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Title: Comparing the nutritional quality of wild-caught salmon to two types of aqualcultured salmon

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Comparing the nutritional quality of wild-caught salmon to two types of aquacultured salmon

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Submitted in partial fulfilment of the requirements for

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Senior Thesis, Ludjelie Manigat

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ABSTRACT

Fish are an important source of protein and lipids. They are a good source of the essential fatty acids needed by the body, some of which cannot be synthesized. With the decrease in the wild-caught supply of fish, and growing world population and demand, aquaculture production has increased greatly. Salmon, a fatty fish, is extensively aquacultured and is consumed extensively. This study to look into the nutritional quality of aquacultured salmon, against that of wild-caught salmon. The results support that aquacultured salmon is at least equally nutritious as wild-caught salmon with respect to macronutrients.

INTRODUCTION

Fish holds many benefits for human nutrition. According to the American Heart Association, fish, especially oily fish, should be consumed at least twice daily. It is not only a good source of protein, it also is an important source of polyunsaturated fatty acids, such as omega-3 and omega-6 fatty acids (Burger & Gochfeld, 2009, Domingo *et al*., 2007). Some fatty acids cannot be synthesized in the body, but are needed by humans for maintaining health and preventing diseases. For example linoleic acid and linoleic acid (both of which are plant scarce) are needed in humans but cannot be synthesized, they must therefore be obtained from the diet. Those fatty acids are used in various way from the phospholipids making up the cell membranes, to playing a role in forming signaling molecules called eicosanoids. Many diseases are associated with the types of fatty acids present or absent in the body – making it important to ensure access to necessary fatty acids (Hunter & Roberts, 2000). For example, studies have shown low incidence of cardiovascular disease in populations with a high intake of fish, such as Alaskan Natives and Japanese people residing in fishing villages (Hu *et al*., 2002). In a clinical study using men with a history of myocardial disease, it was found that in the group that had an increased intake of fish oils, there was a 29% reduction in all-cause mortality (mortality resulting from any reason) observed (Zatsick, 2007). Studies also supports that omega-3 fatty acids (particularly eicosapentaenoic acid or EPA) reduced cholesterol levels and lowered the incidence of stroke, heart disease, coronary heart disease (CHD), arrhythmias, and thrombosis, blood clotting tendency, and of certain cancers both in healthy people and those at risk of suffering cardiovascular diseases, both males and females (Burger & Gochfeld, 2009; Domingo *et al*., 2007, Hu *et al*., 2002).

As fishing has become more industrialized, wild caught fish stocks have declined but demand for fish has remained high due to its positive reviews regarding health. In the past decades (1980-2001), average annual per capita seafood consumption increased from 14 kg to 30 kg per person in France for example. The amount of fish caught worldwide increased from the five million tons at the beginning of the 20^{th} century to almost one hundred million tons in the 1990s. To meet the demands of the growing world population, aquaculture production—fish and shellfish farming—has grown rapidly. While aquacultured fish and shell fish accounted for only 7% of the market supplying 1972, aquaculture currently supplies almost half of the global seafood demand (Henriques *et al.* 2014, Tidwell & Allan, 2001; Cahu, *et al*., 2004). The rate of salmon aquaculture has also gone up due to the increased salmon consumption of a global population (Foran *et al.* 2005). More than half of salmon sold around the world comes from aquaculture (Hites *et al*. 2004).

The story behind aquaculture is far from all positive. Several studies have focused on the negative effects of salmon aquaculture on the environment and their wild counterparts. There is controversy on the subject of salmon aquaculture, with claims that farming salmon is detrimental to wild caught salmon (such as causing them sea lice and disease). For example, while aquacultured salmon gets treated when sick, wild salmon do not, meaning that wild salmon is less likely to survive though the diseases that aquacultured salmon gets treatment for (Buschmann *et al.* 2012, Torrissen *et al.* 2013). There is also the issue of human exposure to various environmental contaminants. Various studies indicated that aquaculured salmon showed consistently higher levels of polychlorinated biphenyl (PCBs), polybrominated diphenyl ethers (PBDEs), and organic pesticides (except toxaphene) than the wild salmon. Those compounds have been linked to a greater risk for a number of health issues such as suppressed immune system and neurological damage. A study by Hites *et al*. showed that consumption of aquacultured salmon could end in consumer exposure to a variety of bio-accumulative chemical contaminants that can pose health risks. The major conclusion of that investigation was that aquacultured salmon contained higher concentration of organic contaminants than wild salmon (Domingo, 2009). Nevertheless, it possesses the potential to bring about great benefits to human nutrition and health. According to a Harvard Center for Risk Analysis, despite the risk of bio-contamination, reduced fish consumption would lead to a negative net public health impact.

