Effect of Fish Consumed on the Level of Mercury in Fishermen Hair

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Abstract

Total and methyl mercury levels were examined in fishermen and non-fishermen in the fishing village of Doha, Kuwait in order to answer the research question posed 'does the amount of fish consumed have an effect on mercury levels in the hair of fishermen'. Previous studies have shown that fish consumption is related to increased levels of mercury in people from different regions throughout the world. I analyse the independent variables that are fish meals eaten per week, parts of the fish eaten, height of subjects, weight of subjects, residence time living in Kuwait, and age of subjects by using a combination of correlation tests, median tests, and constructing a generalised linear model. I find that total and methyl mercury in fishermen are significantly correlated ($\rho = 0.9975$, p < 0.0001), weight is significantly correlated with both forms of mercury, and those who eat more than seven fish meals per week have higher median levels of total mercury (p < 0.10) than those who eat seven meals per week, and those who eat 7 fish meals per week have higher total mercury levels than those who eat less than seven fish meals per week (p < 0.10). Parts of fish eaten was difficult to infer results from. The generalised linear model confirmed that fish meals per week was a significant variable, as well as weight of the subjects. There is enough statistical evidence for us to conclude that fish consumption is related to mercury levels in fishermen. I will pay particular attention to the independent variable residence time, as the research question posed asks to find a relationship between mercury levels amongst all fishermen, not just fishermen in Kuwait. This implies that residence time and mercury levels should ideally be independent.

1 Introduction

Mercury is widely recognized as a harmful substance, causing lasting issues including male impotence, brain damage, and problems with the nervous system (Dickman and Leung, 1997). Methyl mercury, in particular has been recognized as harmful to humans, and has been examined to be found in large amounts in seafood. Many factors have been found to relate to the amount of mercury contained within each fish - this includes the age of the fish, the size of the fish, the breed of the fish, the dietary habits of the fish, and the location the fish resides. Fish that tend to have the highest levels of mercury in other literature were larger, older predatory fish that reside near industrial zones (Myers et al., 2007).

Several studies (e.g. Dickman and Leung, 1997; Myers et al., 2007) have examined the effects of fish consumption on total and methyl mercury levels in humans, and furthermore, the negative effects that are related to having such high levels of mercury. One paper that examined the fish consumption of groups located in both Seychelles and the Faroe Islands, found that the group with higher fish consumption, and less marine mammal consumption had on average higher rates of methyl mercury (Myers et al., 2007).

Another study by Giangrosso et al. (2016) examined total mercury levels of fishermen in Sicily, using similar methods that will be covered in this report. They found that fishermen consuming fish more than five times per week had significantly higher hair mercury concentrations than those who ate fish less than three times per week. Additional findings were that mercury concentrations in fishermen's hair had levels of 6.45 $\pm$ 7.03 μg/g. It further discovered that fishermen from the more industrial areas of Siciliy had higher concentrations of mercury compared to those from the less industrial areas. In addition to this, those consuming seafood containing higher levels of mercury were also shown to have higher concentrations. It also claims that fishermen in Maderia (Portugal) were found to have a mean total mercury level of 39.76 μg/g, and was attributed to their favoring of mercury-rich fish (Giangrosso et al., 2016). A study taking place in Hong Kong by Dickman and Leung (1997) found that individuals consuming fish 4 or more times per week had much higher mercury levels than those consuming fish 4 or less times per week. It also examined a significant relationship between age and mercury levels found in hair samples - claiming that at age 30, the average Hong Kong male will have 3 μg/g of mercury in their hair, but by age 60 this amount would have increased to about 7.5 μg/g .

Given these studies and other publications researching total and methyl mercury in relation to fish consumption, I will conduct median tests to investigate whether fishermen consuming different levels of fish have significant differing medians of total and methyl mercury, as well as examining other important features that may play a role in determining mercury levels. I hypothesize I will find a significant association between fishermen who ate more parts of the fish and total/methyl mercury as well as fishermen who eat fish more often and total/methyl mercury.

2 Methods

2.1 Sampling of Data

100 hair samples were collected from fishermen residing in Doha Fishing Village, Kuwait. A further 35 samples were collected from men working for a local construction company. 2-3 g of were taken from several areas on the scalp. These were then placed in a polyethylene plastic sampling bag accordingly, and stored in a deep freezer ready for the analysis stage. In order to control for hair

length, hair samples were collected from fishermen during the same time period of hair production. The dietary habits of the fishermen were assessed using a questionnaire. The questionnaire asked about their age, nationality, smoking habits, health status with reference to dental problems. These were asked in the case there were any additional relations to methyl and total mercury levels. Questions relevant to fish consumption assessed the number of fish meals per week, quantity of fish meal, source of the fish, and parts of the fish usually eaten. Height and weight were measured as part of the data collection procedure (Al-Majed and Preston, 1999).

