their total body mass compared to cows; therefore *D. carolina* will likely have a similar ratio of LA to ALA to grass-finished beef, but with significantly higher concentrations of both fatty acids Fifth; Aquatic insects frequently consumed by oily fish are likely to have high concentrations of beneficial long-chain fatty acids. Sixth; Insects frequently consumed by fish are likely to have higher concentrations of protein than consumers. Seventh; Aquatic insects are likely to have balanced ratios of omega-6 and omega-3 fatty acids compared to non-graminivore terrestrial insects. The results of this study have supportive of all seven working hypotheses. This study has shown that insects have the potential to be major sources of protein and beneficial LCFAs.

Keywords: animal farming issues; aquatic insects; entomophagy; long-chain fatty acids; protein; terrestrial insects

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TABLE OF CONTENTS

ABSTRACT OF THE THESIS……………………………………………………………ii

ACKNOWLEDGMENTS……………………………………………………………v

LIST OF TABLES…………………………………………………..………….viii

LIST OF FIGURES……………………………………………………………viii

LIST OF INSECT SPECIES TESTED….…………………………….…………………..x

LIST OF FATTY ACIDS……………………..……………………………………..x

SECTION ONE: GENERAL INTRODUCTION…...………………………….……...1

MATERIALS AND METHODS……………………………………………….6

SECTION TWO: TERRESTRIAL SPECIES…………..…………...............................9

OBJECTIVES……………………………………………………………..……9

WORKING HYPOTHESES……………………………………………..….…9

ABSTRACT……………………………………………………………………...10

INTRODUCTION……………………………………………………………11

RESULTS…………………………………………………………………..16

DISCUSSION………………………………………………………………22

SECTION THREE: AQUATIC SPECIES……….…………………………………...24

OBJECTIVES…………………………………………………………………24

WORKING HYPOTHESES…………………………………………………..24

ABSTRACT……………………………………………………………..….24

INTRODUCTION……………………………………………………………....25

RESULTS………………………………….………………………………....29

DISCUSSION………………………………………………………………...36

SECTON FOUR: CONCLUSIONS………..………………..40

REFERENCES……………………………………………………………..………....41

LIST OF TABLES

1. Protein Concentrations of Terrestrial Species ……………………………………...16

2. Long-Chain Fatty Acid Concentrations of Terrestrial Species…………….……..……..18

3. Protein Concentrations of Aquatic Species ……………………………….…….29

4. Long-Chain Fatty Acid Concentrations and Ratios of Aquatic Species ……..…...…31

LIST OF FIGURES

1. Protein Concentrations of Terrestrial Species ……………………………………...16

2. Linoleic Acid (O-6) Concentrations of Terrestrial Species...19

3. α-Linolenic (O-3) Concentrations of Terrestrial Species...19

4. Omega-6 to Omega-3 Ratios of Terrestrial Species……………………….…........19

5. Oleic Acid (O-9) Concentrations of Terrestrial Species………………………………20

6. Palmitic Acid Concentrations of Terrestrial Species……………………………….…20

7. Oleic Acid (O-9) to Palmitic Acid Ratios of Terrestrial Species…………..………….20

8. Palmitoleic Acid (O-7) Concentrations of Terrestrial Species…………..……………21

9. Lauric Acid Concentrations of Terrestrial Species………..,,………………………..21

10. Protein Concentrations of Aquatic Species ……………...29

11. Linoleic Acid (O-6) Concentrations of Aquatic Species …………….…….………..32

12. α-Linolenic (O-3) Concentrations of Aquatic Species ……………………….………..32

13. Eicosapentaenoic acid (EPA) (O-3) Concentrations of Aquatic Species……………32

14. Docosahexaenoic acid (DHA) (O-3) Concentrations of Aquatic Species………...…33

15. Omega-6 to Omega-3 Fatty Acid Ratios of Aquatic Species……………………......33

16. Oleic Acid (O-9) Concentrations of Aquatic Species……………………..…..……..33

LIST OF FIGURES (CONT.)

17. Palmitic Acid Concentrations of Aquatic Species……………………..…..………...34

18. Oleic Acid (O-9) to Palmitic Acid Ratios of Aquatic Species ……............................34

19. Palmitoleic Acid (O-7) Concentrations of Aquatic Species……………………..…..34

21. Lauric Acid Concentrations of Aquatic Species……………………………………..35

LIST OF INSECT SPECIES TESTED

Scientific Name	Common Name	Classification	Life Stage/Caste	Diet	Date, Location and Substrate Collected
Acheta domesticus	House Cricket	Order: Orthoptera Family: Gryllidae	Adult	Omnivore	PetSmart, 111 Nassau Park Blvd, 7/2/19
Tenebrio molitor	Mealworm	Order: Coleoptera Family: Tenebrionidae	Larvae	Detrivore	Kaytee, 521, Clay Street, Chilton, WI, 7/3/19
Hermetia illucens	Black Soldier Fly	Order: Diptera Family: Stratiomyidae	Larvae	Detrivore	TradeKing, 2020 6th Street, Bay City, TX, 7/3/19
Reticulitermes flavipes	Eastern Subterranean Termite	Order: Blattodea Family: Rhinotermitidae	Worker	Xylophagous /Fungivore/ Detrivore	Cook Campus Center, Rotting Log, 9/7/19
Dissosteira carolina	Carolina Grasshopper	Order: Orthoptera Family: Acrididae	Adult	Graminivore	Passion Puddle, Grass, 9/3/19
Diestrammena japanica	Japanese Camel Cricket	Order: Orthoptera Family: Rhaphidophoridae	Adult	Omnivore	Cook Campus Center, Rotting Stump 8/13/19
Acroneuria abnormis	Common Stone	Order: Plecoptera Family: Perlidae	Nymph	Carnivore	Pequest River, Open Water, 605 Pequest Road, Oxford, Warren County, NJ, 6/5/19
Rhyacophila carolina	Green Sedge	Order: Trichoptera Family: Rhyacophilidae	Larvae	Carnivore	Pequest River, Open Water, 605 Pequest Road, Oxford, Warren County, NJ, 6/5/19
Hydropsyche betteni	Common Netspinner	Order: Trichoptera Family: Hydropsychidae	Larvae	Detrivore	Pequest River, Under Rock, 605 Pequest Road, Oxford, Warren County, NJ, 6/5/19
Brachycentrus numerous	American Grannom	Order: Trichoptera Family: Brachycentridae	Larvae	Detrivore	Pequest River, Top of Rock, 605 Pequest Road, Oxford, Warren County, NJ, 6/5/19

LIST OF LONG-CHAIN FATTY ACIDS

Name	Abbreviation	Classification	Lipid Number
Linoleic Acid	LA	Polyunsaturated-omega-6	C18:2n6
α-Linolenic Acid	ALA	Polyunsaturated-omega-3	C18:3n3
Eicosapentaenoic Acid	EPA	Polyunsaturated-omega-3	C20:5
Docosahexaenoic Acid	DHA	Polyunsaturated-omega-3	C22:6
Palmitoleic Acid	PAL	Monounsaturated-omega-7	C16:1
Oleic Acid	OA	Monounsaturated-omega-9	C18:1n9
Palmitic Acid	PA	Saturated	C16
Lauric/Dodecanoic Acid	DA	Saturated	C12

Section One: General Introduction

Global meat production has quadrupled in the last 50 years as the human population reached 7.6 billion people (Ritchie, 2017). However, despite this meat production increase, approximately one billion people worldwide are unable to meet the minimum daily requirements for protein intake. Protein is a vital component of every cell in the body. The minimum daily recommended intake of protein in the human diet is 0.8 grams per kilogram of body weight. This number can double if intense physical activity is performed regularly. A lack of dietary protein can lead to health issues including: stunted growth, anemia, edema, and vascular dysfunction. Dietary protein is required for skeletal muscle growth and proper blood clotting. Children are more vulnerable to malnutrition than adults and protein deficiency has stunted the growth of millions of children worldwide due to lowered skeletal muscle growth (Wu ,2016). Protein deficiency can also lead to Kwashiorkor, a serious condition in children with incapacitating symptoms and protein deficiency can also lead to weakened immune systems as antibodies are proteins (Khan et al., 2017). Proteins in saliva act as buffers to maintain a neutral pH balance of 6.8-7.8 during acidosis, when blood pH drops below 7.35 (Lamanda et al., 2007; Cheaib et al., 2013). A blood pH below 6.8 can be fatal for mammals (Bird et al., 1981).

The omega-6, linoleic acid (LA) and the omega-3, α-linolenic acid (ALA) are both considered essential fatty acids for humans as human/mammal bodies cannot synthesize them and they must be ingested from outside sources (Simopolous., 2016). LA is required for growth and development and has important functions such as reducing blood LDL-cholesterol levels, accelerating wound healing in diabetics, and is important for neurodevelopment (Jandacek., 2017; Yeung et al., 2017; Taha., 2020). However, anything above a 4:1 ratio of omega-6 to omega-3 is considered excessive and can increase the risk of obesity, cardiovascular disease, and insulin resistance (Simopolous., 2016; Taha., 2020). ALA has neuroprotective and homeostatic properties and can reduce the risk of cardiovascular disease (Taha., 2020). The average ratio of omega-6 to omega-3 used to be approximately 1:1 since the Paleolithic period and began to increase to 20:1 and possibly higher during the 20th century (Blasbalg et al., 2011; Simopoulos., 2016). Soybean oil is currently one of the biggest sources of LA in the US and soy consumption increased by one thousand times from 1909-1999 (Blasbalg et al., 2011). Factory farm beef contains high concentrations of LA and low concentrations of ALA due to the lack of grass in the cows' unnatural grain-and-soy-based diet (Elswyck et al., 2014).

Two other important omega-3 fatty acids in the human diet are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These two fatty acids are important for proper fetal development, healthy ageing, and anti-inflammatory processes. Low concentrations of EPA and DHA in the diet are risk factors for cardiovascular and Alzheimer's disease. The current American diet has an excess of saturated fats and a shortage of omega-3 fatty acids due to fast foods. The main sources of these fatty acids in the human diet are oily fish and fish oil supplements (Swanson et al., 2012). The high demand for fish oil supplements leads to overfishing and once EPA and DHA are extracted, the rest of the fish is generally discarded (Nges et al., 2011).

The discarding of the rest of the fish during omega-3 extraction causes consumers to miss out on the omega-7, palmitoleic acid (PAL) (Makoure et al., 2019). This fatty acid is generally used in biodiesel synthesis, but it does have important health benefits

(Cunningham, 2015; Makoure et al., 2019). Palmitoleic acid is not a major contributor to the total fatty acid intake of the human diet and is mainly found in blue-green algae, macadamia nuts, and sea buckthorn (Yang et al., 2001; Cunningham, 2015). PAL is associated with decreased insulin resistance which reduces hyperglycemia and hypertriglyceridemia, which are both risk factors in type-2 diabetes (Yang et al., 2011). It has also been reported to help regulate cholesterol metabolism and hemostasis (Shramko, 2020).

The omega-9, oleic acid is another beneficial fatty acid found in fish which is discarded as waste for biodiesel (Makoure et al., 2019). Maintaining a high ratio of the omega-9, oleic acid to the saturated fat, palmitic acid ratio is also important for human health. Replacing palmitic acid with oleic acid in the diet can also help reduce obesity and insulin resistance. The obesity and type-2 diabetes-reducing Mediterranean diet requires the intake monounsaturated fatty acids to be over 20% of total energy with a low saturated fatty acid intake of 7-8% (Palomer et al., 2017). A high oleic to palmitic ratio is also important in regulating thrombogenesis and fibrinolysis. A low oleic to palmitic ratio increases the risk of atherosclerosis, the buildup of LDL-cholesterol in artery walls. This buildup of cholesterol can rupture the arteries and expose the fibrin in the plaque to blood, leading to thrombosis when the resulting blood clots block the artery (Pacheco et al., 2006).

The unnatural diets from livestock and fish farms along with the crowded conditions cause health problems for the animals, leading the farms to overuse antibiotics (Nges et al., 2012; Abdela, 2016). Antibiotics from manure or uneaten feed leeches into the environment, which leads to an abundance of antibiotics-resistant strains of human pathogens (Steinfeld et al., 2006; Nges et al., 2012). The manure, along with deforestation for grazing lands and feed crops, releases large amounts of greenhouse gasses to the point that livestock have a larger carbon footprint than every type of gasoline-fueled vehicle combined (Steinfeld et al., 2006). Meanwhile aquaculture ironically contributes to overfishing because a major component of fish feed on these farms is in fact, other fish (Yvonne et al., 2017).

Entomophagy can potentially mitigate these problems. Insects have higher feed conversion rates than cows. Producing 1kg of beef requires 10 kg of feed, while producing 1 kg insect protein would require 1.7 kg of feed. This is likely because insects are cold-blooded and do not require feed to maintain body temperature. In the case of *A. domesticus*, an estimated 80% of the insect is edible, while only around 40% of a cow is edible. Detritivore insects such as *T. molitor* and *H. illucens* could potentially convert billions of tons of bio-waste from factory farms and other sources of human-generated waste into edible protein. The bio-waste would then become nitrogen-rich frass which could be used as fertilizer. Mealworms, crickets, and locusts are estimated to produce 100 times less greenhouse gases than comparable masses of cows and pigs (Huis et al., 2013). Around 70% of global freshwater is used in agriculture and one gram of insect protein uses about 20% of the water required for 1g of beef protein and takes up 8-14 times less space than cattle (Huis et al., 2013; Huis et al., 2017). *Acheta domesticus*, *T. molitor*, and *H. illucens* have received the most international attention from organizations such as the Food and Agriculture Organization (FAO) and the USDA as potential alternative food source (Huis et al., 2013).

There is comparatively less research into the potential nutritional value of aquatic insects in the human diet compared to terrestrial insects. Despite the ubiquity of entomophagy in Africa, Columbia, Mexico, China, Thailand, Indonesia, Japan, and Australia, any consumption of aquatic insect species in these countries remains niche at best and has not advanced past the hunter-gather stage (Williams et al., 2017). In 2017, the European Commission enacted act 893 which allowed the use of seven insect species as components of fish feed. These seven species were: black soldier fly, common housefly, yellow mealworm, lesser mealworm, house cricket, banded cricket, and field cricket, all of which are terrestrial species (Council directive 2017/EC). Several studies discussing the potential of insects as feed to aquaculture fish species such as Atlantic salmon and rainbow trout only discuss terrestrial species (Arru et al., 2019; Biancarosa et al., 2019; Józefiak et al., 2019). The irony here is that juvenile Atlantic salmon and rainbow trout already eat a variety of aquatic insects including Ephemeroptera, Plecoptera, and Trichoptera. These aquatic insects are important for the growth and development of salmonids and a lack of aquatic insects can lead to increased mortality rates in juvenile salmonids (Dedual et al., 1995; Feltmate et al., 1989; Johansen et al., 2010; Shustov et al., 2012). The presence of salmonid carcasses is associated with increased biomass of Trichoptera (Minakawa et al., 2002). Which are, in turn, eaten by other salmonids, thereby facilitating the transfer of nutrients between generations of salmonids (Minakawa et al., 2002).

In this study, I compared the overall protein concentrations and long-chain fatty acid concentrations and ratios of selected aquatic and terrestrial insect species and compared them to each other and to commercial beef and fish samples.

Materials and Methods

Vertebrate Groups

Grain/soy-fed, grass-fed, and grass-finished ground beef were selected for testing as representing terrestrial meat sources. The grain/soy-fed beef was purchased from Mccaffrey's Food Market, 301 N Harrison St, Princeton, NJ 08540. The grass-fed beef was purchased from the Farm Store at Dairy Barn at Rutgers University, College Farm Rd, New Brunswick, NJ08901. The grass-finished beef was purchased from Beachtree Farms, 105 Crusher Rd, Hopewell, NJ 08525. Pacific sardines, European anchovies, and separate samples of wild-caught and farm-raised Atlantic salmon meat were purchased from Mccaffrey's Food Market 301 N Harrison St, Princeton, NJ 08540.

Insects Used

House crickets (*Acheta domesticus*) were purchased from PetSmart, 111 Nassau Park Blvd, Princeton, NJ in July, 2019. Mealworms (*Tenebrio molitor*) were purchased online from Kaytee, 521, Clay Street, Chilton, WI and black soldier fly larvae (*Hermetia illucens*) were purchased online from TradeKing, 2020 6th Street, Bay City, TX in June, 2019. *Reticulitermes flavipes* and *D. japanica* were collected from rotting logs at the Cook Campus Center, 59 Biel Rd, New Brunswick NJ in September, 2019. *D. carolina* were collected from the field around Passion Puddle, by Lipman Drive on Cook Campus, New Brunswick, NJ in September 2019.

Preparing Specimens

All specimens were stored and frozen at -75^{o}C. All specimens were completely dehydrated at 60^{o}C for nineteen and a half hours. Dehydrated insects were then ground into powders using a porcelain mortar and pestle. Dehydrated beef or fish meat were ground into powder using a coffee grinder.

BCA Protein Assay

Eight milligrams of powder were weighed out and lysed with a 5% SDS detergent solution in order to release the nutrients from the cells. The resulting slurry was centrifuged for 30 min at 13,450 rpm with a BC-16 Centrifuge. Protein was estimated with the Pierce method according to the manufacturer's instructions. Absorbance was measured using a Persee T6U uv/vis spectrometer set at 562nm. Protein concentration was estimated using a standard curve constructed with bovine serum albumin from the Pierce assay kit.

Lipid Extraction

Lipids were prepared for analysis by the method of Bligh and Dyer (Bligh et al., 1959). A sample of 30 mg of powder was weighed out and mixed with 2ml of 2:1 chloroform: methanol solution and 1ml of acidified saline solution (0.9% NaCl in 0.1M HCL). The mixture was centrifuged at 295xg for ten minutes, after which, the top methanol layer containing salts and carbohydrates and the middle layer containing cellular debris were discarded. The bottom chloroform layer containing the lipids was extracted using a Pasteur pipette and dehydrated with nitrogen gas. The concentrations of long-chain fatty acids were measured using GC-MS analysis.

GC-MS Analysis

The lipid fraction was dried with the addition of pentadecanoic acid as a relative reference standard. Fatty acids were then trans-methylated in the presence of 14% boron trifluoride in methanol. The resulting methyl esters were extracted with hexane and analyzed by gas-liquid chromatography. The analyses were performed in triplicates in an Agilent 7870A gas chromatograph coupled with an Agilent 5977 mass spectrometer (Agilent Technologies Santa Clara, CA) equipped with a DB-WAX capillary column (30m, 0.250mm diameter, 0.25uM film, J&W scientific, Folsom, CA, USA). GC-MS analyses were performed in triplicate on each sample. The samples were introduced via split injection with the port heated to 250ºC. Helium was used as the carrier gas with 1ml/min constant flow. Oven temperature was initially held at 50ºC for 1min, increased to 200ºC at a 25ºC/min rate, and then raised to 230ºC at 3ºC/min, where it was held for 35min. The mass spectrometer interface temperature was set to 230ºC and the mass spectral data were collected in full scan mode with a mass range of 50-500 m/z.27.

Statistical Analysis

The statistical significance of the mean protein and long-chain fatty acid concentration differences between species was calculated in SAS with analysis of variance (ANOVA). The statistical significance of protein and long-chain fatty acid concentration differences along with omega-6 to omega-3 fatty acid ratios and oleic acid to palmitic acid ratios of terrestrial and aquatic species were also measured with ANOVA.

Section 2: Terrestrial Insects and Factory Farming

Objective: To determine which species of terrestrial insects have high concentrations of protein and beneficial long-chain fatty acids.