Some species of fish, such as cod or turbot, have less than 1% of muscle fat and are (considered lean fish), whereas other species, such as Atlantic salmon, have more than 10% muscle fat and are considered fatty fish (Cahu *et al*., 2004). Since fish contain essential fatty acids that are needed for proper human development, the fattiest fish would correlate with the highest content of essential fatty acids. Indeed, when compared to fourteen species of fish salmon showed of the highest levels of EPA and docosahexaenoic acid (DHA) (Domingo *et al*., 2007). This information pinpoints salmon as a very nutritious fish, and makes this project relevant to potential impact on human nutrition and health of the switch from wild-caught to aquacultured salmon.

Aquacultured salmon are often subjected to specific formulated diets, meant to optimize growth while minimizing cost. Wild salmon on the other hand have diets based on marine fish and crustaceans. Some other projects have also have studied the effect of various diets on the resulting fish growth and composition (Hatlen *et al.* 2013, Azevedo *et al.* 2002), especially pertaining to the resulting fatty acid composition because of the health benefits associated with salmon and other fish (Schlechtriem *et al.* 2009). This project attempts to look at possible differences between wild-caught salmon and aquacultured salmon from two locations of the world (the Atlantic Ocean and the Pacific Ocean). While it does not focus on any specific factor that would lead to the possible differences, it should provide some insight into how much better (or worse) the aquacultured salmon option is compared to wild-caught salmon.

MATERIALS AND METHODS

The salmon was obtained from a local fishmonger: one that was aquacultured in the Pacific Ocean, one that was aquacultured in the Atlantic Ocean, and one wild-caught salmon. The pacific salmon was fed on an organic diet while the Atlantic salmon was fed a commercial feed bland. The fish were sliced into pieces approximately 0.5 cm x 2 cm x 1 cm in size. They were then lyophilized for 2 days. The freeze-dried samples were then crushed into a powder and stored in scintillation vials in the 4 degree C fridge until processed. Seven samples of each category (aquacultured – Pacific/Atlantic, and wild) were prepared. Small (0.3-1.0 g) amounts were aliquoted in aluminum foil weighing boat, dried at 60 °C overnight and then burned in a furnace at 500 °C overnight. The respective ashes were then weighed. The organic content or each sample was then calculated according to the following equation:

(Equation 1) $\% organic\ matter = \frac{dry\ mass - ash\ mass}{dry\ mass}$

A bomb calorimeter was used for calorimetric analysis of samples the three types of fish. Benzoic acid pellets were used as standard. Pellets the samples of the different categories (seven sample from each) were pressed using the crushed fish samples and burned following the bomb calorimetry protocol. The average was then calculated.

The elemental analysis was done using a Perkins Elmer 2400 EA. Small tin foil packets were prepared according to the standard protocol: Ten tin packets holding nothing to count blanks, fifteen packets containing about ~2mg of the K standard (acetanilide) to count as standards to calibrate the Elemental Analysis machine. Twelve packets of each experimental group, each containing ~2mg of fish sample, were then made. After calibrating the instrument, the samples were loaded into the carousel and auto-ran by a program. In programing the auto run, each packet was characterized by an ID (blank standard or sample), by the carousel position, by the Run ID and number, and by its mass except with the blanks. The instrument was blanked every twelve readings. The instrument automatically sent the data (compiled by Run ID and number) and computed the corresponding C/N ratio into a spreadsheet.

For protein extraction, buffer was first prepared from Tris HCl and Tris base, along with NaCl salt. The pH was set at 7.5. After aliquoting 50 mg of the fish power of the respective three categories, and adding 2.5 mL of 70% EtOH, and 7.5 mL of buffer, the mixture was vortexed, sonicated, and centrifuged so as to collect the supernatant where protein would end up. The extraction supernatant were then stored in the 4 ºC fridge until ready to be used.