2.2 Sample Preparation

Hair samples were cut into short segments and washed successively with acetone and water. Samples were separated by centrifugation and dried in a laminar flow hood (Al-Majed and Preston, 1999).

2.3 Analysis of Total Mercury

Around 0.2 g of the dry sample was digested with 4 ml of nitric acid and 2 ml of sulfuric acid, for 2 hours at room temperature and then 3 hours at 90°C. The final determination was carried out using a PSA Hydride Vapor Generator connected to an Atomic Fluorescence Detector Model 10.023. A 20% Tin(II) Chloride solution was used as a reducing agent and 1% nitric acid solution was used as a blank. Argon was used as the carrier gas. The accuracy of analysis was checked by running four samples of Sample Reference Material (SRM) with each batch of samples (set of 25 samples). Recovery was between 94.6% and 98.9%. Six blank samples were also run with each set. The results show undetectable levels of total mercury in all of the analysed blank samples. In order to check the replicability of the analysis, 23% of the samples were analysed in triplicate and one SRM out of four was also treated in the same manner. The coefficient of variation was between 0.08% and 2.95% (Al-Majed and Preston, 1999).

2.4 Analysis of Methyl Mercury

The accuracy of 0.2 g of dry sample was analysed in accordance with the UNEP (1987) method. The final determination was carried out on an HP Gas Chromatograph Model 5890 Series II, equipped with a glass column of 1.6 m length and 2 mm internal diameter, packed with 5% DEGS-PS (diethylene glycol succinate modified with phosphoric acid) on 100-120 mesh (Supelco) and an electron capture detector. Nitrogen was used as the carrier gas. The accuracy of analysis was checked by running two SRMs with each batch of samples (set of eight samples). The recovery was in the range 92.4-105.8%. Two blank samples were run with each set. The results show undetectable levels of methyl mercury. In order to check the replicability of the analysis, 18% of the samples were analysed in triplicate. The coefficient variation was 0.40-4.01% (Al-Majed and Preston, 1999).

2.5 Statistical Methodology

I disregard the sampled control group of 35 individuals that were not fishermen, as the research question seeks to examine a relationship between fish consumption and fishermen only. First, I perform a Shapiro-Wilk normality test on the dependent variables methyl and total mercury. I conducted this to see whether I would be able to perform statistical tests assuming a normal distribution. I then perform exploratory data analysis on the variables weight, residence time, parts of fish eaten, and fish meals per week. I disregard age and height as I believe that they are unrelated to fish consumption, and are not irrelevant to the research question. If applicable for each of the considered variables, I examine Spearman's correlation coefficient to examine the amount of effect this variable has on the level of methyl mercury, and how statistically significant this effect is by testing using a a rank-order correlation. I use this method rather than Pearson's correlation as the dependent variables (methyl and total mercury) do not follow a normal distribution as stated. I then perform Mood's median test to examine a statistically significant relationship between the amount of fish consumed and methyl and total mercury levels. Following this, I construct an optimised generalised linear model, with a gamma link function to look at which independent variables are most significant when predicting mercury levels, to further examine findings from the data analysis and statistical tests performed. Finally I construct a matrix of Spearman's correlation values, to gain further insight in how the independent variables may be related to eachother, and their subsequent effect on mercury levels.

3 Results

The questionnaire results provided showed that the fishermen were in good health, and all Egyptian. None had dental problems or fillings, however 99% of them smoke 3-5 cigarettes per day. It was stated that there is no favourite type of fish, as they usually eat what is available from their daily catch. I perform statistical analysis on all 8 independent variables and how they relate to the 2 dependent variables (total mercury and methyl mercury).

3.1 Total vs Methyl Mercury

Looking at total and methyl mercury levels, in figure 1, they appear follow a very similar distribution.