Working Hypotheses:

- Termites eaten in Africa have very high protein concentrations, therefore the local species of termites, *Reticulitermes flavipes,* is likely to also be high in protein
- Camel crickets can jump much higher than the house cricket, *Acheta domesticus,* therefore, the Japanese camel cricket, *Diestrammena japanica* is likely to have a higher protein concentration than *A. domesticus* due to larger and stronger muscles
- Graminivore insects such as the Carolina grasshopper, *Dissosteira carolina* are likely to have high concentrations of alpha-linolenic acid and a balanced ratio of omega 6 to omega 3 fatty acids based on the comparison of beef raised on grass-only diets with beef raised on grain and soy diets
- The food intake of Grasshoppers is far higher relative to their total body mass compared to that of cows; therefore *D. carolina* will likely have a similar ratio of LA to ALA as grass-finished beef, but with significantly higher concentrations for both fatty acids

Abstract

Current sources of protein have become unsustainable economically, environmentally, and nutritionally. The factory farming practices employed for livestock and poultry in order to meet increasing demands for meat negatively impact the environment, contributing to global climate change, acid rain, and runoff among other issues. The factory farming paradigm also negatively affects the quality of the meat itself. The artificial and unnatural grain and soy livestock diets used to streamline the process results in omega-6 to omega-3 ratios higher than the maximum nutritionally safe ratio, 4:1. This contributes to obesity and other health issues. The large amounts of corn required for these diets means rising gasoline prices which leads to higher beef prices. Here, I compared the overall protein concentrations and long-chain fatty acid contents of six local, terrestrial insect species; *Acheta domesticus, Tenebrio molitor, Hermetia illucens, Reticulitermes flavipes, Dissosteira carolina* and *Diestrammena japanica,* and three ground beef samples; grain-fed, grass-fed, and grass-finished beef. *Diestrammena japanica* had twice the protein concentration of *A. domesticus* while *R. flavipes* had about three times more protein. The graminivorous *D. carolina*, was the only insect out of the seven species to have a significant concentration of the omega-3 fatty acid, alpha-linolenate (ALA), an essential fatty acid found in higher concentrations in grass than in flaxseed oil. *Dissosteira carolina* had the same near 1:1 O6-O3 ratio as grass-finished ground beef, but the two polyunsaturated fatty acids were present in far higher concentrations. The other six insect species all had O6-O3 ratios above 4:1. *D. carolina* was the only graminivore of the seven. *Reticulitermes flavipes* had the highest ratio of

O6-O3, but also a 3:1 ratio of oleic acid to palmitic acid. The data obtained support the working hypotheses.

Introduction

Rapidly rising beef prices illustrate the urgent need to find more cost-effective protein sources. According to the USDA, beef prices have climbed 53% from 2006 to 2016 (Barret, 2015; Westcott et al., 2016). One of the factors contributing to the increase in feed costs is the overreliance on "cheap" ingredients such as corn, wheat, and soybeans to increase productivity without having to spend large amounts of money on grazing lands. However, the expansion of the fuel industry has also increased demand for corn, wheat, and soybeans, among other plants with significant concentrations of precursors for ethanol and also used in livestock feeds, thereby increasing the prices of these plants and therefore defeating the main purpose of using them for feed instead of grasses to save money (Hofstrand, 2009). The rising feed costs have made consumption of livestock products increasingly expensive to those with limited financial resources to consistently consume these products. Grass-fed livestock products are even more prohibitive and are relegated to a smaller consumer population as switching from conventional beef to organic grass-fed beef, can almost double the price (Consumer Report, 2015).

There is no significant difference in protein quality or concentration between livestock reared on grass-based or grain-based diets (Elswyck et al., 2014). However, the two different diets do alter the fatty acid content in livestock (Elswyck et al., 2014). Some factory farms add extenders and fillers to meat products, which can include flour, starch and soy (Pearson, 1976). Soybean oil is currently one of the biggest sources of the omega-6, linoleic acid (LA) in the US and soybeans have a higher concentration of protein than beef (Blasbalg et al., 2011).

The lack of grass in the grain and soy diet of cows leads to higher omega-6 to omega-3 ratios, as the cows are not receiving the high concentrations of the omega-3, α-linolenic acid (ALA) found in grasses (Elswyck et al., 2014). The grain and soy based diet also causes sub-acute acidosis, a potentially fatal condition for cattle that results from them being fed starchy grains and soy instead of properly grazing on fibrous grasses (Abdela, 2016). The term "grass-fed beef" means that grass is only part of the cow's diet or the animal was started on grass before switching to an all grain diet (Elswyck et al., 2014). ALA makes up approximately 55% of the total fatty acid content of flaxseed, which is considered one of the richest sources of ALA in the human diet, (Rodriguez et al., 2010; McCullough et al., 2011). In comparison, ALA accounts for approximately 62% of the total fatty acid content of grass on average (Clapham et al., 2005).

The biggest source of water pollution in the US is nitrogen and phosphorous runoff from fertilizer and animal waste from factory farms. Factory farm runoff creates algal blooms which deplete the water of dissolved oxygen, creating dead zones in which all but the most tolerant aquatic organisms (such as leeches and mosquito larvae) die. Excrement from livestock that seeps into groundwater through wet soil during storms contains nitrates and antibiotic-resistant pathogens which may contaminate drinking water. Ingesting nitrate-contaminated drinking water can lead to blue baby syndrome which causes infant deaths and abortions (Steinfeld et al., 2006). Throughout the U.S., livestock excrement from factory farms has contaminated groundwater in 17 states and polluted 35,000 miles of rivers in 22 states. According to the EPA, a single cow can produce 120 pounds of wet manure per day, which is equal to the average waste produced by 20-40 people. In total, this is approximately 130 times the amount of human excrement produced annually by the entire human population (Adkins et al., 2019).

Livestock excrement from factory farms is not processed the same way as human waste (Steinfeld et al., 2006).Therefore, it ends up being 500 times more concentrated and contains antibiotic resistant strains of pathogens such as the gram-negative bacteria, *Salmonella* (Lignières), *Escherichia coli* (Migula) and *Campylobacter* (Sebald & Véron) (Steinfeld et al., 2006). The overuse of antibiotics in cattle threatens public health by facilitating the emergence of hyper antibiotic-resistant strains of bacteria such as *Salmonella* and *Campylobacter*, one of the major causes of foodborne bacterial illness in the United States (Acar et al., 2003). Outbreaks of super-resistant strains of pathogens have been severe enough in the past to warrant the attention of the Centers for Disease Control (CDC, 2011). One such outbreak in 2011 of a strain of *Salmonella* Lignières resistant to four different antibiotics used in animal farming was linked to 136 cases of illness and one death (CDC, 2011). There is evidence that employees of these farms are 32 times more likely to carry hyper-resistant strains of *E. coli* (Price et al., 2007). These resistant bacteria can easily be spread through a variety of routes including airborne, delivery vehicles, and, especially, runoff (Chee-Sanford et al., 2003; Chapin et al., 2005; Neyra et al., 2014). Bacteria possessing antibiotic-resistant genes are 10,000 times more common downriver from feedlots than upstream from these same feedlots (Pruden et al., 2012).

This hyper-resistance spreads rapidly throughout populations of bacteria because of their ability to exchange DNA in the process of conjugation. The conjugative transfer of bacterial pathogens is one of the main factors in the increasing numbers of bacteria demonstrating resistance to multiple antibiotics. Bacterial conjugation involves the transfer of DNA between a donor and recipient bacterium by way of fusing their membranes and multi-protein complexes with components from both bacteria (Grohmann et al., 2003).

Factory farms also cause severe air pollution due to the various gases released by livestock excrement. The particulate matter and bacterial toxins released by livestock waste can cause respiratory and cardiac disorders (Brigham and Meyrick., 1986). The factory farms all have anaerobic lagoons, also known as waste slurry lagoons, where the animal excrement is dumped. The toxic ammonia fumes released by the excrement combine with nitrous oxide from fertilizers to form nitric acid. The resulting acid rain leeches nutrients from the soil, despoils forest habitats, and releases toxic waste minerals into aquatic ecosystems (Steinfeld et al., 2006). Nitrous oxide is also 300 times more effective at trapping heat in the atmosphere than carbon dioxide (Pachauri et al., 2007).

According to the Food and Agriculture Organization of the United Nations (FAO), livestock is responsible for 9% of human-induced emissions of carbon dioxide, 37% of emissions of methane, which has more than 20 times the global warming potential (GWP) of CO_2 and 65% of emissions of nitrous oxide (N_2O), which has nearly 300 times the GWP of CO_2. The corn and other high-energy feeds utilized by factory farms rely on large amounts of chemical fertilizer. The production of these fertilizers releases 41 million tons of CO_2 annually. Factory farms require large amounts of heating, cooling and ventilation, which rely heavily on fossil fuels. The amounts required release over 90 million tons of CO_2 annually. Livestock slaughter, meat packaging, and transport release several million tons of CO_2 annually. The clearing of forests for the growth of feed crops and for grazing releases 2.4 billion tons of CO_2 annually. Cultivating land for feed crops releases 28 million tons of CO_2 globally. The overgrazing of pastures can induce desertification, causing the release of 100 million tons of CO_2 annually (Steinfeld et al., 2006). Even more disturbing, emissions of methane, a gas twenty times more potent than $CO_{2,}$ from livestock manure have increased by 58.7 percent from 1990 to 2018 (Bailey et al., 2014).

In this study, I compared the overall protein concentration and long-chain fatty acid content of the three ground beef samples; grain-fed, grass-fed, and grass-finished, with *A. domesticus, T. mollitor,* and *H. illucens* and with three other local species: *D. carolina, R. flavipes,* and *D. japanica*

Results

Protein Concentrations in Terrestrial Specimens Estimated by the Pierce's BCA Assay with Bovine Serum Albumin as Reference Standard

The grain-fed ground beef had the highest protein concentration of the ground beef samples (F = 259.9; df = 2; $P < 0.0001$) (Table 1). *Acheta domesticus* had a higher protein concentration than grain/soy-fed ground beef (F = 32.67; df = 1; $P < 0.01$) (Table 1). *Diestrammena japanica* had more than twice the protein concentration as *A. domesticus* (F = 1812.44; df = 1; $P < 0.0001$) (Table 1). *Reticulitermes flavipes* had the highest protein concentration of all the terrestrial specimens (F = 424.11; df = 1; $P < 0.0001$) (Table 1). *Tenebrio molitor* and *H. illucens* had lower protein concentrations than the beef samples (F=229.14; df = 2; $P < 0.0001$) (Table 1). *Dissosteira carolina* had a higher protein concentration than grass-fed and grass-finished ground beef (F = 276.62; df = 2; $P < 0.0001$), but lower than grain-fed ground beef (F = 7.94; df = 1; $P < 0.01$) (Table1).

Table 1. Protein Concentrations of Terrestrial Species, Dry Weight, N=3.

Specimen	Life Stage/Caste	Mean Concentration (µg/mg) ± SD
Grain/Soy-Fed Beef	N/A	1.06 ± 0.02d
Grass-Fed Beef	N/A	0.60 ± 0.01e
Grass-Finished Beef	N/A	0.60 ± 0.01e
Acheta domesticus	Adults	1.2 ± 0.014c
Diestrammena japanica	Adults	2.70 ± 0.05b
Dissosteira carolina	Adults	1.00 ± 0.05d
Reticulitermes flavipes	Workers	3.33 ± 0.01a
Tenebrio molitor	Larvae	0.36 ± 0.01f
Hermetia illucens	Larvae	0.22 ± 0.05g

Protein concentrations were measured as described with dehydrated tissue samples against bovine serum albumin standards. All beef samples were ground beef. Means with the same letters are not statistically significant ($P \geq 0.05$). Means with different letters are statistically significant ($P < 0.05$).

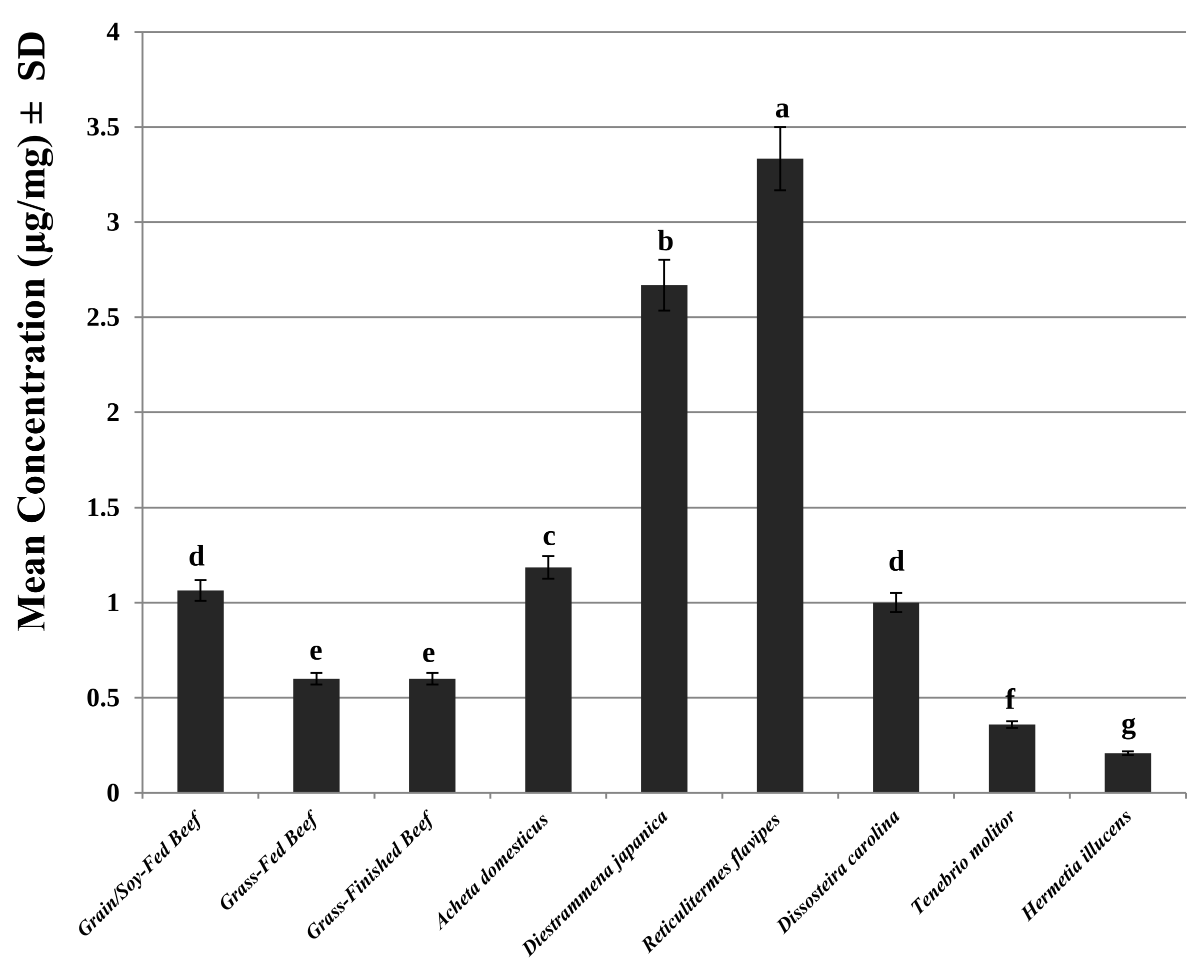

Figure 1. Protein Concentrations of Terrestrial Species, Dry Weight, N=3. Means with the same letters are not statistically significant ($P \geq 0.05$). Means with different letters are statistically significant ($P < 0.05$).

Long-Chain Fatty Acid Analysis in Terrestrial Specimens

Grain-fed ground beef had a 49:1 ratio of linoleic acid (LA), and α -linolenic acid (ALA) ($F = 318.62$; df = 1; $P < 0.0001$) (Table 2). Grass-fed ground beef had an 8:1 LA/ALA ratio ($F = 238.72$; df = 1; $P < 0.0001$) while grass-finished ground beef had a 1:1.4 LA/ALA ratio ($F = 17.76$; df = 1; $P < 0.05$) (Table 2). *Dissosteira carolina* had the same omega-6 to omega-3, LA/ALA ratio as grass-finished ground beef ($F = 16.92$; df=1; $P < 0.05$), but with concentrations almost forty times higher for both linoleic acid ($F = 292.08$; df = 1; $P < 0.0001$) and α-linolenic acid ($F = 310.55$; df = 1; $P < 0.0001$) (Table 2). *Dissosteira carolina* had a 2:1 ratio of oleic to palmitic acid ($F = 54.41$; df = 1; $P < 0.01$). *Reticulitermes flavipes* had the highest ratio of omega-6 to omega-3 at 144:1 ($F = 300.01$; df = 1; $P < 0.0001$), but also the highest ratio of oleic acid to palmitic acid at 3.4:1 ($F = 141.65$; df = 1; $P < 0.001$) (Table 2). All of the terrestrial insect species tested except *D. carolina* had omega-6 to omega-3 ratios higher than 4:1 (Table 2). *Hermetia illucens* had the highest concentrations of palmitoleic acid ($F = 10.28$; df = 1; $P < 0.05$) and lauric acid ($F = 229.28$; df = 1; $P < 0.0001$).

Table 2. Long-Chain Fatty Acid Concentrations of Terrestrial Species, Dry Weight, N=3

Long-Chain Fatty Acid Mean Concentrations (µg/g) ± SD / Specimen	Linoleate	α -Linolenate	Omega-6 to Omega-3 Ratio	Oleate	Palmitate	Oleate to Palmitate Ratio	Palmitoleate	Dodecanoate	Total Long-Chain Fatty Acids
Grain/Soy-fed Beef	1,473 ± 114f	29.9 ± 2.4g	49:1****	15,706 ± 1,224c	11,517 ± 898c	1.36:1*	2,011 ± 40.8	1,572 ± 123h	52, 262 ± 4,082c
Grass-Fed Beef	3,483 ± 278e	427 ± 34.3d	8:1****	37,710 ± 2,449a	27,126 ± 2,205a	1.4:1*	4,524 ± 367b	4,447 ± 327e	113,666 ± 8,165b
Grass-Finished Beef	189 ± 15.4g	269 ± 21.9e	1.4:1*	15,879 ± 1,225c	9,684 ± 784d	1.6:1**	2,592 ± 204c	10,777 ± 817c	40,730 ± 3,450e
Acheta domesticus	29,236 ± 1,632b	991 ± 73.5c	30:1****	17,465 ± 1,388c	19,463 ± 1,551b	1:1.1 N.S.	1,547 ± 123d	1,319 ± 106i	82,020 ± 6,532d
Diestrammena japanica	1,294 ± 93.9f	62.4 ± 4.9f	21:1****	1,283 ± 98e	1,372 ± 106g	1:1.1 N.S.	137 ± 24.5f	1,816 ± 147g	7,901 ± 645f
Dissosteira carolina	7,493 ± 604d	10,447 ± 984a	1.4:1*	11,150 ± 910d	5,946 ± 408f	2:1**	1,133 ± 90e	2,802 ± 229f	43,391 ± 3,266e
Reticulitermes flavipes	8,459 ± 686d	58.7 ± 4.1f	144:1****	26,591 ± 2,123b	7,796 ± 572e	3.4:1***	1,785 ± 139d	9,708 ± 480d	63,294 ± 4,903c
Tenebrio mollitor	35,754 ± 2,858a	1,427 ± 114b	25:1****	26,798 ± 2,122b	17,623 ± 1,388b	1.5:1**	2,413 ± 196c	15,842 ± 1,224b	108,610 ± 8,165b
Hermetia illucens	16,207 ± 1,306c	1,248 ± 98b	13:1****	28,027 ± 2,287b	18,868 ± 1,470b	1.5:1**	5,267 ± 408a	615,310 ± 49,808a	706,684 ± 30,243a

Mean fatty acid concentrations in the same column with the same letters are not statistically significant ($P \geq 0.05$). Means in the same column with different letters are statistically significant ($P < 0.05$). For fatty acid ratios in the same column; N.S. = $P \geq 0.05$ (*) = $P < 0.05$, (**) = $P < 0.01$, (***) = $P < 0.001$. (****) = $P < 0.0001$.