Protein analysis was performed using a Pierce™ BCA Protein Assay Kit along with a microplate reader (Multiskan™ FC Microplate Photometer, from Fisher scientific). Standards (bovine serum albumin or BSA) of varying concentrations were prepared according to the protocol of the kit, after which the extraction samples made previously were used as unknowns for the assay. The stock protein solution (2 mg/µL) was provided by the kit, and the standards were prepared from the stock using serial dilution. Triplicates of each sample (five samples) and standards were mixed with bicinchoninic acid (BCA) according to the protocol of the kit, plated, and read on the microplate reader (filter 550nm). The absorbance of the standards were used to make a standard protein concentration curve (absorbance vs. concentration) which was then used to calculate the concentration of each sample. Equation 2 was used to calculate the concentration of the samples form the aquacultured fish and equation 3 was used to calculate the concertation of the samples from the wild-caught fish. The calculated concentrations were then averaged.

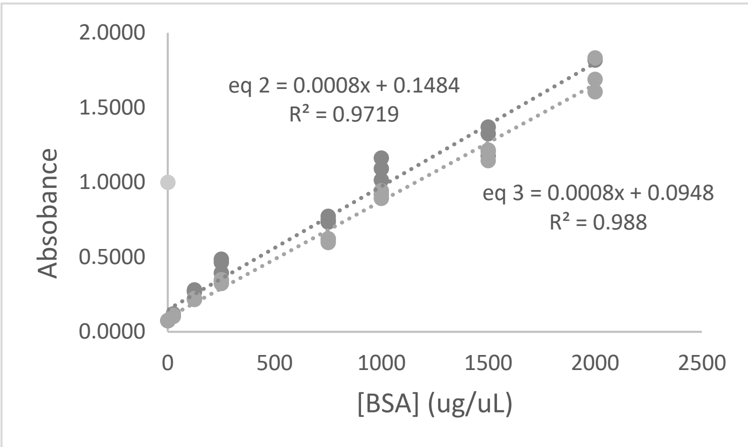

Figure 1. Standard curve generated using the stock BSA provided by the protein analysis kit. Two curves were generated – equation 2 for the aquacultured samples and equation 3 one for the wild caught samples. See equation 2 and 3.

(Equation 2) $y = 0.0008x + 0.1484$

(Equation 3) $y = 0.0008x + 0.0948$

Where x = absorbance read by microplate reader, and y = the calculated concentration

RESUTLS

A. Organic Content

Results show that the Atlantic aquacultured fish had on average a slightly higher organic content per mass of fish (~96.9 %), followed by the wild caught fish (~96.0 %) and then the Pacific aquacultured fish (~95.8 %) (Figure 2). There was a significant difference between the organic content of the three groups (Anova, df=20, F=5.520279, P-value= 0.013502).

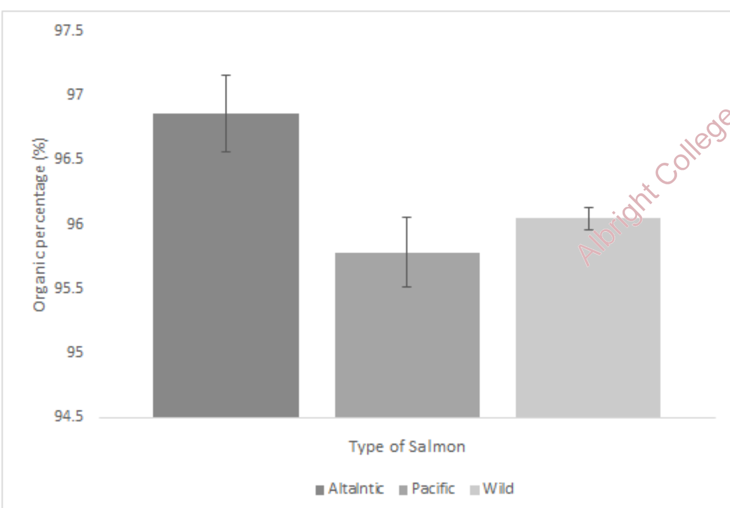

Figure 2. The average organic content of seven small freeze-dried samples of salmon categorized as wild-caught, aquaculture in the Pacific Ocean, aquaculture in the Atlantic Ocean.

B. Caloric Content

The instruments adjusted the heat reading for variation of mass of the pellets. On average, the samples from the Atlantic aquacultured group showed a higher calorie reading. The average for the wild-caught fish sample was slightly higher than that of the Pacific aquacultured fish sample (Figure 3). However, Anova analysis shows that there was not a significant difference in the calorie content between the three salmon groups (Anova, df=20, F=2.101949, P-value=0.151213).

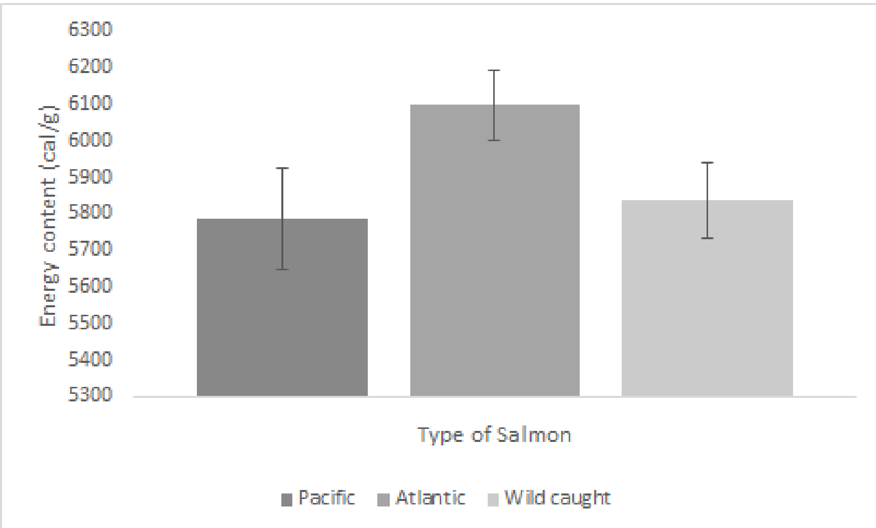

Figure 3. The average caloric content of seven small freeze-dried samples of salmon categorized as wild-caught, aquaculture in the Pacific Ocean, aquaculture in the Atlantic Ocean.

C. Elemental Analysis

Results show that there was not much difference in the relative amounts of carbon, nitrogen and hydrogen. In each case, the carbon amount was by far the highest value, followed then by the amount of nitrogen and then of hydrogen (Figure 4). Anova analysis supports that there was a significant difference in the carbon, nitrogen, and hydrogen (Anova, carbon - df=35, F=13.18548, P-value=6.19E-05; nitrogen - df=35, F=23.86403, P-value=3.89E-07; hydrogen - df=35, F=6.149592, P-value=0.00537).

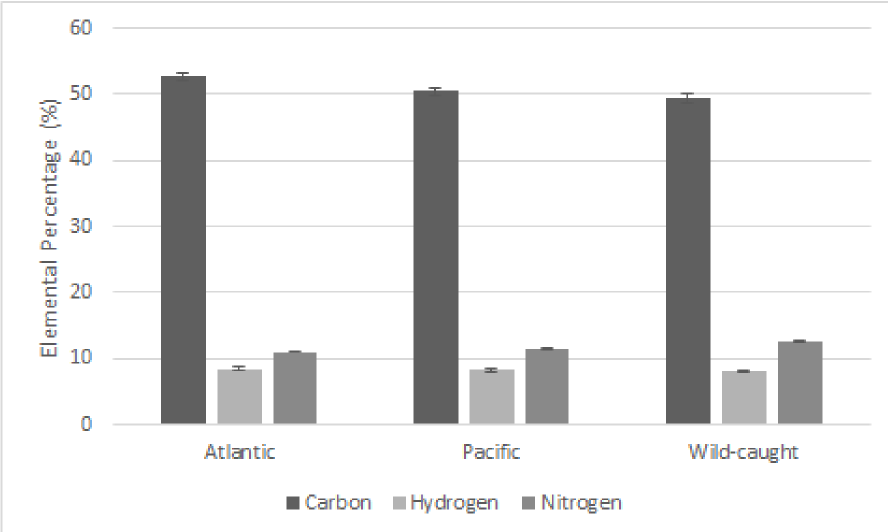

Figure 4. Average Amounts of Carbon, Nitrogen, and Hydrogen in the samples from the three different groups. 12 samples were analyzed in total.