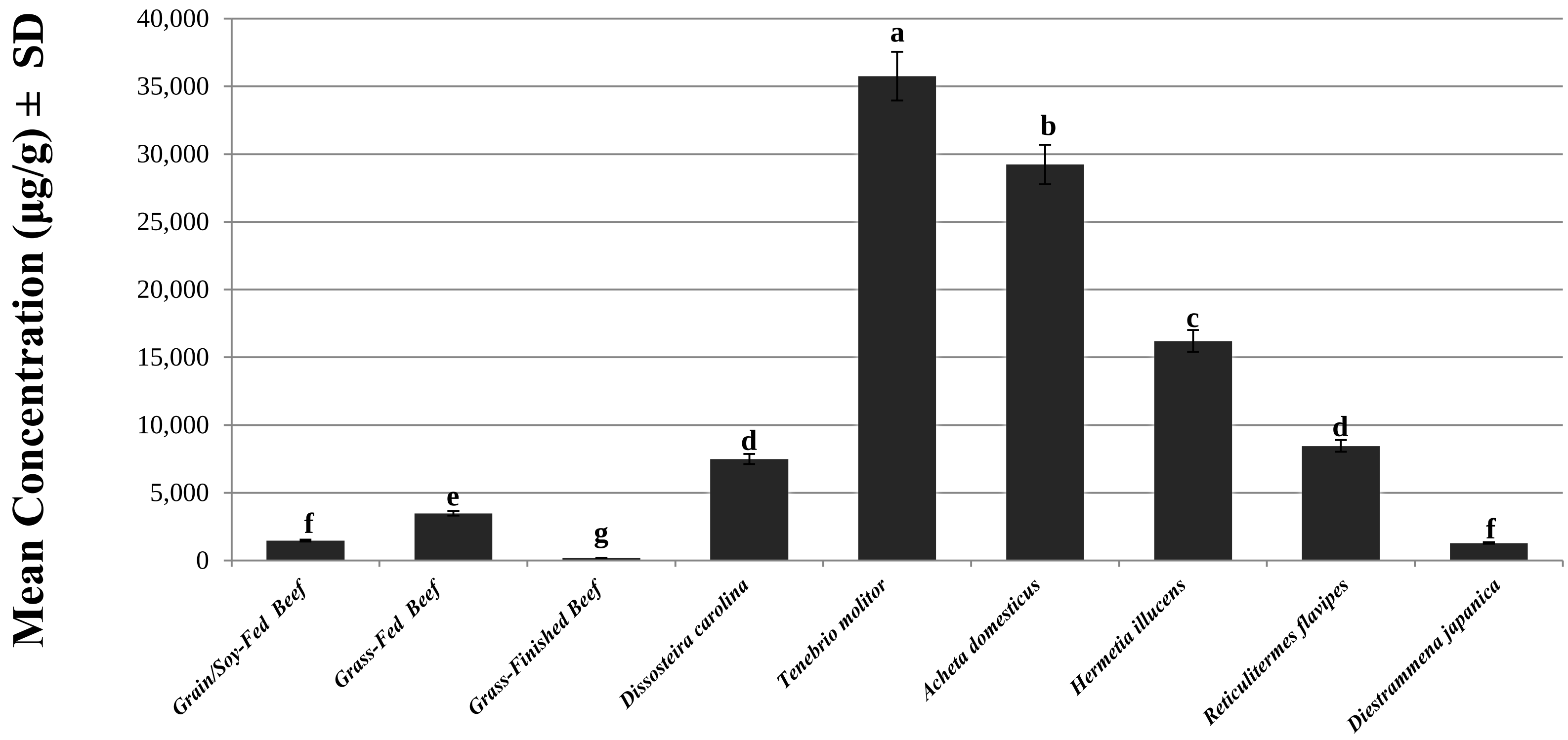

Figure 2. Linoleic Acid Concentrations of Terrestrial Species, Dry Weight, N=3. Means with the same letters are not statistically significant ($P \geq 0.05$). Means with different letters are statistically significant ($P < 0.05$).

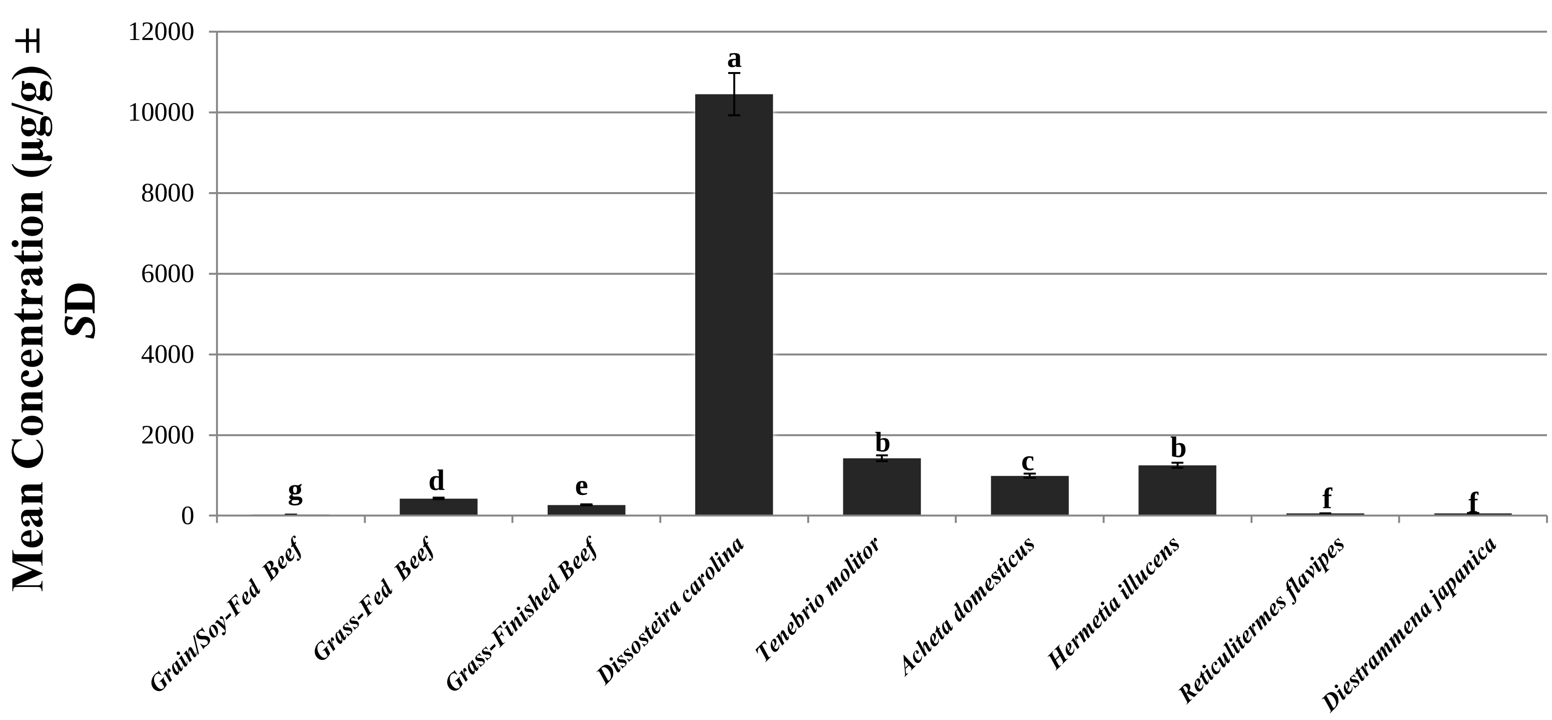

Figure 3. α-Linolenic Acid Concentrations of Terrestrial Species, Dry Weight, N=3. Means with the same letters are not statistically significant ($P \geq 0.05$). Means with different letters are statistically significant ($P < 0.05$).

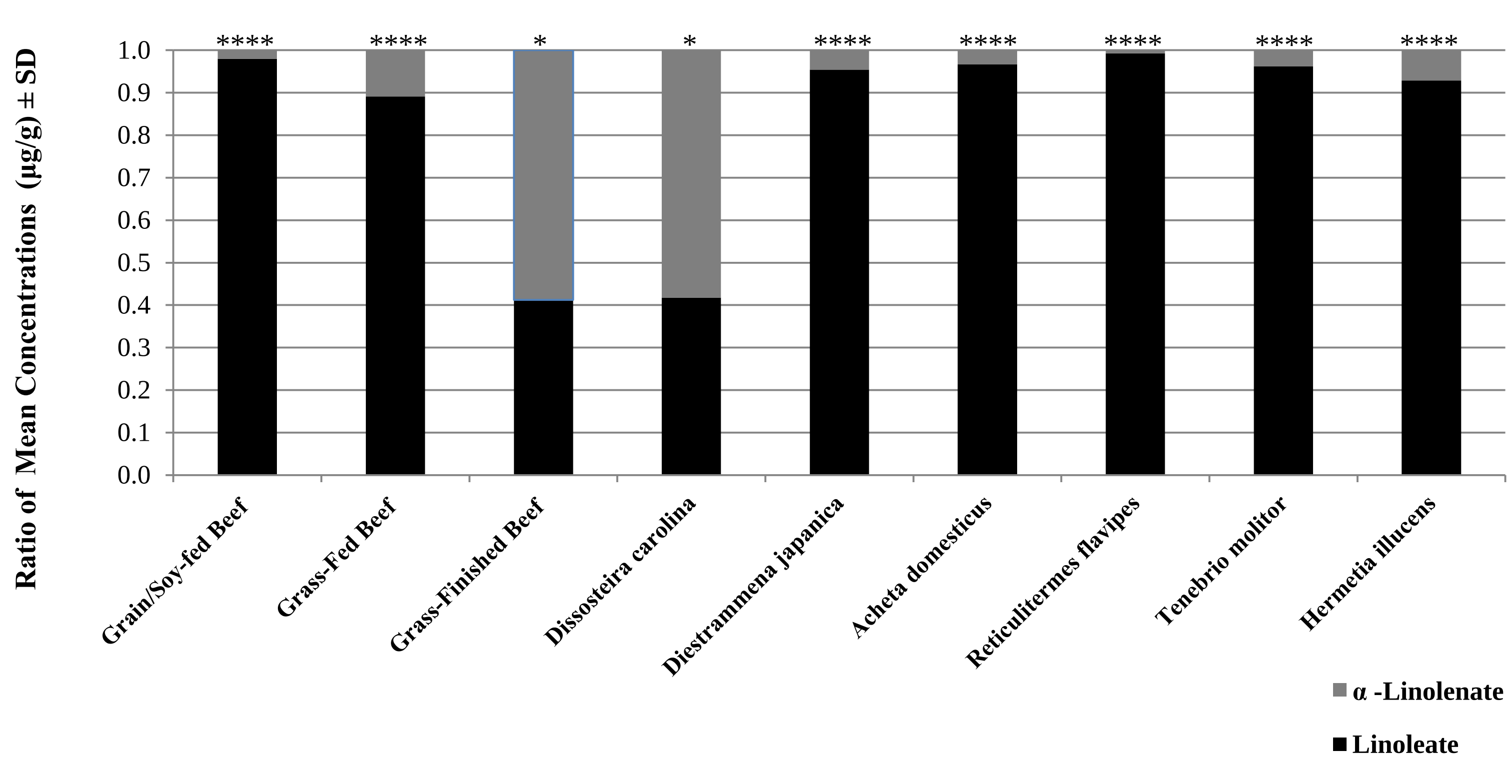

Figure 4. Omega-6 to Omega-3 Ratios in Terrestrial Species, Dry Weight, N=3. (*) = $P < 0.05$, (****) = $P < 0.0001$.

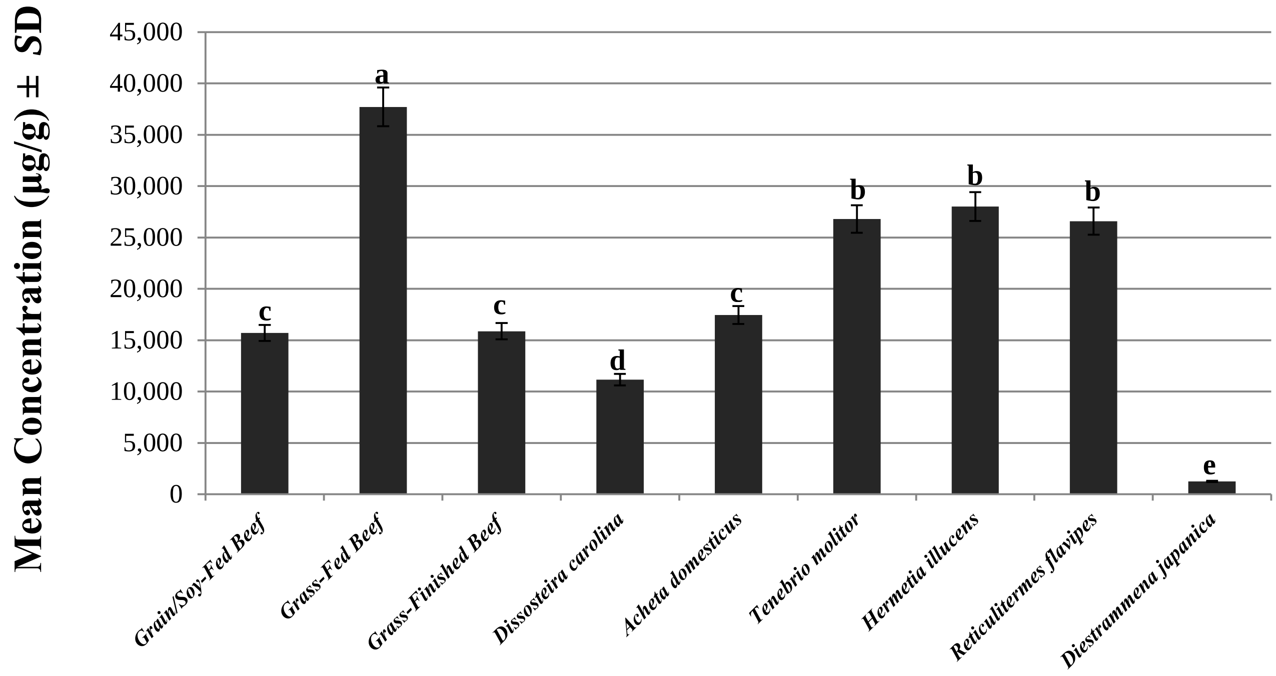

Figure 5. Oleic Acid Concentrations of Terrestrial Species, Dry Weight, N=3. N.S. Means with the same letters are not statistically significant ($P \geq 0.05$). Means with different letters are statistically significant ($P < 0.05$).

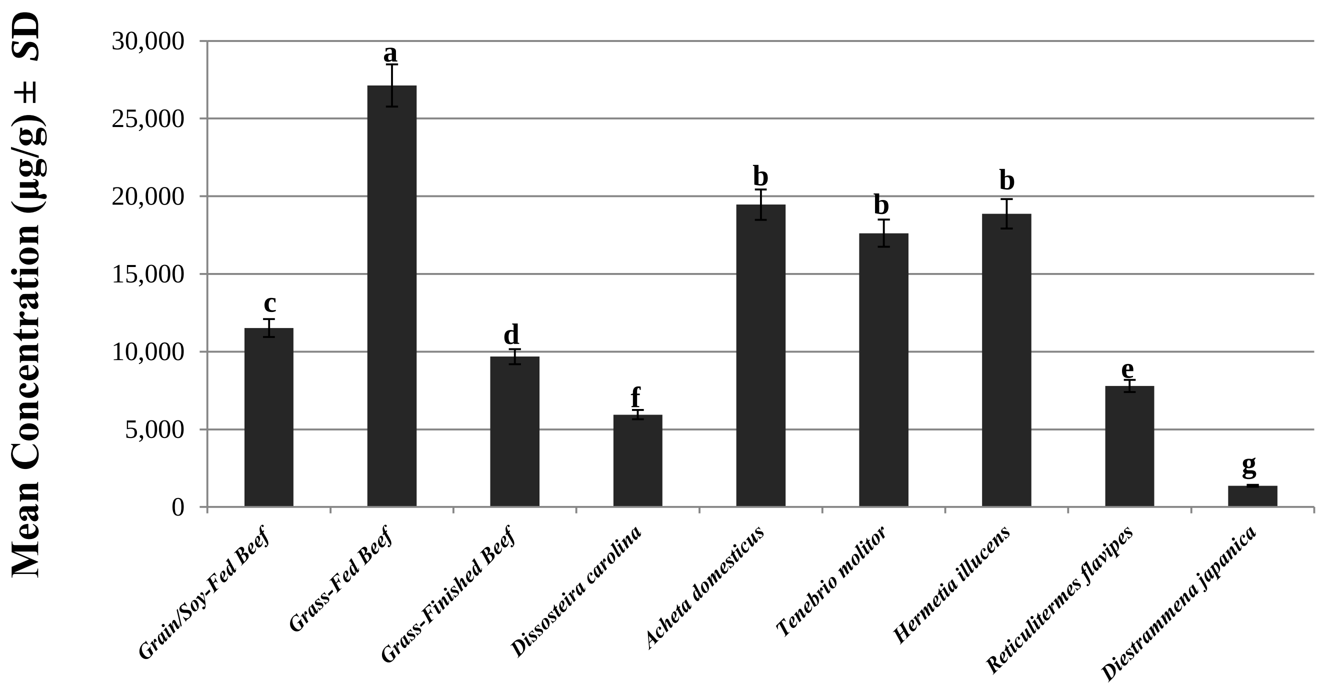

Figure 6. Palmitic Acid Concentrations of Terrestrial Species, Dry Weight, N=3. N.S. Means with the same letters are not statistically significant ($P \geq 0.05$). Means with different letters are statistically significant ($P < 0.05$).

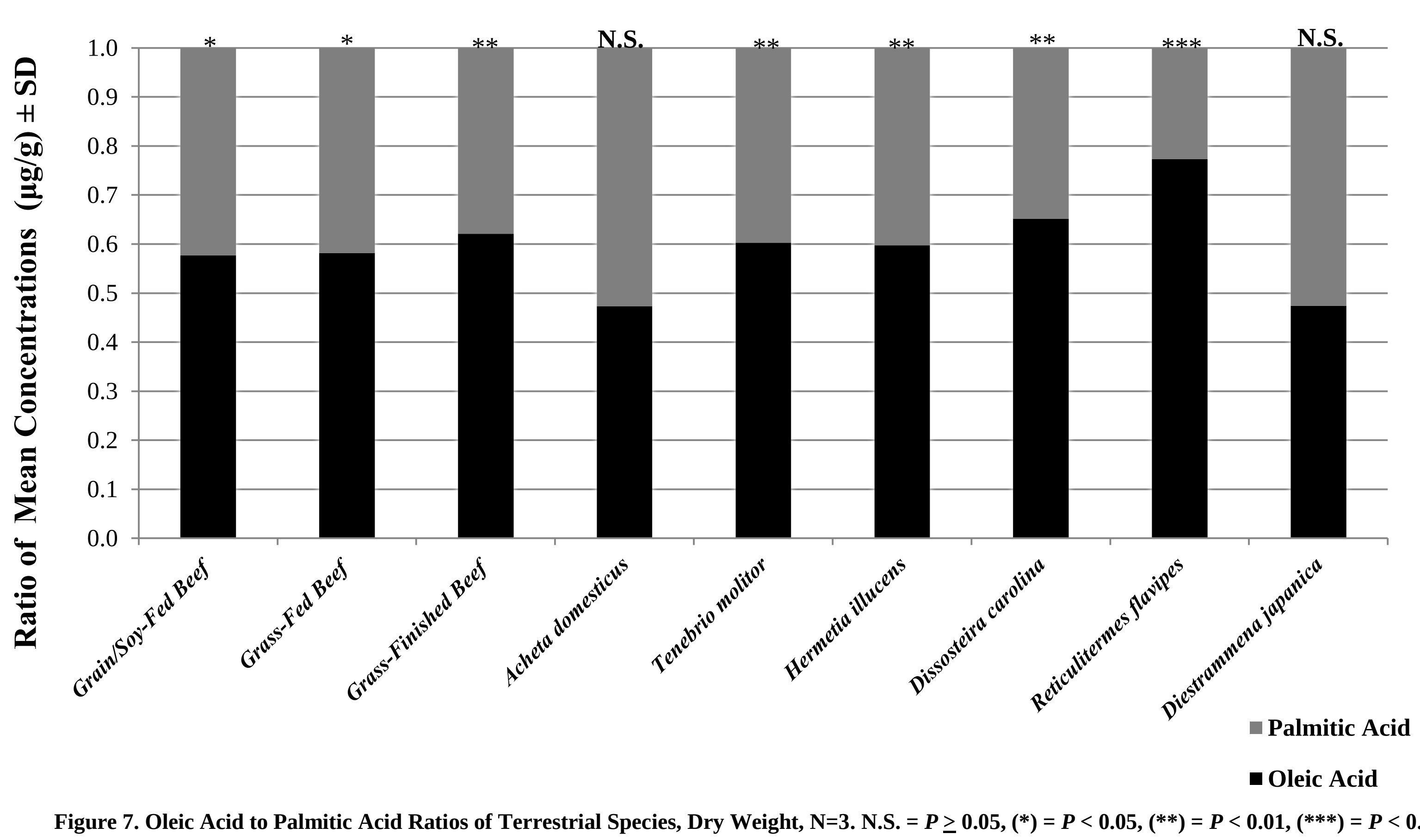

Figure 7. Oleic Acid to Palmitic Acid Ratios of Terrestrial Species, Dry Weight, N=3. N.S. = $P \geq 0.05$, (*) = $P < 0.05$, (**) = $P < 0.01$, (***) = $P < 0.001$.

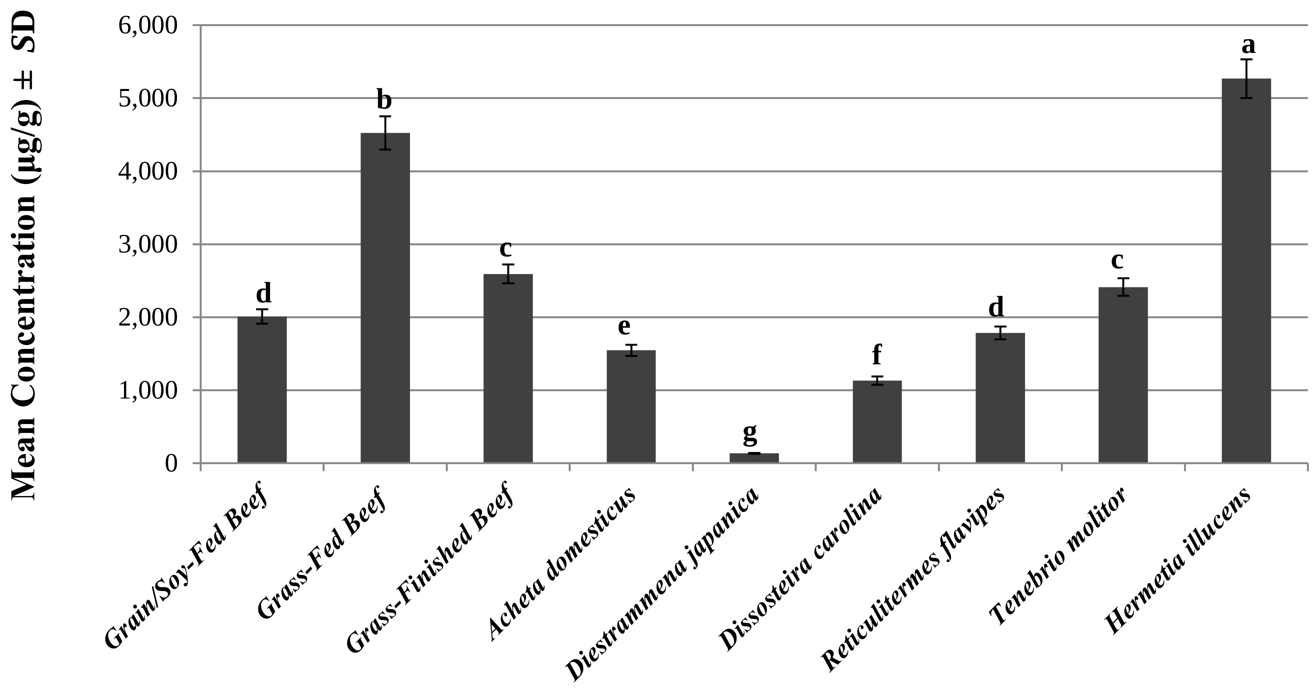

Figure 8. Palmitoleic Acid Concentrations of Terrestrial Species, Dry Weight, N=3. Means with the same letters are not statistically significant ($P \geq 0.05$). Means with different letters are statistically significant ($P < 0.05$).

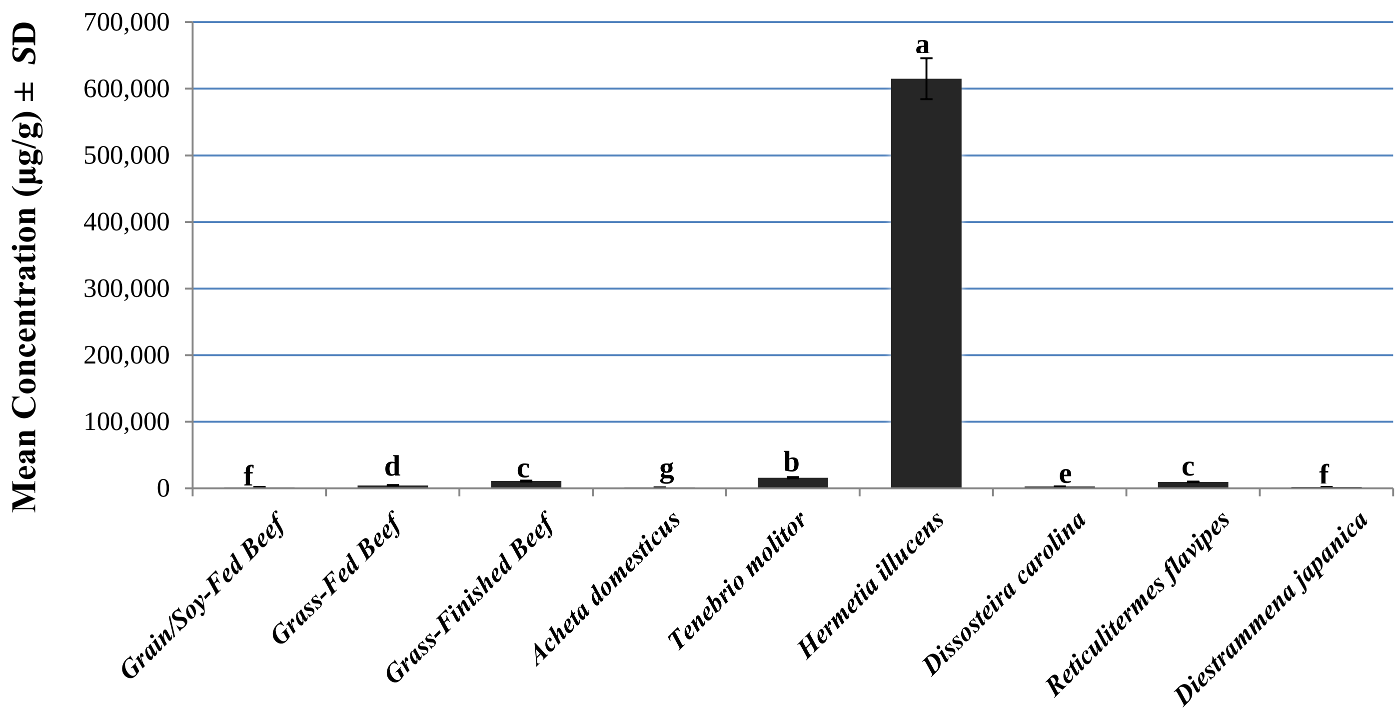

Figure 9. Lauric Acid Concentrations of Terrestrial Species, Dry Weight, N=3. Means with the same letters are not statistically significant ($P \geq 0.05$). Means with different letters are statistically significant ($P < 0.05$).

Discussion

The protein and LCFA data from the three beef samples in Tables 1 and 2 and Figures 1 and 2 is consistent with existing research. The grass-fed and grass-finished beef samples both had around the same concentrations of protein despite the differences in LCFA content. This is consistent with existing research that demonstrates that livestock diets do not significantly impact the protein concentration of the meat (Elswyck et al., 2013). However, the grain-fed beef did have a higher concentration of protein than the other two beef samples and was comparable to that of *A. domesticus* (Table 1). Unfortunately, the grain-fed beef also had a 49:1 omega-6 to omega-3 ratio (Table 2). To reiterate, any omega-6 to omega-3 ratio over 4:1 is a risk factor for obesity and cardiovascular disease (Simopolous., 2016; Taha., 2020). According to the USDA, soybeans have a higher concentration of protein than beef and as stated above, soy is also one of the richest sources of LA in the US and factory farms tend to add processed soybeans to ground beef as filler (Heinz et al., 2007; Blasbalg et al., 2011). This could explain both the higher concentration of protein and the 49:1 omega-6 to omega-3 ratio of the grain-fed beef compared to the grass-fed and grass-finished beef. Other studies have also observed that processed meat has comparable total concentrations of protein to *A. domesticus* (Payne et al., 2016). As mentioned, the term, “grass-fed beef” either indicates that grass is only part of the cow’s diet or that the animal was started on grass before being switched to an all grain diet (Elswyck et al., 2013). This is consistent with the “grass-fed” ground beef sample having an 8:1 ratio of omega-6 to omega-3, which is in between the omega-6 to omega-3 ratios of grain-fed and grass-finished beef (Table 2). *Acheta domesticus* had over3 times more protein than *T. mollitor and H. illucens* (Table 1). In other studies the protein concentrations of the three species have been closer together (Finke., 2002; Finke., 2012). However, *T. mollitor and H. illucens* are both scavengers which colonize a variety or organic materials so there nutrient content will likely vary with different diets (Zheng et al., 2013). *Diestrammena japanica* had more than twice the protein concentration of *A. domesticus* (Table 1). This could be attributed to their powerful muscles which allow them to jump over 60 times their body length (Palmer et al., 2017).

All of the tested terrestrial insects had omega-6 to omega-3 ratios above 4:1 with the exception of *D. carolina* (Table 2). Other studies have also found terrestrial insects including *A. domesticus, T. mollitor,* and *H. illucens* all had O6-O3 ratios above 4:1 (Finke., 2002; Finke., 2012) The Carolina grasshopper had the same nearly 1:1 ratio of linoleic acid to ALA acid as the grass-finished beef did, but the concentrations of the two fatty acids were approximately forty times higher in the grasshopper (Table 2). This is consistent with other studies which have observed ratios of O6-O3 in other species of grasshopper less than 4:1 (Womeni et al., 2009; Torruco-Uco et al., 2018). While grasshoppers and cows are both graminivores, cows eat approximately 1.5-2% of their total body mass per day, while grasshoppers eat approximately 25-50% of their total body mass per day, and thus have far higher concentrations of the nutrients from grass relative to their body mass than cows (Royer., 2018).

Hermetia illucens larvae had the highest concentration of lauric acid, (DA) by far (Table 2), which is consistent with other research (Finke., 2012; Gasco et al., 2018; Ewald., 2020). Lauric acid has anti-bacterial and anti-viral properties, and is particularly effective against gram-positive bacterial pathogens, such as *Streptococcus*, which causes strep throat and *Clostridium perfringens,* one of the leading causes of food poisoning in the USA (Spranghers et al., 2017; Gasco et al., 2018).

Despite a very poor 143:1 LA to ALA ratio (Figure 3), *R. flavipes* had the highest concentration of protein of all insects tested by a wide margin (Table 1), and has the highest ratio of oleic acid to palmitic acid at over 3:1 (Table 2). Oleic acid serves as important chemical signal which triggers undertaking behavior in eusocial insects such as ants, bees, wasps and termites (Sun et al., 2013). Other studies analyzing African species of termites have demonstrated that these species of termites have the same or higher concentrations of protein than *A. domesticus* (Finke., 2012; Kinyuru et al., 2013). OA builds up in termite corpses during the first 48 hours after death which signals the termites to cannibalize the corpses for the purpose of nutrient recycling (Sun et al., 2013). African termite species also have high concentrations of OA (Kinyuru et al., 2013). This could possibly explain why *R. flavipes* has such a high OA/PA ratio compared to the other insects.

Section 3: Aquatic Insects vs. Aquaculture and the Reduction Industry

Objective: To determine which species of aquatic insects have the highest concentrations of protein and beneficial long-chain fatty acids.

Working Hypotheses:

- Aquatic Insects frequently consumed by fish are likely to have higher concentrations of protein than the fish consuming them.
- Aquatic Insects frequently consumed by oily fish are likely to have high concentrations of beneficial long-chain fatty acids.
- Aquatic insects are likely to have balanced ratios of omega-6 and omega-3 fatty acids compared to non-graminivorous terrestrial insects.

Abstract

Current fish-based sources of protein and omega-3 fatty acids for human consumption are becoming unsustainable. The fish farming methods used to meet the increasing demand for fish protein have led to severe environmental issues as well as reductions in the nutritional value of the fish meat. The demand for omega-3 fatty acid supplements, a billion dollar industry, has led to over-fishing of populations of oily fish for the purpose of extracting the omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), while discarding the rest of the body, including other beneficial fatty acids. Here I compared the concentrations of protein and long-chain fatty acids of the following aquatic insect species; the common stonefly, *Acroneuria abnormis* (Newman,), *Rhyacophila carolina* (Banks), a free-living caddisfly, *Hydropsyche betteni* (Ross), a net-spinning caddisfly, and *Brachycentrus numerous* (Say), a case-maker caddisfly, to four commercial fish groups; farm-raised and wild-caught Atlantic salmon, *Salmo salar* and wild-caught European anchovy, *Engraulis encrasicolus* (L.) and Pacific sardine, *Sardinops sagax* (Jenyns), as sources of beneficial LCFAs. All four aquatic insect species also had higher concentrations of protein than all of the commercial fish

control groups and are all frequent prey of trout, an oily fish, which, so far, supports the first working hypothesis. The farm-raised *S. salar* had a 3:1 O6-O3 ratio and higher concentrations of LA and ALA compared to the 1:10 O6-O3 ratio of wild-caught *S. salar*. However, the farm-raised *S. salar* also had 1,000 times less EPA and around a twenty-three percent less protein compared to the wild-caught *S. salar*. This is likely the result of the aquaculture diet and crowded rearing conditions in small enclosures. *Rhyacophila carolina* and *A. abnormis* demonstrated higher concentrations of EPA than those of all four of the fish groups. *Hydropsyche betteni* and *B. numerous* demonstrated lower concentrations of EPA than that of *S. sagax,* but demonstrated comparable concentrations to the wild-caught *S. salar* and *E. encrasicolus.* However, *H. betteni* and *B. numerous* also had high concentrations of LA and ALA without sacrificing EPA, unlike the farm-raised *S. salar.* Therefore, the results support the second working hypothesis, so far. All four aquatic insects also had balanced ratios of omega-6 to omega-3 fatty acids while only *D. carolina* had a balanced O6-O3 ratio thus supporting the third working hypothesis, thus far.

Introduction

The environmental issues caused by fish farming practices are nearly identical to those caused by factory farms as discussed in section one, especially in cases where fish farms use livestock factory farm waste as fertilizer in fish culture ponds (Okocha et al., 2018). These issues include fish parasites, hyper-antibiotic resistant strains of pathogenic bacteria, and heavy metals (Aly, 2014; Okocha et al., 2018). High concentrations of zinc and copper have been detected in the waste produced by salmon farms (Yeats et al., 2005). The eggs and immature stages of salmonid fish such as *Salmo salar* are vulnerable to harmful pathogens due to their immature immune systems and therefore, are constantly treated with antibiotics (Nges et al., 2012). Antibiotics are usually administered through the feed, resulting in large amounts of antibiotics leaching into the environment through feces and uneaten feed (Nges et al., 2012; Okocha et al., 2018). Increased numbers of hyper-resistant pathogenic bacteria have been found in bodies of water near fish farms (Nges et al., 2012). These pathogens include the human pathogens; *Vibrio cholera* (Pacini & Koch), *Vibrio parahaemolyticusi* (Sakazaki et al), *Vibrio vulnificus* (Farmer), *Shigella* spp (Castellani & Chalmers), *Salmonella* spp (Lignières), and the opportunistic pathogens; *Aeromonas hydrophila* (Stanier), *Plesiomonas shigelloides* (Habs and Schubert), *Edwardsiella tarda* (Ewing et al), *Streptococcus iniae* (Pier), *Piscirickettsia salmonis* (Fryer et al), *Escherichia coli* (Migula) and *Tetracapsuloides bryosalmonae* (Canning et al) (Fryer & Hedrick, 2003; Aly et al., 2014; Mo et al., 2017; Miranda et al., 2018). Antimicrobial resistance genes have been detected in sediment bacteria world-wide (Han et al., 2017). *Vibrio* (Pacini) is a genus of gram-negative anaerobic, bacteria, which can cause gastroenteritis, cholera, and septicemia in humans (Logue et al., 2017). Foodborne *Vibrio* bacteria are responsible for over 50,000 deaths annually in the US with *V. parahaemolyticus* in particular responsible for approximately 65% of those deaths (Logue et al., 2017). *V. vulnificus* is the leading cause of seafood-related deaths in the US, causing over 95% of them (Elmahdi et al., 2017; Heng et al., 2017). *Streptococcus iniae*, is a gram-positive anaerobic bacterium which can cause kidney inflammation in immunocompromised individuals. *Plesiomonas shigelloides* and *Aeromonas hydrophila* are gram-negative anaerobic bacteria which can cause gastroenteritis and, in rare cases, necrosis in humans with weakened immune systems. *Aeromonas hydrophila* strains detected in meat products often demonstrate antibiotic resistance (Logue et al., 2017). *Edwardsiella tarda* is a gram-negative intracellular bacterium that has been recognized as one of the most dangerous pathogens of aquaculture, with outbreaks causing severe economic damage since 1962. *Edwardsiella tarda* causes hernia, lesions of internal organs, and abnormal buildup of fluid in the abdomens of many species of fish and can cause diarrhea in humans (Xu et al., 2017). *Tetracapsuloides bryosalmonae* is a malacosporean parasite which causes swelling of the kidneys and spleen along with anemia in salmonid fish including *S. salar* (Skovgaard et al., 2012; Mo et al., 2017). *Tetracapsuloides bryosalmonae* is detected more often in farmed *S. salar* than in wild-caught *S. salar* (Mo et al., 2017). *Shigella* is a genus of gram-negative, anaerobic bacteria that cause abdominal pain, fever, and bloody diarrhea. *Shigella* is responsible for approximately 130,000 deaths annually in the US and 1.1 million deaths globally. *Shigella* is also found in beef and chicken products and the mode of transmission is fecal to oral from water sources (Logue et al., 2017). Chile produces one third of total global farmed salmon which have one of the highest antibiotics to mass concentrations (Miranda et al., 2018). This has been attributed to the high mortality rates in the marine phase caused by the aerobic, gram-negative intracellular pathogen, *P. salmonis* (Miranda et al., 2018). *Piscirickettsia salmonis* causes anemia, lesions, ulcers, and swollen kidneys and spleen in salmonid fish and mortality rates can range from 20-90% (Fryer et al., 2003). There is evidence that the Chilean salmon farms had been falsely reporting the amounts of antibiotics used to combat *P. salmonis* until 2016 and illegally using quinolones, a class of antibiotics only approved for human medical use. Resistance genes for quinolones have been detected in urinary *E. coli* from people living in close proximity to Chilean aquaculture sites (Miranda et al., 2018).

Aquaculture actually contributes to overfishing as 49% of all trawler catches consist of so-called trash fish used to make fishmeal to feed the fish-eating farmed fish. Trash fish are defined as fish not fit for human consumption because they are completely inedible, not fully grown, or not economically viable for mass marketing (Yvonne et al., 2017). In fact, a significant percentage of the fish species used to make fishmeal are food-grade for humans and approximately three fourths of those fish are juveniles (Cashion et al., 2017; Yvonne et al., 2017). China accounts for 16% of global fish production volume and 76% of the species of fish they farm rely on fishmeal for feed, which translates to 7.17 million tons of trash fish, a figure which exceeds the entire annual catch of Indonesia (Yvonne et al., 2017). In fact, most fisheries also target fish for use in fishmeal as well as fatty acid extraction (Cashion et al., 2016). The overfishing of low-trophic-level fish such as anchovies and sardines has disrupted the transfer of energy through the food web from phytoplankton to zooplankton to higher trophic level predators (Essington et al., 2015; Yvonne et al., 2017). This leads to scenarios such as large numbers of sea lion pups starving to death on southern California beaches due to declining anchovy and sardine populations (McClatchie et al., 2016).

Another factor contributing to overfishing is the demand for omega-3 fatty acid supplements made from extracting EPA and DHA from oily fish. The market value of omega-3 supplements was US$ 33.04 billion USD in 2016 and the demand is predicted to more than double by 2025 (Grand View Research., 2017). Approximately 64 million tons of fish waste is generated from omega-3 extraction annually (Nges et al., 2011). It is estimated that if the minimum daily intakes of EPA and DHA recommended by various governments were followed worldwide, the global fish population would collapse by the 2050s (Greene et al., 2013). This situation is likely even more precarious than this figure implies, because the process of extraction and reduction into supplements actually reduces the bioavailability EPA and DHA compared to consuming the entire fish (Visioli et al., 2003). On average, only 38% of the unsaturated fats in salmon are polyunsaturated while the other 62% is monounsaturated. In sardines, on average it is 35% polyunsaturated and 65% monounsaturated fatty acids. The discarded monounsaturated fatty acids include the beneficial omega-7, palmitoleic acid and the omega-9, oleic acid (Makoure et al., 2019). Several brands of omega-3 supplements were observed by the USDA to have lower concentrations of EPA and DHA than their labels reported (Mason et al., 2016). Many of these brands contain equal concentrations of saturated fats, such as palmitic and myristic acid, as they did omega-3 (Mason et al., 2016). Even more concerning, many of these supplements also contained concentrations of oxidized lipids at levels higher than recommended by international industry standards (Cameron-Smith et al., 2015; Mason et al., 2016). The products of lipid oxidation such as, peroxides and aldehydes can cause a wide variety of health problems, including, but not limited to, inflammation, endothelial dysfunction, insulin resistance, increase in low-density lipoprotein levels, and increased cardiovascular risk, all issues for which people are taking omega-3 supplements to mitigate in the first place (Mason et al., 2016).