Similarly to the average respective values of carbon, nitrogen and hydrogen, the average ratio of carbon to nitrogen of the 3 groups of sample were similar (Figure 5). Avova supported that there was a significant difference between the samples groups (Anova, df=35, F=18.02743, P-value=5.11E-06).

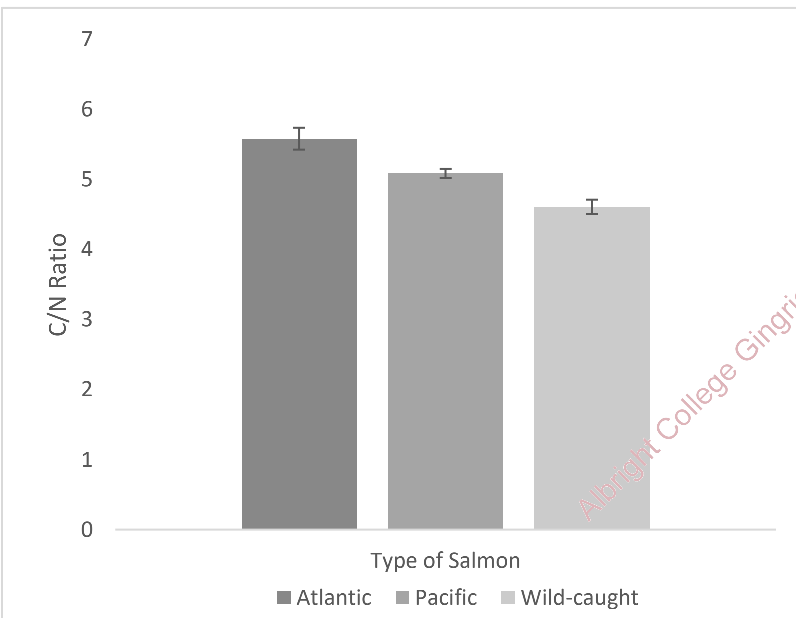

Figure 5. Average carbon to nitrogen ratio for each of the three sample groups

D. Protein Content

Results show that there was not much variation in the protein concentration of the sample from the Atlantic and Pacific aquacultured fish. The protein concentration of the wild-caught samples were consistently lower than those of the aquacultured fish samples. Accordingly, the average reflected those results (Figure 6). The difference in the results were significant (Anova, df= 44, F=2918549, P-value= 1E-108).

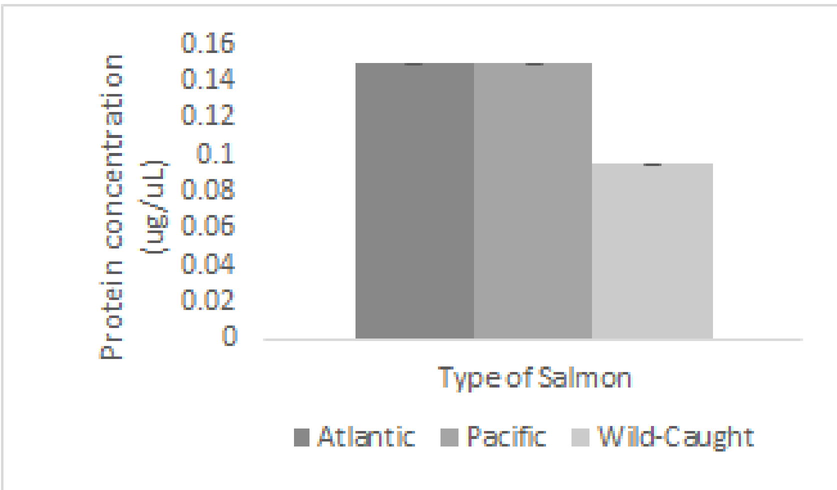

Figure 6. The average protein content (in ug/uL) of five small freeze-dried samples of salmon categorized as wild-caught, aquaculture in the Pacific Ocean, aquaculture in the Atlantic Ocean.