In this study, I tested four aquatic insect species for overall protein concentration and long-chain fatty acid content. All four of these species are frequent trout prey and each has its own nickname and unique imitation lure used by fly fishermen; *A. abnormis,* a species of stonefly in the Perlidae family known as the “common stone,” *R. carolina,* a Rhyacophilid caddisfly is known as the “green sedge,” *H. betteni,* a species of net-spinning caddisfly is known as the “spotted sedge,” *B. numerous,* a species of tube-making caddisfly in the Brachycentridae family known as the “American grannom

Results

Protein Concentrations in Aquatic Specimens Estimated by the Pierce's BCA Assay with Bovine Serum Albumin as Reference Standard

Farm-Raised *Salmo salar* had a lower protein concentration than wild-caught *S. salar* (F= 29.10; df = 1; $P < 0.0001$) (Table 3). *Salmo salar* had a higher protein concentration than *S. sagax* and *E. encrasicolus* (F= 71.16; df = 2; $P < 0.0001$) (Table 3). All four aquatic insect specimens had more protein than the commercial fish specimens (F = 769.72; df = 7; $P < 0.0001$) (Table 3). The detrivorous filter feeders, *H. betteni* and *B. numerous* had more protein than the carnivorous *R. carolina* and *A. abnormis* (F = 393.94; df = 3; $P < 0.0001$) (Table 3).

Table 3. Protein Concentrations of Aquatic Species, Dry Weight, N=3.

Specimen	Life Stage/Caste	Mean Concentration (µg/mg) ± SD
Salmo salar (Farm-Raised)	N/A	0.49 ± 0.02f
Salmo salar (Wild-Caught)	N/A	0.63 ± 0.01e
Sardinops sagax	N/A	0.42 ± 0.03g
Engraulis encrasicolus	N/A	0.39 ± 0.03g
Acroneuria abnormis	Nymphs	1.10 ± 0.009c
Rhyacophila carolina	Larvae	0.90 ± 0.03d
Hydropsyche betteni	Larvae	1.40 ± 0.06b
Brachycentrus numerous	Larvae	1.70 ± 0.01a

Protein concentrations were obtained from pulverized, dehydrated tissue samples using methods described above. All beef samples were ground beef. Means with the same letters are not statistically significant ($P \geq 0.05$). Means with different letters are statistically significant ($P < 0.05$).

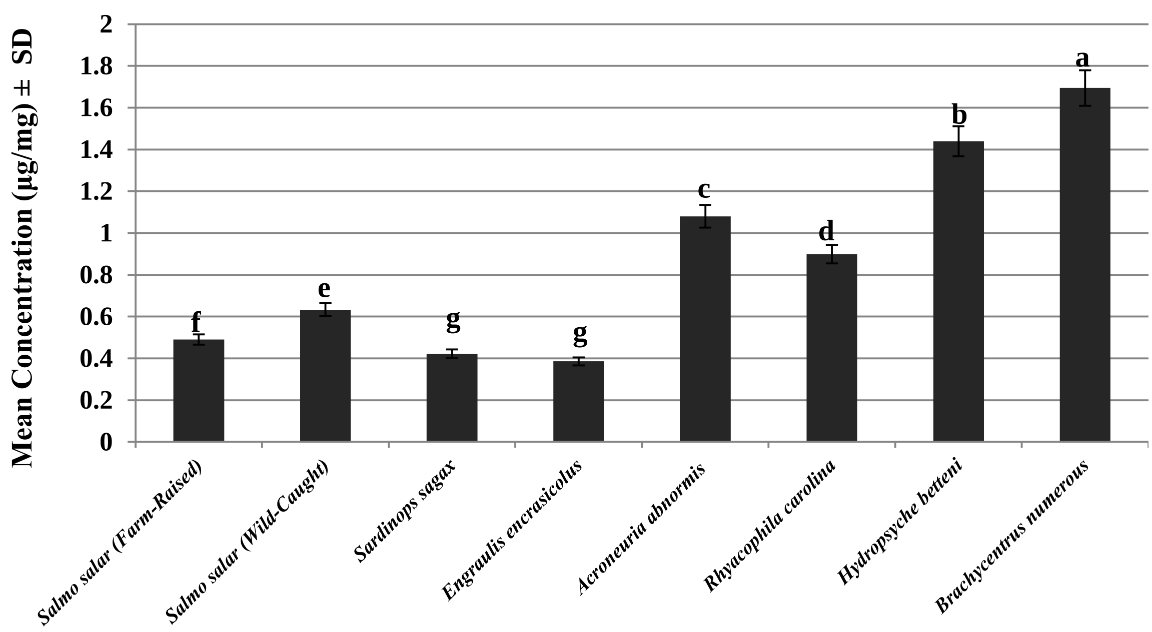

Figure 10. Protein Concentrations of Aquatic Species, Dry Weight, N=3. Means with the same letters are not statistically significant ($P \geq 0.05$). Means with different letters are statistically significant ($P < 0.05$)

Long-Chain Fatty Acid Analysis of Aquatic Specimens

Farm-raised *Salmo salar* has approximately ten times higher concentrations of linoleic acid (F = 245.00; df =1; *P* < 0.0001) and α–linolenic acid (F = 249.2; df = 1; *P* < 0.0001), than wild-caught *S. salar* (Table 4). Farm-raised *S. salar* had a 3:1 omega-6 to omega-3 ratio (F = 119.24; df = 1; *P* < 0.001) while wild-caught *S. salar* had a 1:6 omega-6 to omega-3 ratio (F = 208.42; df = 1; *P* < 0.0001) (Table 4). Farm-raised *S. salar* had a 1.5:1 ratio of oleic acid to palmitic acid (F = 38.13; df = 1; *P* < 0.01) while wild-caught *S. salar* had a 1:1 ratio of oleic acid to palmitic acid (F = 0.02; df = 1; $P \geq 0.05$) (Table 4). Wild-caught *S. salar* had approximately forty-thousand times more EPA than farm-raised *S. salar* (F = 307.99; df = 1; *P* < 0.0001) (Table 4). Despite this, farm-raised *S. salar* had a slightly higher DHA concentration than wild-caught *S. salar* (F = 8.11; df = 1; *P* = 0.0465) (Table 4). *Sardinops sagax* had the highest concentration of EPA out of the commercial fish samples, (F = 44.13; df = 1; *P* < 0.01) (Table 4). *Acroneuria abnormis* had a higher concentration of EPA than *S. sagax* (F = 24.81; df = 1; p < 0.01) and *R. carolina* had an even higher EPA concentration, nearly twice that of *S. sagax* (F = 54.11; df = 1; *P* < 0.01). *Acroneuria abnormis* (F = 107.97; df = 1; *P* < 0.001), *R. carolina* (F = 141.40; df = 1; *P* < 0.001), and *B. numerous* (F = 47.45; df = 1 *P* < 0.01) had higher concentrations of palmitoleic acid than farm-raised *S. salar* (Table 4). *Brachycentrus numerous* had the highest concentration of α–linolenic acid of the aquatic specimens, more than twice that of the next highest, *A. abnormis* (F = 65.86; df = 1; *P* < 0.001) (Table 4). *Brachycentrus numerous* also had, by far, the highest concentration of DA of the aquatic specimens by far (F = 170.56; df = 1; *P* < 0.001).

Table 4. Long-Chain Fatty Acid Concentrations and Ratios of Aquatic Species, Dry Weight, N=3

Long-Chain Fatty Acid Mean Concentrations (µg/g) ± SD / Species	Linoleate	α -Linolenate	EPA	DHA	Omega-6 to Omega-3 Ratio	Oleate	Palmitate	Oleate to Palmitate Ratio	Palmitoleate	Dodecanoate	Total Long-Chain Fatty Acids
***Salmo salar* farm-raised**	7,123 ± 572a	1,690 ± 149d	0.404 ± 0.03g	595 ± 48b	3:1***	16,523 ± 1,306a	9,978 ± 735b	1.7:1**	3,056 ± 245d	15,283 ± 1225e	69,051 ± 5,634e
***Salmo salar* wild-caught**	760 ± 62.1f	182 ± 14.7f	3,952 ± 318d	471 ± 38c	1:6****	8,575 ± 694c	8,670 ± 653c	1:1[N.S.]	2,333 ± 188e	48,062 ± 3,919c	87,394 ± 7,103cd
Engraulis encrasicolus	363 ± 29.4g	135 ± 10.6g	3,240 ± 261e	558 ± 45b	1:11****	1,006 ± 86f	5,624 ± 408d	1:5.5****	1,174 ± 90f	1,056 ± 82g	18,638 ± 1,470g
Sardinops sagax	819 ± 66.1f	429 ± 34.3e	6,992 ± 563c	702 ± 57a	1:10****	2,804 ± 229e	7,983 ± 645c	1:3***	2,252 ± 180e	1,606 ± 131f	31,766 ± 2,449f
Acroneuria abnormis	4,462 ± 359c	3,633 ± 294b	10,486 ± 816b	14.15 ± 1.1e	1:3***	15,526 ± 1,225a	11,125 ± 898b	1.4:1*	8,407 ± 686b	63,877 ± 4,899b	131,148 ± 10,614b
Rhyacophila carolina	3,537 ± 286d	3,209 ± 261b	12,871 ± 980a	16.67 ± 1.3d	1:4.5***	8,140 ± 653c	11,520 ± 898b	1:1.4*	10,487 ± 849a	19,261 ± 1,551d	81,634 ± 6,532d
Hydropsyche bettini	2,340 ± 188e	2,149 ± 171c	3,828 ± 245d	5.62 ± 0.4f	1:2.5***	5,746 ± 465d	6,191 ± 489d	1:1.1[N.S.]	2,584 ± 204e	61,838 ± 4,900b	90,234 ± 7,348c
Brachycentrus numerous	5,347 ± 433b	7,531 ± 612a	2,415 ± 196f	0.147 ± 0.01g	1:2**	12,859 ± 980b	19,397 ± 1,306a	1:1.5**	5,375 ± 408c	221,319 ± 16,330a	284,349 ± 12,097a

Mean fatty acid concentrations in the same column with the same letters are not statistically significant ($P \geq 0.05$). Means in the same column with different letters are statistically significant ($P < 0.05$). For fatty acid ratios in the same column; N.S. = $P \geq 0.05$ (*) = $P < 0.05$, (**) = $P < 0.01$, (***) = $P < 0.001$. (****) = $P < 0.0001$.

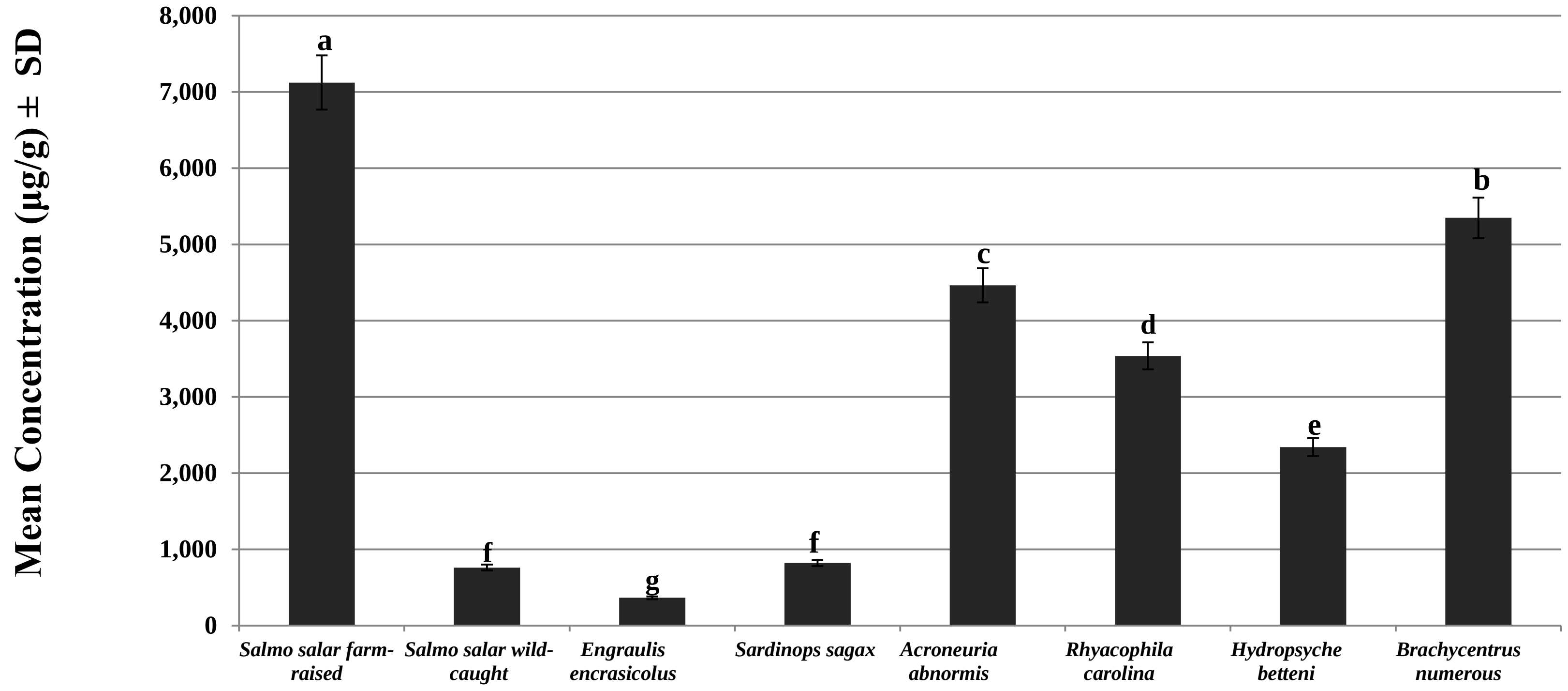

Figure 11. Linoleic Acid Concentrations of Aquatic Species, Dry Weight, N=3. Means with the same letters are not statistically significant ($P \geq 0.05$). Means with different letters are statistically significant ($P < 0.05$).

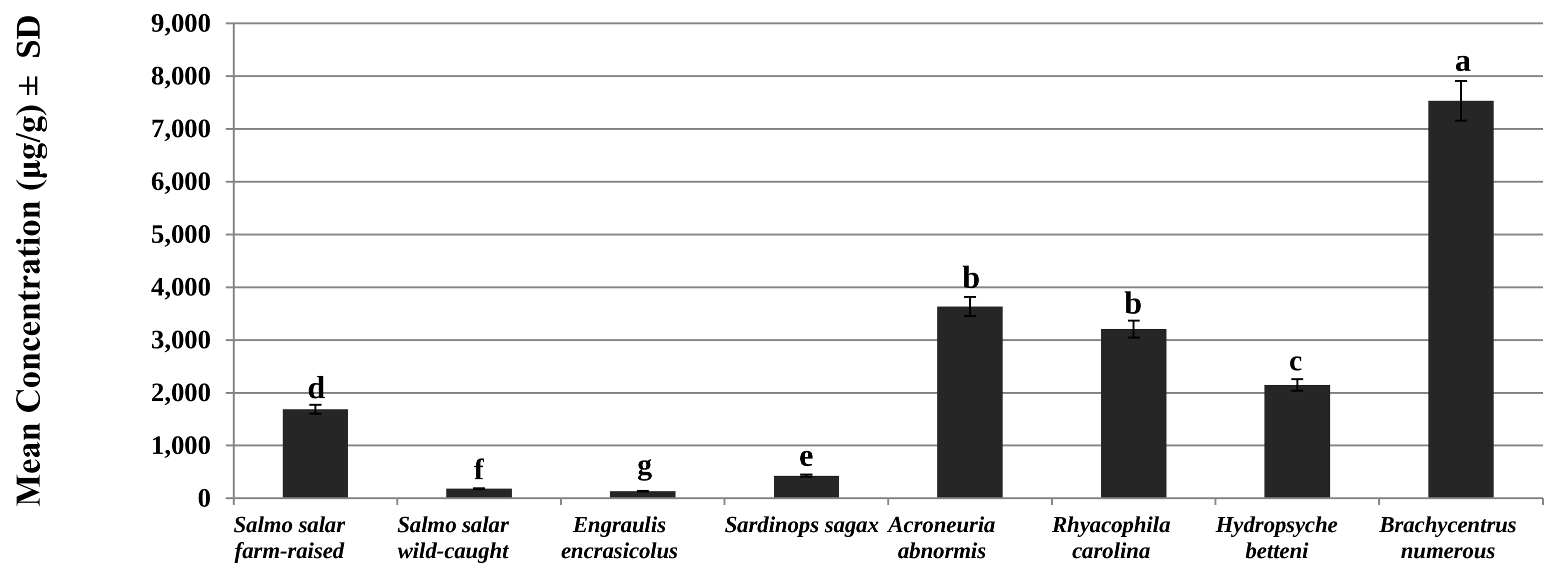

Figure 12. α–Linolenic Acid Concentrations of Aquatic Species, Dry Weight, N=3. Means with the same letters are not statistically significant ($P \geq 0.05$). Means with different letters are statistically significant ($P < 0.05$).

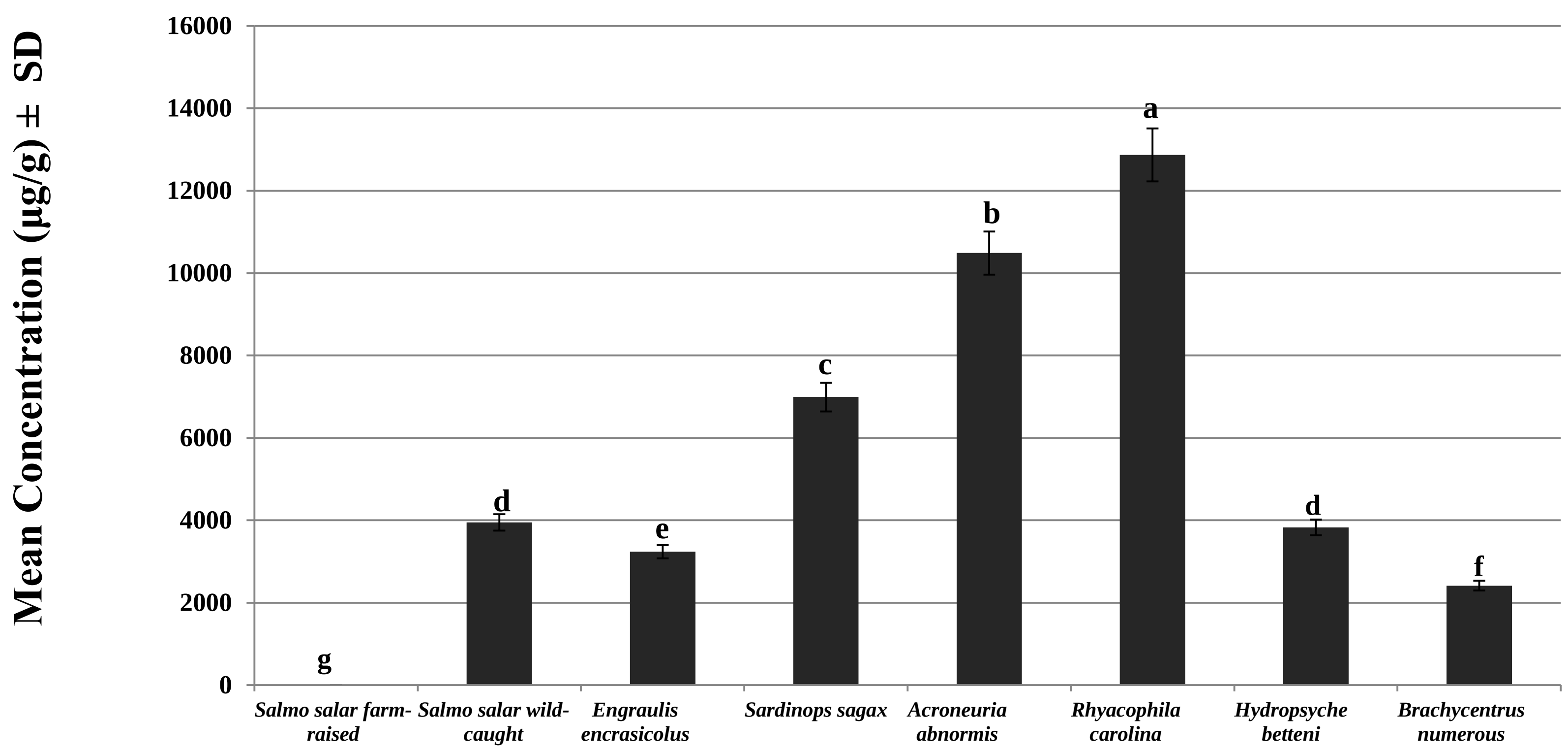

Figure 13. Eicosapentaenoic Acid (EPA) Concentrations of Aquatic Species, Dry Weight, N=3. Means with the same letters are not statistically significant ($P \geq 0.05$). Means with different letters are statistically significant ($P < 0.05$).

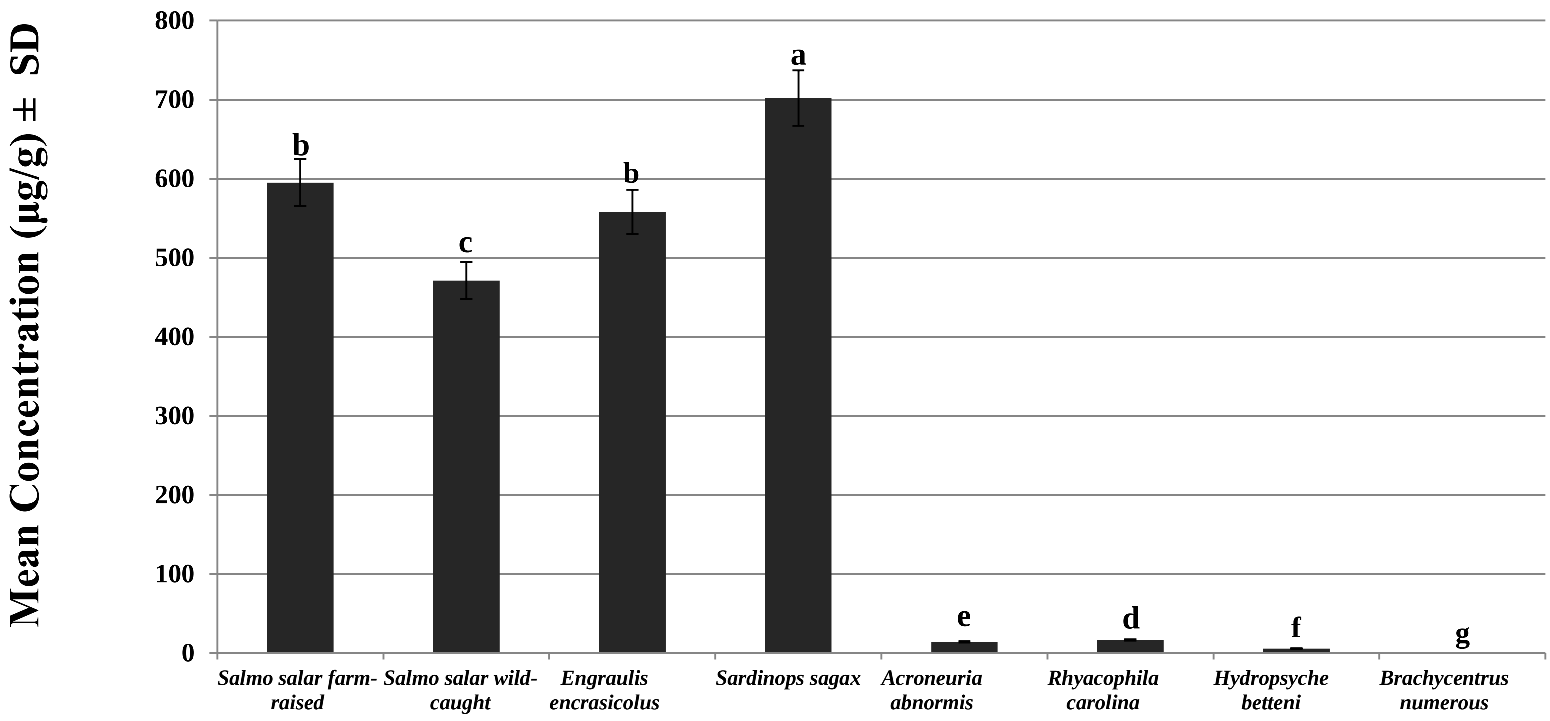

Figure 14. Docosahexaenoic Acid (DHA) Concentrations of Aquatic Species, Dry Weight, N=3. Means with the same letters are not statistically significant (*P* ≥ 0.05). Means with different letters are statistically significant (*P* < 0.05).

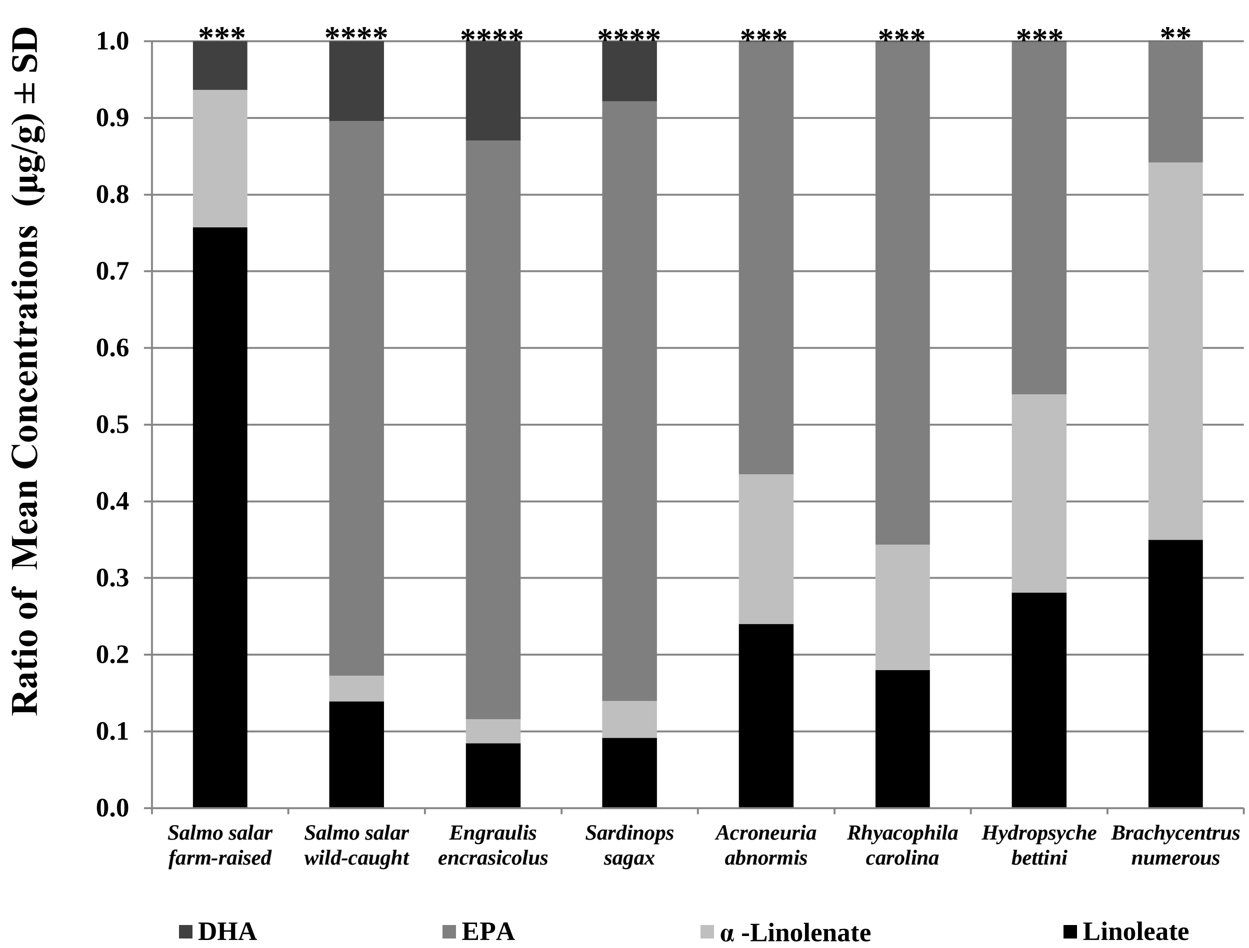

Figure 15. Omega-6 to Omega-3 Fatty Acid Ratios of Aquatic Species, Dry Weight, N=3. N.S. = (**) = *P* < 0.01, (***) = *P* < 0.001, (****) = *P* < 0.0001.

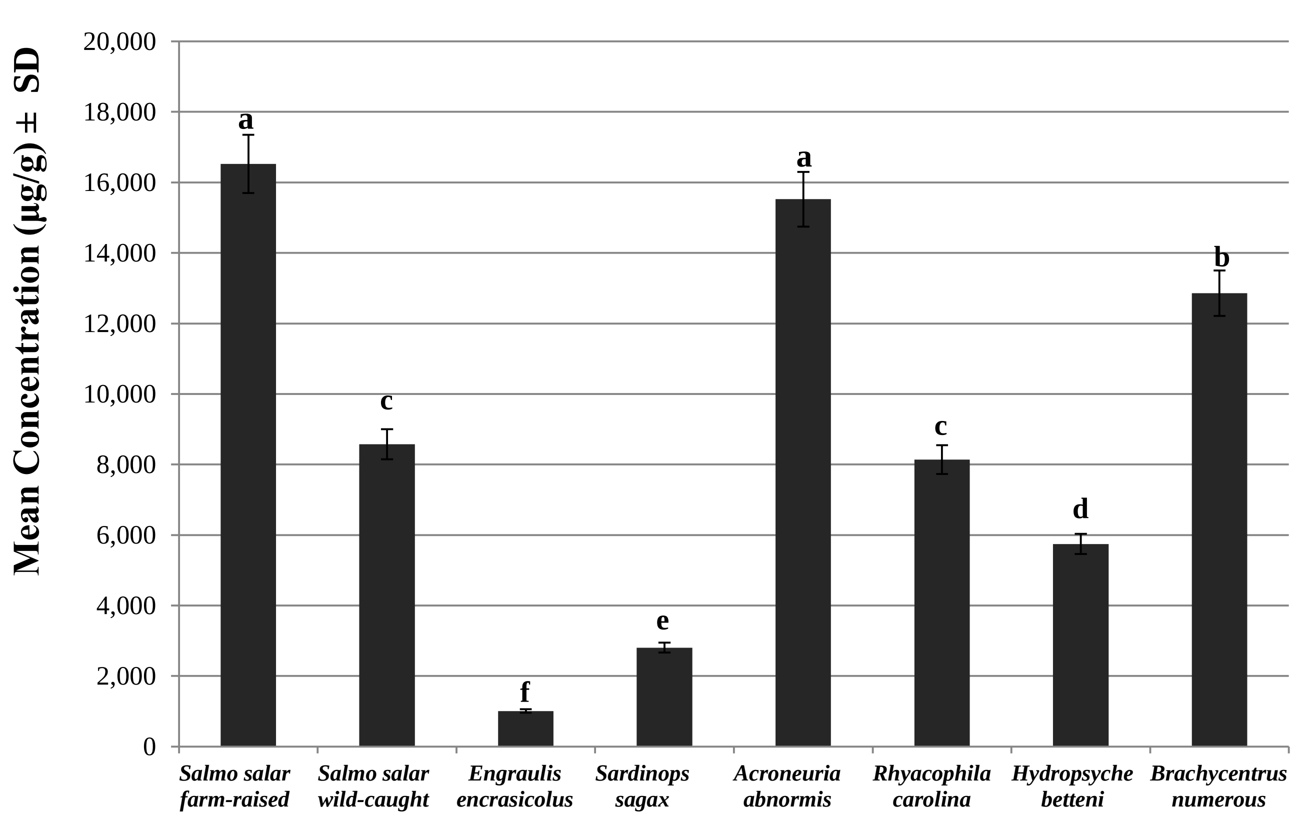

Figure 16. Oleic Acid Concentrations of Aquatic Species, Dry Weight, N=3. Means with the same letters are not statistically significant (*P* ≥ 0.05). Means with different letters are statistically significant (*P* < 0.05).

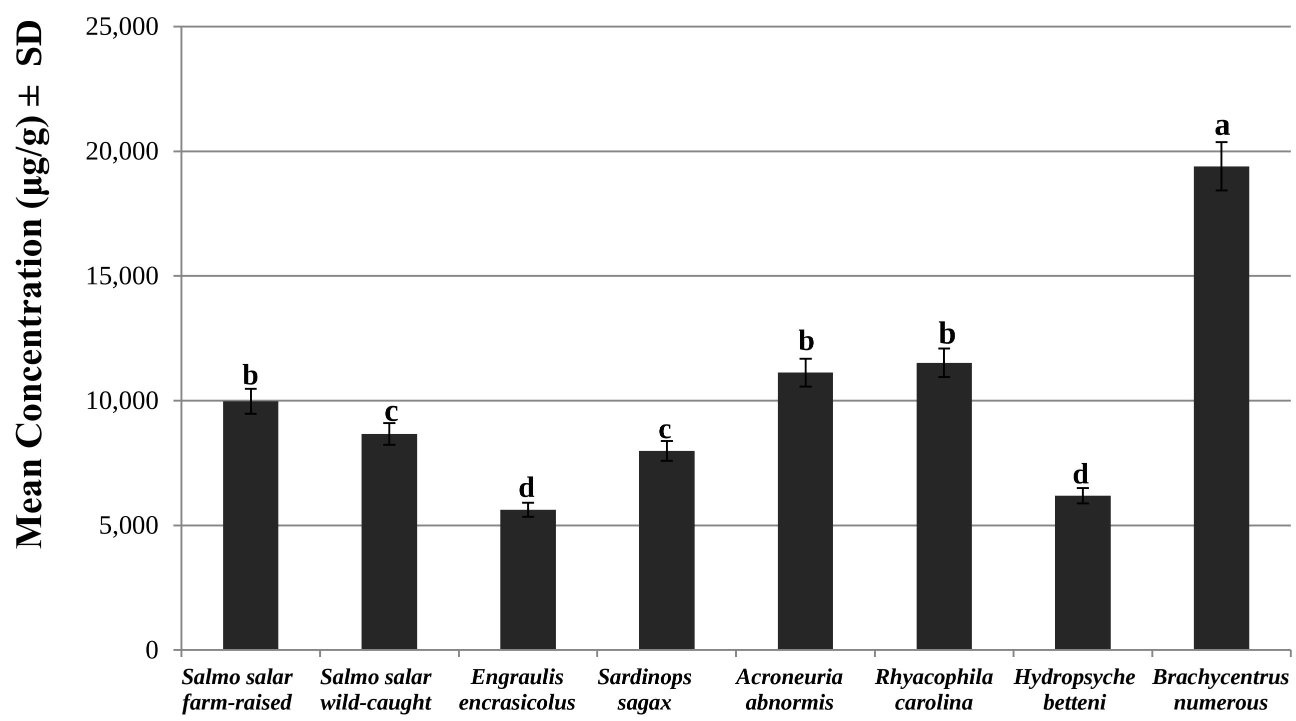

Figure 17. Palmitic Acid Concentrations of Aquatic Species, Dry Weight, N=3. Means with the same letters are not statistically significant ($P \geq 0.05$). Means with different letters are statistically significant ($P < 0.05$).

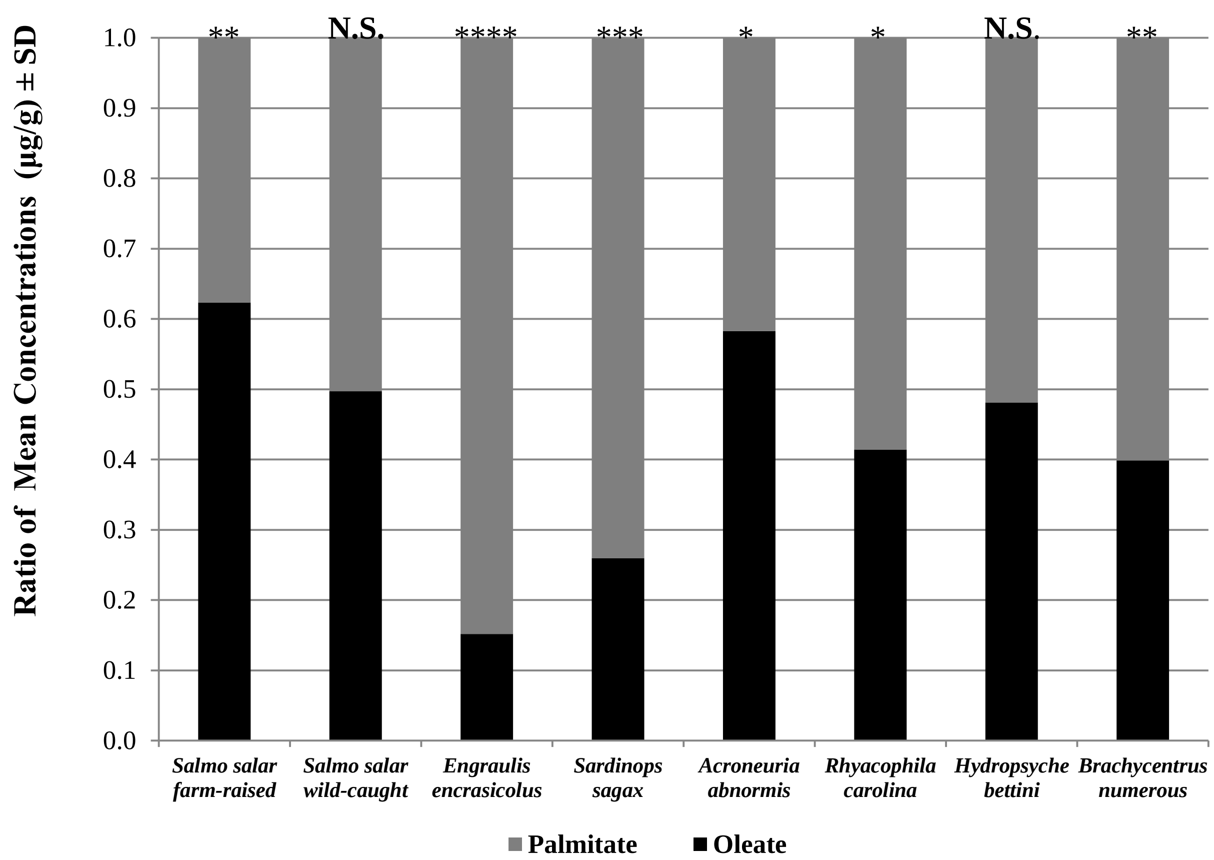

Figure 18. Oleic Acid to Palmitic Acid Ratios of Aquatic Species, Dry Weight, N=3. N.S. = $P \geq 0.05$, (*) = $P < 0.05$, (**) = $P < 0.01$, (***) = $P < 0.001$, (****) = $P < 0.0001$.