DISCUSSION

On average, the organic macromolecule content of the three types of fish analyzed was very close, with the salmon aquacultured in the Atlantic having a slightly higher percentage in organic content. As expected, the samples from the salmon aquacultured in the Atlantic also showed the highest average caloric content. Those results are consistent with each other since more organic matter means more fuel to be burned is present. The samples from the wild-caught fish showed the second highest caloric content and organic percentage per mass, with the samples from the fish aquacultured in the Pacific last. The anova test showed that only the organic content was the significant. This could imply that other factors affect the calorie content. The error bars indicated a great deal of variation, which is consistent with that possibility.

The Elemental Analysis results indicate that the variation in relative amounts of carbon (C), nitrogen (N), and hydrogen (H) in the samples from the three groups compared is significant. The ratio of carbon to nitrogen (C/N) data was also significant, and the C/N ratio was slightly higher in the Atlantic group, which is in accordance with the organic content results: more organic matter (molecules based on C) would correlate with higher amounts of carbon in the fish. This data further supports the benefits of aquacultured salmon, especially which raised in the Atlantic Ocean. In terms of elemental composition, aquacultured fish thus seem to provide more (especially the Atlantic salmon) than the wild-caught fish.

The differences in protein concentration results were significant. According to the protein concentration data the wild-caught salmon consistently showed the least amount of protein per mass. Fish are considered a valuable source of protein which is an important dietary requirement. Therefore, the higher the protein content, the better the product (provided everything else is kept the same). This data points to the aquaculture salmon providing the most benefits (both aquacultured fish essentially had the same average protein content). The higher protein content is also reflected in the C/N ratio data, which showed that the Atlantic aquacultured salmon had the highest values, followed by the Pacific aquacultured salmon and then the wild-caught salmon. Since Atlantic aquacultured fish are fed a specially formulated diet, it is likely that the formula used to nourish the Atlantic aquacultured salmon is richer in protein than what the wild caught salmon ate while free (wild salmon feeds on plankton, benthos and other fish, and Pacific aquacultured salmon is fed an organic diet). The main ingredients of these feeds is fishmeal manufactured from species that are most used for human consumption and so protein content is likely to be high (Cahu *et al*., 2004). When raised in captivity (or aquacultured) humans have much better control on the dietary intake of the fish, which will be reflected in the macromolecules present in the fish.

So far, the samples from the Atlantic salmon displayed the highest organic matter, caloric content, and protein content per mass of fish, all of which speak in the favor of the quality of such fish. A lipid analysis would be a next logical step of this study. With the increased consumption of aquacultured fish, it is necessary to verify that the aquaculture process does not significantly affect the fatty acid composition of the produced fish. Exposure to different diets, could lead to different fatty acid profiles. As mentioned before, fatty acids are required for proper human metabolism, and fish have be known to provide those fatty acids. A study by Cahu *et al*. (2004) showed that while aquacultured fish expressed generally higher total lipid content, they also expressed generally lower EPA and DHA levels in aquacultured fish, when these values are expressed relatively to total fatty acids. That data also supports that aquacultured fish can provide consumers a nutritional composition that is at least as beneficial as that provided by wild fish (Cahu *et al.*, 2004). In terms of macromolecules, the content of the Atlantic salmon were significantly higher but not necessarily biologically relevant. In other words, while the differences are present, in the long run, all three types fulfill the dietary requirement.

Micronutrients (such as minerals and vitamins) provided by the fish need to be analyzed as well. Micronutrients are also crucial to proper human development and so contribute to the overall nutritional quality of the fish. For example, iron, a micronutrient, if an essential part of red blood cells and the process of oxygenating our blood. The next step would be to increase the sample size of each type of see if the trends remain or if new trends appear. Further research also need to be done on the lipid analysis and the effects on diet on lipid composition but so far it seems aquacultured fish is just as good, if not better, than wild-caught fish in terms of nutritional quality.

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