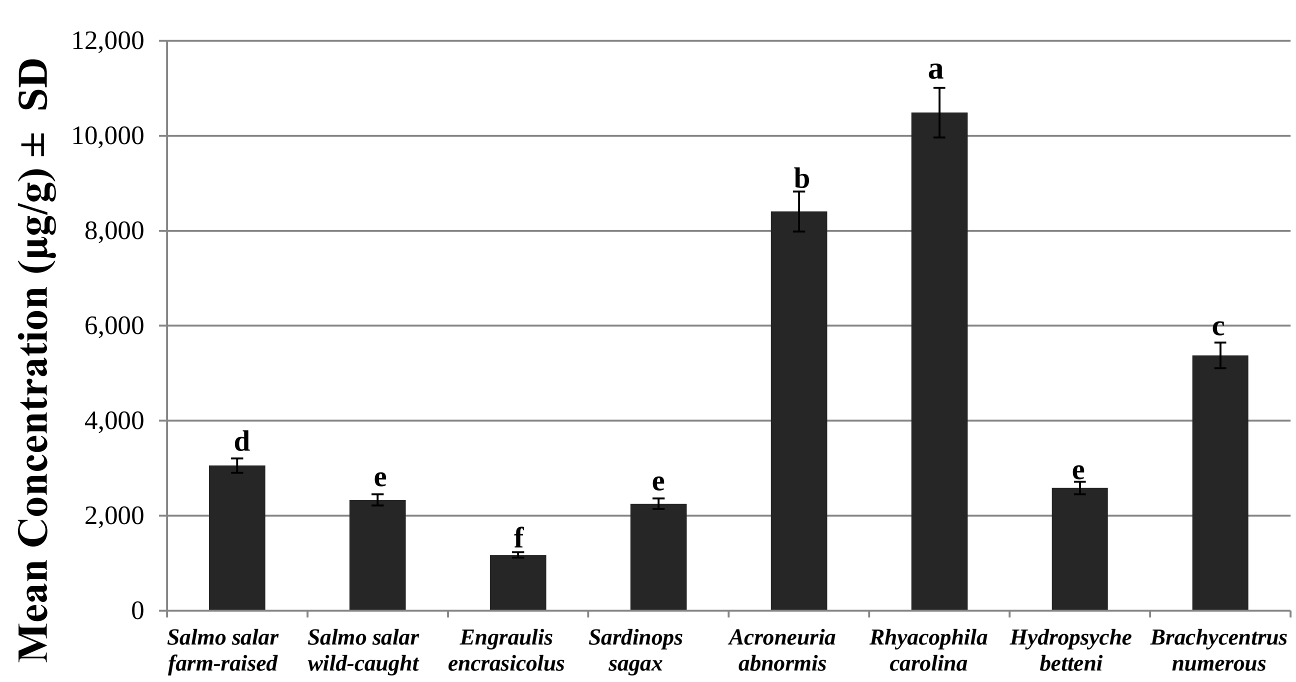

Figure 19. Palmitoleic Acid Concentrations of Aquatic Species, Dry Weight, N=3. Means with the same letters are not statistically significant ($P \geq 0.05$). Means with different letters are statistically significant ($P < 0.05$)

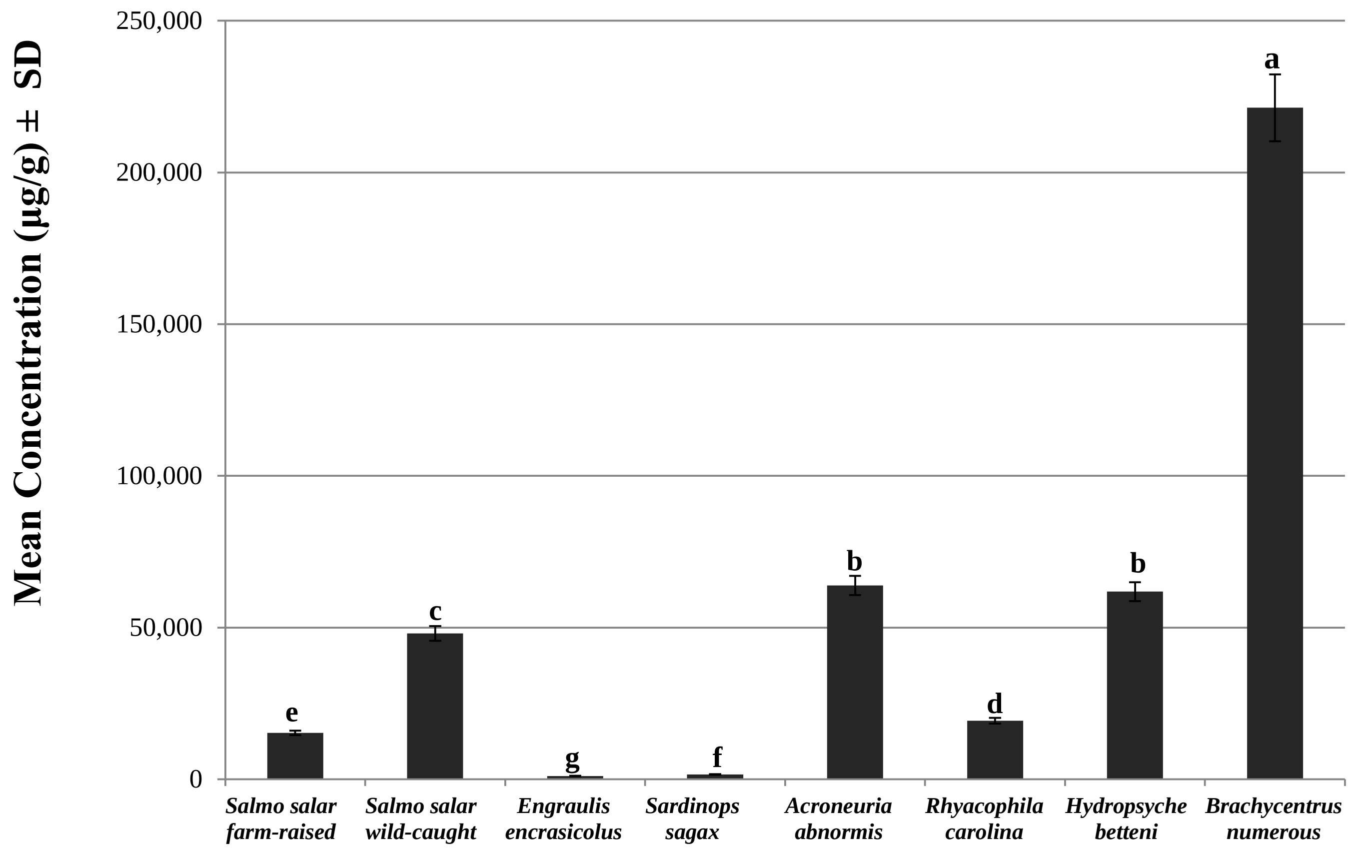

Figure 20. Lauric Acid Concentrations of Aquatic Species, Dry Weight, N=3. Means with the same letters are not statistically significant ($P \geq 0.05$). Means with different letters are statistically significant ($P < 0.05$).

Discussion

This research substantially expands our knowledge, as information about aquatic insects and their potential to contribute to the human diet, has so far been afforded minimal scientific attention (Williams et al., 2017). Low trophic level fish in saltwater ecosystems, such as sardines and anchovies, play in an important role in the transfer of energy and nutrients to higher trophic levels on the food web. The basis of my three working hypotheses for this portion of my research was; aquatic insects play similar roles in the food webs of freshwater ecosystems as anchovies and sardines do in saltwater ecosystems; they should have at least comparable nutrient content relations to their own predators.

The results from the commercial fish samples show clearly, the consequences of the unnatural diets of farmed fish, especially when comparing the long-chain fatty acid content of farm-raised and wild-caught *S. salar*. Ironically, the farm-raised salmon does have higher concentrations and a more balanced ratio of LA and ALA and also a slightly better OA to PA ratio (Table 4). However, this comes at the cost of having less protein than wild-caught salmon (Table 3) and having an EPA concentration thousands of times lower than the wild-caught *S. salar* (Table 4). Studies have shown that farmed fish only have high concentrations of omega-3 fatty acids if they are actually fed fish oil (Bibus., 2015). Since the farm-raised *S. salar* had almost no EPA they were likely not fed any fish oil. The fact that the farm-raised salmon had 10 times the LA concentration compared to the wild-caught salmon may be due to the soy in the aquaculture diet. Salmon are carnivores, so feeding them plants such as maize and soy can cause intestinal inflammation in the salmon and even have carcinogenic effects (Dale et al., 2009). When comparing *E. encrasicolus* and *S. sagax*, *S. sagax* had over twice as much EPA and slightly more DHA compared to *E. encrasicolus* (Table 4). This difference can be explained by *E. encrasicolus* mainly feeding on carnivorous zooplankton and decapod larvae while *S. sagax* favors a primary producer, phytoplankton (van der Lingen., 1994; Plounevez et al., 2000).

All four aquatic insects also have higher concentrations of protein than the commercial fish samples; therefore, the first working hypothesis has been supported so far (Table 3). In contrast to the carnivorous *S. salar*, *H. betteni* and *B. numerous*, are omnivorous, detritivore filter feeders which inhabit flowing, freshwater habitats (McCafferty, 1981). Since ALA is primarily derived from consuming terrestrial plants (Brenna, 2002), these caddisfly larvae are likely consuming a mixture of aquatic and terrestrial detritus. The terrestrial detritus would be derived from any organic matter that falls into the stream such as grass, leaves, twigs, dead insects, etc. As a result, these insects can naturally have significant concentrations of LA and ALA without sacrificing EPA, while maintaining balanced O6-O3 ratios. Out of all of the terrestrial insect species only the graminivore, *D. carolina,* had less than a 4:1 omega-6 to omega 3 ratios (Table 2). In contrast all four aquatic insect species had less than a 4:1 omega-6 to omega 3 ratios. This is consistent with existing research that reports aquatic insect generally have lower omega-6 to omega 3 ratios than terrestrial insects (Twining et al., 2018). It should be pointed out that none of the fish samples had any significant concentrations of ALA and none of the beef samples or terrestrial insects had any significant concentration of EPA and DHA. All four aquatic insect species had relatively high concentrations of both ALA and EPA (Table 4).

A. abnormis and *R. carolina* had the highest concentrations of EPA out of all of the aquatic specimens which also supports the second and third working hypothesis (Table 4). *A. abnormis* and *R. carolina* are both carnivorous species, so they have likely been consuming other insects which have fed on a mixture of terrestrial and aquatic detritus, since they also both had higher concentrations of LA and ALA than the fish samples (Table 4) (McCafferty, 1981). It is unlikely they were exclusively preying on *H. betteni* and *B. numerous*, since they have four to five times the concentrations of EPA compared to the two filter-feeding trichoptera, despite their higher trophic levels (Table 4). At least one of the prey species of *A. abnormis* and *R. carolina* could potentially have even higher concentrations of EPA (Table 4).

Despite surpassing the fish specimens in ALA and EPA concentrations, the aquatic insect specimens were lacking in DHA compared to the commercial fish specimens (Table 4). Even the farm-raised *S. salar* had over ten times the concentration of DHA compared to the aquatic insects despite its nearly nonexistent EPA concentration. *S. salar* are able to maintain high concentrations of DHA in their muscle tissues and blood regardless of their diet. In contrast, the concentration of EPA in *S. salar* muscle tissues and blood is dependent on their diet (Seternes et al., 2020). This would explain why there was no significant difference in the DHA concentrations of farm-raised and wild-caught *S. salar* despite the massive difference in EPA concentrations (Table 4).

B. numerous had the highest concentration of DA out of the aquatic specimens and the second highest concentration overall only behind *T. mollitor* (Table 4). Despite this, *B. numerous* could arguably be considered a better source of DA than *H. illucens* because *H. betteni* also has a more balanced O6-O3 ratio and approximately seven times the protein concentration compared to *H. illucens*, which had the lowest protein concentration out of all the specimens (Table 1). Although *H. betteni* had a lower DA concentration, it is still in the hundreds of thousands, far more than any other specimen (Table 4).

Section 4: Conclusions

The current methods of meeting increasing demands for protein and long-chain fatty acids are not environmentally, economically, and nutritionally viable. Insects have potential as sources of protein and long-chain fatty acids; however it is important to be mindful of fatty acid ratios. The nutritional value of insects varies more depending on their diets compared to other animals because they consume larger amounts relative to their overall body mass. *A. domesticus,* and *T. molitor,* are the only insect species generally accepted in the US for entomophagy; however, my results reveal they all have omega-6 to omega-3 ratios above the maximum 4:1 ratio, increasing the risk of obesity. So far, the graminivore and aquatic insects I tested have shown balanced omega-6 to omega-3 ratios lower than 4:1. Further testing of more graminivore and aquatic insect species for long-chain fatty acids is a priority. Identifying and assaying all of the prey species of the carnivorous *A. abnormis* and *R. carolina* could lead to detecting aquatic insect species with even higher concentrations of EPA.

A majority of the insect species I tested have higher concentrations of protein than the commercial beef and fish samples. Further studies are needed to determine protein quality of insects compared to commercial beef and fish. Protein quality is important because foods with higher protein quality provide more essential amino acids. This study is the first step in finding more viable insect species for entomophagy and will help introduce higher quality and more sustainable sources of proteins and fatty acids into mainstream human diets.

References

Abdela, N. (2016). Sub-acute Ruminal Acidosis (SARA) and its Consequence in Dairy Cattle: A Review of Past and Recent Research at Global Prospective.*Achievements in the Life Sciences*. 10(2), 187-196. At https://doi.org/10.1016/j.als.2016.11.006

Acar, J. (2003). Joint FAO/OIE/WHO Expert Workshop on Non-Human Antimicrobial. Usage and Antimicrobial Resistance: Scientific Assessment At http://www.fao.org/3/a-bq500e.pdf

Adkins, R., & Ross, J. (2019). Environmental Biotechnology. *Edtech Press*. 54, Sun Street, Waltham Abbey Essex, United Kingdom, EN9 1EJ.pp127-130.

Aly M. S., & Albutti, A. (2014). Antimicrobials Use in Aquaculture and their Public Health Impact. *J. Aquac. Res. Dev*, 5:4 doi:10.4172/2155-9546.1000247

Arru, B., Furesi, R., Gasco, L., Madau, F., & Pulina, P. (2019). The Introduction of Insect Meal into Fish Diet: The First Economic Analysis on European Sea Bass Farming. *Sustainability*, 11 (6), 1697. doi:10.3390/su11061697

Bailey, R., Froggatt, A., & Wellesley, L. (2014) Energy, Environment and Resources Livestock Climate Change's Forgotten Sector Global Public Opinion on Meat and Dairy Consumption. *Chatham House. The Royal Institute of International Affairs.* At https://www.chathamhouse.org/sites/default/files/field/field_document/20141203LivestockClimateChangeForgottenSector BaileyFroggattWellesleyFinal.pdf

Barret, R. (2015). With Demand Outstripping Supply, Beef Prices Hit Record. *Milwaukee Wisconsin Journal Sentinel.* At http://archive.jsonline.com/business/with-demand-outstripping-supply-beef-prices-hit-record-b99471967z1-298075441.html

Biancarosa, I., Sele, V., Belghit, I., Ørnsrud, R., Lock, E.-J., & Amlund, H. (2019). Replacing fish meal with insect meal in the diet of Atlantic salmon (*Salmo salar*) does not impact the amount of contaminants in the feed and it lowers accumulation of arsenic in the fillet. *Food Additives & Contaminants: Part A*, 1–15. doi:10.1080/19440049.2019.1619938

Bibus, D. M. (2015). Long-chain omega-3 from Low-trophic-level Fish Provides Value to Farmed Seafood. *Lipid Technol*, 27(3), 55–58. At https://doi.org/10.1002/lite.201500006

Bird B.R., & Forrester F. T. (1981). Basic Laboratory Techniques in Cell Culture. *US. HHS*, Atlanta, Georgia.

Blasbalg, L. T., Hibbeln, R. J., Ramsden, E. C., Majchrzak, F. S., & Rawlings, R. R. (2011). Changes in Consumption of Omega-3 and Omega-6 Fatty Acids in the United States during the 20th Century. *Am. J. Clin. Nutr.* 93(5): 950–962. doi:10.3945/ajcn.110.006643

Bligh E. G., & Dyer, W. J. (1959) A Rapid Method of Total Lipid Extraction and Purification. *Can J Biochem Physiol*. Aug; 37(8):911-7. doi: 10.1139/o59-099. PMID: 13671378.

Brenna, J. T. (2002) Efficiency of Conversion of Alpha-linolenic Acid to Long Chain n-3 Fatty Acids in Man. *Curr Opin Clin Nutr Metab Care.* Mar; 5(2):127-132. doi:10.1097/00075197-200203000-00002.

Brigham, K., and Meyrick, D. (1986). Endotoxin and Lung Injury. *Am Rev Respir Dis*.133(5):913-27. PMID: 3085564.

Cameron-Smith, D., Albert, B. B., & Cutfield, S. W. (2015). Fishing for Answers: is Oxidation of Fish Oil Supplements a Problem? *J. Nutr. Sci.* 4 (36): 1-2 doi:10.1017/jns.2015.26

Cashion, T., Le Manach, F., Zeller, D., & Pauly, D. (2017). Most Fish Destined for Fishmeal Production are Food-grade Fish. *Fish Fish*. doi: 10.1111/faf.12209.

CDC. **(**2011**)**. Multistate Outbreak of Human *Salmonella* Heidelberg Infections Linked to Ground Turkey. At https://www.cdc.gov/salmonella/2011/ground-turkey-11-10 2011.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fsalmonella%2Fheidelberg%2F111011%2Findex.html

Chapin, A., Rule, A., Gibson, K., Buckley, T., & Schwab, K. Airborne Multidrug -resistant Bacteria Isolated from a Concentrated Swine Feeding Operation. *Environ Health Perspect*. 2005 Feb; 113 (2):137-42. doi: 10.1289/ehp.7473.

Cheaib, Z., & Lussi, A. (2013). Role of Amylase, Mucin, IgA and Albumin on Salivary ProteinBuffering Capacity: a Pilot Study. *J. Biosci*; 38(2):259-265. doi:10.1007/s12038-013-9311-

Chee-Sanford, J. C., Mackie, R. I., Koike, S., Krapac, I. G., Lin, Y. F., Yannarell, A. C., Maxwell, S., & Aminov, R. I. (2009). Fate and Transport of Antibiotic Residues and Antibiotic Resistance Genes Following Land Application of Manure Waste. *J Environ Qual*. Apr 27; 38(3):1086-108.doi: 10.2134/jeq2008.0128. PMID: 19398507.

Chapin, A., Rule, A., Gibson, K., Buckley, T., & Schwab, K. Airborne Multidrug-resistant Bacteria Isolated from a Concentrated Swine Feeding Operation. Environ Health Perspect. 2005 Feb; 113 (2):137-42. doi: 10.1289/ehp.7473. PMID: 15687049; PMCID: PMC1277855.

Clapham, W. M., Foster, J. G., Neel, J. P. S., & Fedders, J. M. (2005). Fatty Acid Composition of Traditional and Novel Forages. Journal of Agricultural and Food Chemistry, 53(26), 10068–10073. doi:10.1021/jf0517039

Consumer Report. (2015). Why Grass-fed Beef Costs More. You'll pay a Little Extra, but Consumer Reports' Tests of Ground Beef Show Grass-fed is less likely to Harbor Dangerous Bacteria. At http://www.consumerreports.org/cro/magazine/2015/08/why-grass-fed-beef-costs-more/index.htm

'Council directive 2017/EC amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No 142/2011 as regards the provisions on processed animal protein' (2017) *Official Journal*. L 138, P. 92.

Cunningham, E. (2015). What Are n-7 Fatty Acids and Are There Health Benefits Associated with Them? *J Acad Nutr Diet*, 115(2), 324.doi:10.1016/j.jand.2014.11.021

Dale, B.O., Tørud, B., Kvellestad, A., Koppang, S. H.,& Koppang, O. E. (2009). From Chronic Feed-Induced Intestinal Inflammation to Adenocarcinoma with Metastases in Salmonid Fish. *Cancer Res*. 15; 69 (10):4355-62. doi:10.1158/0008-5472.CAN-08-4877

Dedual, M., & Collier, K. J. (1995). Aspects of Juvenile Rainbow Trout (*Oncorhynchus mykiss*) Diet in Relation to Food Supply during summer in the lower Tongariro River, New Zealand. *N. Z. J. Mar. Freshwater Res*, 29(3), 381–391. doi:10.1080/00288330.1995.9516673

Elmahdi, S., Parveen, S., Ossai, S., DaSilva, L. V., Jahncke, M., Bowers, J., & Jacobs, J. (2018). *Vibrio parahaemolyticus* and *Vibrio vulnificus* Recovered from Oysters during an Oyster Relay Study. *Appl. Environ. Microbiol*, 84(3), e01790-17. At https://doi.org/1 0.1128/AEM.01790-17

Elswyk M. E., & McNeill S. H. (2014). Impact of Grass/Forage Feeding Versus Grain Finishing on Beef Nutrients and Sensory Quality: the U.S. Experience. *Meat Sci.* 96(1):535-540. doi:10.1016/j.meatsci.2013.08.010

Essington, T. E., Moriarty, P. E., Froehlich, H. E., Hodgson, E. E., Koehn, L. E., Oken, K. L., Siple, M. C., & Stawitz. C. C. (2015). Fishing Amplifies Forage Fish Population Collapses. *Proc Natl Acad Sci U S A.* 112(21):6648-52. doi: 10.1073/pnas.1422020112.

Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, Sabine., & Lalander, C. **(**2020**).** Fatty Acid Composition of Black Soldier Fly Larvae (*Hermetia illucens*) Possibilities and Limitations for Modification through Diet**.** *Waste Management*.102: 40-47. At https://doi.org/10.1016/j.wasman.2019.10.014

Feltmate, B. W., & Williams, D. D. (1989). Influence of Rainbow Trout (*Oncothynchus mykiss*) on Density and Feeding Behavior of a Perlid Stonefly. *Can. J. Fish. Aquat* 46(9), 1575–1580.doi:10.1139/f89-200

Finke, M. D. (2002). Complete Nutrient Composition of Commercially Raised Invertebrates used as Food for Insectivores. *Zoo Biology*, 21(3), 269–285. doi:10.1002/zoo.10031

Finke D. M. (2012). Complete Nutrient Content of Four Species of Feeder Insects. *Zoo Biology*. 32(1):27-36. doi: 10.1002/zoo.21012.

Food Empowerment Project. (2016). Pollution (Water, Air, Chemicals). At http://www.foodispower.org/pollution-water-air-chemicals

Fryer, J. L., & Hedrick, R. P. (2003). Piscirickettsia salmonis: a Gram-negative Intracellular Bacterial Pathogen of Fish. *J. Fish Dis.*251-262. doi*:*10.1046/j.1365-2761.2003.00460.x

Gasco, L., Finke, M., & Huis, A., (2018). Can Diets Containing Insects Promote Animal Health? *J. Insects as Food Feed*. 4: 1-4. doi: 10.3920/JIFF2018.x001.

Grand View Research. **(**2017). Omega 3 Supplement Market Size, Share & Trends Analysis Report by Source (Fish Oil, Krill Oil), by Application (Infant Formula, Food & Beverages, Nutritional Supplements, Pharmaceutical), and Segment Forecasts, 2018 –2025. At https://www.grandviewresearch.com/industry-analysis/omega-3-supplement-market

Greene, J., Ashburn, M. S., Razzouk, L., & Smith, D. A. (2013). Fish Oils, Coronary Heart Disease, and the Environment. *Am. J. Public Health*, 103(9), 1568–1576. At https://doi.org/10.2105/AJPH.2012.300959

Grohmann, E., Muth, G., & Espinosa, M. (2003). Conjugative Plasmid Transfer in Gram-Positive Bacteria. *MMBR*. 67(2): 277–301. At https://doi.org/10.1128/mmbr.67.2.277-301.2003

Han, Y., Wang, J., Zhao, Z., Chen, J., Lu, H., & Liu G. (2017). Fishmeal Application Induces Antibiotic Resistance Gene Propagation in Mariculture Sediment. *Environ. Sci. Technol*; 51(18):10850-10860. doi:10.1021/acs.est.7b02875

Heinz, G.; Hautzinger, P. (2007). Meat Processing Technology for Small to Medium-Scale Producers. *FAO.*

Heng, S. P., Letchumanan, V., Deng, C. Y., Ab Mutalib, N. S., Khan, T. M., Chuah, L. H., Chan, K. G., Goh, B. H., Pusparajah, P., & Lee, L. H. (2017). *Vibrio vulnificus*: An Environmental and Clinical Burden. *Front. Microbiol*, 8: 997. https://doi.org/10.3389/fmicb.2017.00997

Hofstrand, Don (2009). Impact of Rising Feed Prices on Cattle Finishing Profitability.*AgMRC*. At http://www.agmrc.org/renewable-energy/ethanol/impact-of-rising-feed-prices-on-cattle-finishing-profitability

van Huis**,** A., Itterbeeck, Van, J., Esther, K. H., Halloran, A. M., Muir, G., & Vantomme P. **(**2013). Edible Insects: Future Prospects for Food and Feed Security. *FAO.* At http://www.fao.org/3/i3253e/i3253e.pdf

van Huis, A., & Oonincx, D.G.A.B. (2017). The Environmental Sustainability of Insects as Food and Feed. A Review. *Agron. Sustain. Dev*. 37(5): 1-9. doi:10.1007/s13593-017-0452-8.

Jandacek R. J. (2017). Linoleic Acid: A Nutritional Quandary. *Healthcare (Basel, Switzerland)*. 5(2), 25. https://doi.org/10.3390/healthcare5020025

Johansen, M., Thorstad, E. B., Rikardsen, A. H., Koksvik, J. I., Ugedal, O., Jensen, A. J., Skasgard, L., & Næsje, T. F. (2010). Prey Availability and Juvenile Atlantic Salmon Feeding During Winter in a Regulated Subarctic River Subject to Loss of Ice Cover. *Hydrobiologia*, 644(1), 217–229. doi:10.1007/s10750-010-0118-x

Józefiak, A., Nogales-Merida, S., Mikołajczak, Z., Rawski, M., Kierończyk, B., & Mazurkiewicz, J. (2019). The Utilization of Full-Fat Insect Meal in Rainbow Trout (*Oncorhynchus mykiss*) Nutrition: The Effects on Growth Performance, Intestinal Microbiota and Gastrointestinal Tract Histomorphology. *Ann. Anim*. 19. doi: 10.2478/aoas-2019-0020.

Khan, A., Khan, S., Jan, A. A., & Khan, M. (2017). Health Complication Caused by Protein Deficiency. *Food Sci. Nutr.*; 1(1):1-2. doi: 10.35841/food-science.1.1.1-2

Kinyuru, J. N., Konyole, S. O., Roos, N., Onyango, C. A., Owino, V. O., Owuor, B. O., Estamable, B. B., Friis, H., Aagaard-Hansen, J., Kenj, G. M. (2013). Nutrient Composition of Four Species of Winged Termites Consumed in Western Kenya. *J Food Compost Anal*, 30(2), 120–124. doi:10.1016/j.jfca.2013.02.008

Lamanda, A., Cheaib, Z., Turgut, M. D., & Lussi, A. (2007). Protein Buffering in Model Systems and in Whole Human Saliva. *PLOS ONE*. 2(2): e263. At https://doi.org/10.1371/journal.pone.0000263

Logue, C. M., Barbieri, N. L., &Nielsen D.W. (2017). Pathogens of Food Animals: Sources, Characteristics, Human Risk, and Methods of Detection. *Adv Food Nutr Res* 82:277-365. doi: 10.1016/bs.afnr.2016.12.009.

Makoure, D., Arhaliass, A., Echchelh, A., & Legrand, J. (2019). Fish Oil Chemical Composition for Biodiesel Production. *J. Mater. Environ. Sci.* 10(12): 1221-1229. At erenvironsci.com/Document/vol10/vol10_N12/JMES-2019-10-121-Makoure.pdf

Mason R.P., & Sherratt S. C. R. (2016). Omega-3 Fatty Acid Fish Oil Dietary Supplements Contain Saturated Fats and Oxidized Lipids that may Interfere with their Intended Biological Benefits. *Biochem.Biophys. Res. Commun*. 483(1):425-429. doi:10.1016/j.bbrc.2016.12.127

McCafferty, P. W., & Provonsha, V. A. (1981). Aquatic Entomology: The Fisherman's and Ecologist's Illustrated Guide to Insects and Their Relatives: Fishermen's and Ecologists' Illustrated Guide to Insects and Their Relatives. *Jones and Bartlett Publishers Inc*.

McClatchie, S., Field, J., Thompson, A. R., Gerrodette, T., Lowry, M., Fiedler, P. C., Watson, W., Nieto, K. M., & Vetter, R. D. (2016). Food Limitation of Sea Lion Pups and the Decline of Forage off Central and Southern California. *R. Soc. Open Sci.*, 3(3):150628.https://doi.org/10.1098/rsos.150628

McCullough, R. S., Edel, A. L., Bassett, C. M., Lavallée, R. K., Dibrov, E., Blackwood, D. P., Ander, B. P., & Pierce, G. N. (2011). The Alpha Linolenic Acid Content of Flaxseed is Associated with an Induction of Adipose Leptin Expression. *Lipids*. 46(11): 1043–1052. At https://doi.org/10.1007/s11745-011-3619-0

Minakawa, N., Gara, R. I., &Honea, J. M. (2002). Increased Individual Growth Rate and Community Biomass of Stream Insects Associated with Salmon Carcasses. *J N AM BENTHOL SOC*, 21(4): 651–659. doi:10.2307/1468436

Miranda, D. C., Godoy, A. F., & Lee, R. M., (2018). Current Status of the Use of Antibiotics and the Antimicrobial Resistance in the Chilean Salmon Farms. *Front. Microbiol*.9:1284. doi:10.3389/fmicb.2018.01284

Mo, T. A., & Jørgensen A. (2017). A Survey of the Distribution of the PKD-parasite *Tetracapsuloides bryosalmonae* (Cnidaria: Myxozoa: Malacosporea) in Salmonids in Norwegian Rivers - Additional Information Gleaned from Formerly Collected Fish. *J. Fish Dis*. 40(5):621-627.doi:10.1111/jfd.12542

Neyra, C. R., Frisancho, A., J. Rinsky, L. J., Resnick, C., Carroll, C. K., Rule, M. A., Ross, T., You, Y., Price, B. L., & Silbergeld, K. E., (2014). Multi-Drug Resistant and Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Hog Slaughter and Processing Plant Workers and Their Community in North Carolina (USA). *Environ. Health Perspect*. 122(5): 471-477.doi:10.1289/ehp.1306741

Nges, A. I., Mbatia, B., & Björnsson, L., (2012). Improved Utilization of Fish Waste by Anaerobic Digestion following Omega-3 Fatty Acids Extraction. *J. Environ . Manage*.110:159-165. https://doi.org/10.1016/j.jenvman.2012.06.01

Okocha, R. C., Olatoye, I. O., & Adedeji, O. B. (2018). Food Safety Impacts of Antimicrobial use and their Residues in Aquaculture. *PHR*. 39: 21.https://doi.org/10.1186/s40985-018-0099-2

Pachauri, R., K. & Reisinger, A. (2007). Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. *IPCC*. pp104. At https://www.ipcc.ch/site/assets/uploads/2018/02/ar4_syr_full_report.pdf

Pacheco Y. M., Bermúdez, B., López, S., Abia, R., Villar, J., & Muriana, F.J., (2006). Ratio of Oleic to Palmitic Acid is a Dietary Determinant of Thrombogenic and Fibrinolytic factors During the Postprandial State in Men. *Am. J. Clin. Nutr*. 84(2):342-349 doi: 10.1093/ajcn/84.1.342

Palmer, E. H., Deshler, N., & Mittal, R. (2017). "Aeromechanics of Long Jumps in Spider Crickets: Insights from Experiments and Modeling." *Proceedings of the ASME 2017 International Mechanical Engineering Congress and Exposition*. *Volume 7: Fluids Engineering*. Tampa, Florida, USA.doi:10.1115/imece2017-73498

Palomer, X., Pizarro-Delgado, J., Barroso, E., & Vázquez-Carrera, M. (2017). Palmitic and Oleic Acid: The Yin and Yang of Fatty Acids in Type 2 Diabetes Mellitus. *Trends Endrocrinol.Metab.* 29(3):178-190. doi:10.1016/j.tem.2017.11.009

Payne, C. L., Scarborough, P., Rayner, M., & Nonaka, K. (2016). Are Edible Insects more or less Healthy' than Commonly Consumed Meats? A Comparison using Two Nutrient Profiling Models Developed to Combat Over- and Under Nutrition. *Eur. J. Clin. Nutr*, 70(3): 285–291. https://doi.org/10.1038/ejcn.2015.149

Pearson, A. M. (1976) "Meat Extenders and Substitutes." BioScience, vol. 26, no. 4, pp. 249–256. JSTOR, www.jstor.org/stable/1297347.

Plounevez, S., & Champalbert, G. (2000). Diet, Feeding Behaviour and Trophic Activity of the Anchovy (*Engraulis encrasicolus* L.) in the Gulf of Lions (Mediterranean Sea). *Oceanologica Acta*, 23(2): 175–192. doi:10.1016/s0399-1784(00)00120-1

Price, B. L., Graham, P. J., Lackey, G. L., Roess, A., Vailes, R., & Silbergeld, (2007). Elevated Risk of Carrying Gentamicin-Resistant *Escherichia coli* among U.S. Poultry Workers*. Environ. Health Perspect.*115 (12): 1738–1742. https://doi.org/10.1289/ehp.10191

Pruden, A., Arabi, M., & Storteboom, N. H. (2012). Correlation between Upstream Human Activities and Riverine Antibiotic Resistance Genes. *Environ. Sci. Technol.* At https://doi.org/10.1021/es302657r

Ritchie, H. (2017) - "Meat and Dairy Production". *Our World In Data.* At https://ourworldindata.org/meat-production

Rodriguez-Leyva D., Dupasquier C. M., McCullough, R., & Pierce G. N. (2010) The Cardiovascular Effects of Flaxseed and its Omega-3 Fatty Acid, alpha-Linolenic Acid. *CJC*. 26(9):489-496. doi:10.1016/s0828-282x(10)70455-4

Royer, T. (2018). Grasshoppers Swarm the State. *Examiner Enterprise*. At https://www.examiner-enterprise.com/news/20180812/grasshoppers-swarm-state

Seternes, T., Johansson, G. S., Evje, I., & Olsen, R. L. (2020). The Level of Eicosapentaenoic Acid (EPA), but not Docosahexaenoic acid (DHA), in Blood of Atlantic salmon (*Salmo salar L*.) is related to Formulation and Concentration of EPA or DHA in feed. *Aquaculture*.735407. doi:10.1016/j.aquaculture.2020.735407

Shramko, Viktoriya S et al. (2020). The Short Overview on the Relevance of Fatty Acids for Human Cardiovascular Disorders." *Biomolecules*. doi:10.3390/biom10081127

Shustov, Y. A., Baryshev, I. A., & Belyakova, E. E. (2012). Specific Features of the Feeding of Juvenile Atlantic Salmon (*Salmo salar L.)* in the Subarctic Varzuga River and its Small Tributaries (Kola Peninsula). *Inland Water Biol*, 5(3), 288–292. doi:10.1134/s1995082912030121

Simopoulos A. P. (2016). An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. *Nutrients*. 8(3): 128. At https://doi.org/10.3390/nu8030128

Skovgaard, A., & Buchmann, K. (2012). *Tetracapsuloides bryosalmonae* and PKD in Juvenile Wild Salmonids in Denmark. *Dis Aquat Organ*. 101(1):33-42. doi: 10.3354/dao02502.

Spranghers, T., Michiels, J., Vrancx, J., Ovyn, A., Eeckhout, M., De Clercq, P., & De Smet, S. (2017). Gut Antimicrobial Effects and Nutritional Value of Black Soldier Fly (*Hermetia illucens L*.) prepupae for weaned piglets. *Anim. Feed Sci. Technol*. 235: 33-42 At https://doi.org/10.1016/j.anifeedsci.2017.08.012

Steinfeld, H., (2006). Livestock's long shadow: environmental issues and options. *Rome: FAO*. At http://www.fao.org/3/a0701e/a0701e.pdf

Sun, Q. & Zhou, X. (2013).A Comprehensive Understanding of Corpse Management in Termites. *Int J Biol Sci*. 9(3):313-321. doi:10.7150/ijbs.5781

Swanson, D., Block, R., & Mousa, S. A. (2012). Omega-3 Fatty Acids EPA and DHA: Health Benefits Throughout Life. *Adv Nutr,* 3(1): 1–7. At https://doi.org/10.3945/an.111.000893

Taha A. Y. (2020). Linoleic Acid-Good or Bad for the Brain? *NPJ Sci. Food*.4:1. At https://doi.org/10.1038/s41538-019-0061-9

Torruco-Uco, J. G., Hernández-Santos, B., Herman-Lara, E., Martínez-Sánchez, C. E., Juárez-Barrientos, J. M., & Rodríguez-Miranda, J. (2018). Chemical, Functional and Thermal Characterization, and Fatty Acid Profile of the Edible Grasshopper (*Sphenarium purpurascens Ch*). *Eur. Food Res. Technol.* doi: 10.1007/s00217-018-3160-y

Twining, W. C., Lawrence, P., Winkler, W. D., Flecker, S. A., & Thomas, J. (2018) Conversion Efficiency of α-Linolenic Acid to Omega-3 Highly Unsaturated Fatty Acids in Aerial Insectivore Chicks. *J. Exp. Bioly.* 221: jeb165373 doi: 10.1242/jeb.165373

van der Lingen, C. D. (1994). Effect of Particle Size and Concentration on the Feeding Behavior of Adult Pilchard *Sardinops sagax*. *Mar. Ecol. Prog. Ser.* 109: 1–13.doi: 10.3354/meps109001.

Visioli, F., Risé, P., Barassi, C. M., Marangoni, F., & Galli, C. (2003). Dietary Intake of Fish vs. Formulations Leads to Higher Plasma Concentrations of n-3 Fatty Acids. *Lipids*. 38 (4):415-8. doi: 10.1007/s11745-003-1077-x.

Westcott, P., & Henson, J. (2016). USDA Agricultural Projections to 2025.*USDA.* At https://www.ers.usda.gov/publications/pub-details/?pubid=37818

Williams, D. D., & Williams, S. S. **(**2017). Aquatic Insects and their Potential to Contribute to the Diet of the Globally Expanding Human Population. *Insects*.8(3). pp72. doi: 10.3390/insects8030072.

Womeni, H. M., Linder, M., Tiencheu, B., Mbiapo, F. T., Villeneuve, P., Fanni, J., & Parmentier, M. (2009). Oils of Insects and Larvae Consumed in Africa: Potential Sources of Polyunsaturated Fatty Acids. *OCL*, 16(4-5-6): 230–235. doi:10.1051/ocl.2009.0279

Wu, G. (2016). Dietary Protein Intake and Human Health. *Food Funct*. 7(3): 1251–1265. doi:10.1039/c5fo01530h

Xu, T., & Zhang, X.-H. (2014). *Edwardsiella tarda*: An Intriguing Problem in Aquaculture. *Aquaculture*. 431, 129–135. doi:10.1016/j.aquaculture.2013.12.001

Yang, B., & Kallio, H. P. (2001). Fatty acid composition of Lipids in Sea Buckthorn (*Hippophaë rhamnoides L*.) Berries of Different Origins. *J Agric Food Chem*. Apr; 49(4): 1939-47.doi: 10.1021/jf001059s. PMID: 11308350.

Yang, Z. H., Miyahara, H., & Hatanaka, A. (2011). Chronic Administration of Palmitoleic Acid Reduces Insulin Resistance and Hepatic Lipid Accumulation in KK-Ay Mice with Genetic Type 2 Diabetes. *Lipids Health Dis.* 10: 120. https://doi.org/10.1186/1476-511X- 10-120

Yeats, P. & Milligan, Timothy & Sutherland, Terri & Robinson, Shawn & Smith, J. & Lawton, Peter & Levings, Colin. (2005). Lithium-Normalized Zinc and Copper Concentrations in Sediments as Measures of Trace Metal Enrichment Due to Salmon Aquaculture. doi:10.1007/b136011.

Yeung, S. E., Hilkewich, L., Gillis, C., Heine, J.A., & Fenton, T. R. (2017). Protein intakes are associated with reduced length of stay: a comparison between Enhanced Recovery after Surgery (ERAS) and conventional care after elective colorectal surgery. *Am. J. Clin. Nutr.* 106 (1):44-51. doi:10.3945/ajcn.116.148619

Yvonne S. M., (2017). Research Report on China's Trash Fish Fisheries Greenpeace East-Asia 2017. *SWIMS.* At greenpeace.de/sites/www.greenpeace.de/ files/publications/2017_greenpeace_studie_china__jungfische_aus_ beifang_als_fischfutter_in_aquakulturen_engl.pdf

Zheng, L., Hou, Y., Li, W., Yang, S., Li, Q., & Yu, Z. (2013). Exploring the Potential of Grease from Yellow Mealworm Beetle (*Tenebrio molitor*) as a Novel Biodiesel Feedstock. *Appl. Energy*. 101:618–621. doi:10.1016/j.apenergy.2012.06.067