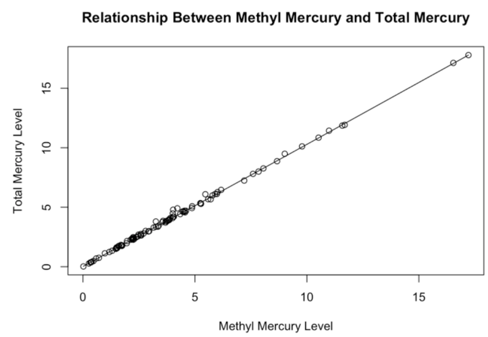
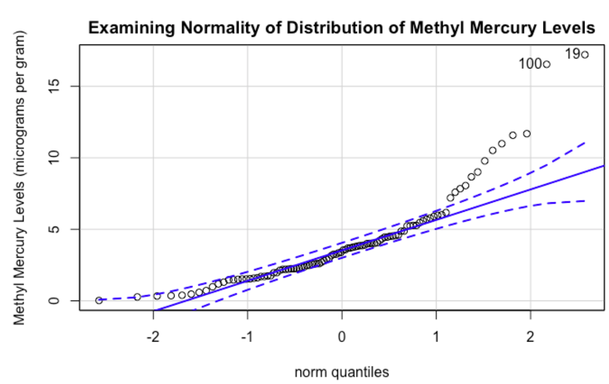


Figure 1: Statistical Relationship between Methyl and Total Mercury

The mean of methyl mercury in the fishermen group was found to be 4.015 ± 3.13 μg/g, while the mean total mercury was found to be 4.181 ± 3.22 μg/g. From viewing figure 2 it is clear that these two variables do not follow a normal distribution. I conducted a Shapiro-Wilk normality test to further confirm this ($p < 0.0001$ for both methyl and total mercury).



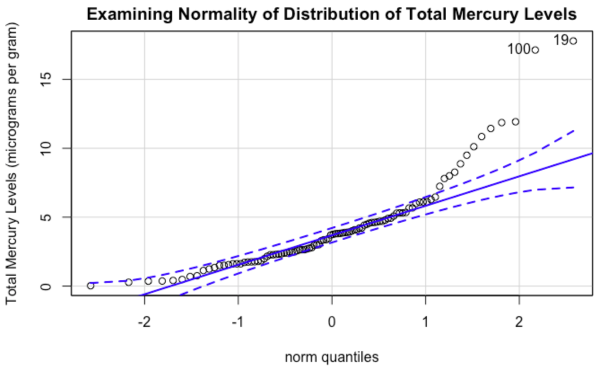


Figure 2: Q-Q plots Showing a Lack of Normal Distribution in Methyl and Total Mercury Levels

The two variables were found to have a significant relationship ($p < 0.0001$), with 99.75% of fluctuation in the levels of methyl mercury being explained by the level of total mercury ($\rho = 0.9975$) after examining Spearman's correlation. There are two distinct outliers in terms of both methyl and total mercury levels - with both having over 16 μg/g in both areas. For the first anomaly, the fisherman consumed large quantities of fish (21 fishmeals per week), while the second only consumed 4 fish meals per week but ate the whole fish. Given that these two variables are so highly correlated, I will mainly examine just methyl mercury when considering the independent variables.

3.2 Residence Time

Residence time measures the time that the sample person has been residing in Kuwait. The relationship between residence time and mercury levels is important to consider, as we are interested in the mercury levels of fishermen in the general population, rather than in the data provided. If I was to discover a significant relationship between residence time and mercury levels, we would not be able to use the results from this study as a point of general inference for all fishermen. Examining the data shows it has huge range, with a minimum of 0 years and maximum of 25 years. The median is 3.00 years and mean is 5.36 ± 5.80 (2dp).

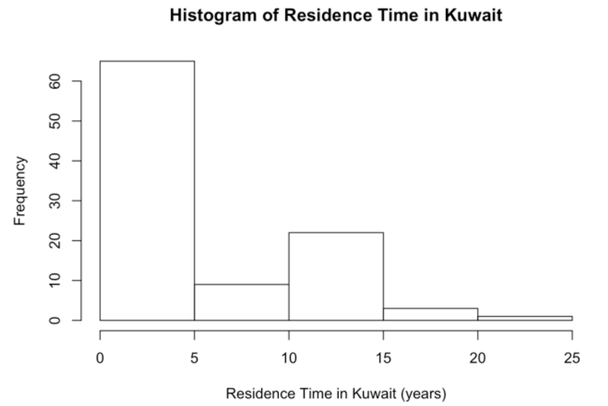


Figure 3: Distribution of Residence Time in Kuwait (Not Normally Distributed)

There does not appear to be any association between duration of residence and levels of methyl mercury in the scatterplot below. Because residence time is not normally distributed, I am unable to use Pearson's method of testing correlation using a t-statistic. Instead, I use Spearman's rank-order correlation. Results of this showed that mercury levels and residence time in Kuwait were not correlated (p-value = 0.6999 (4dp) for methyl mercury, and p-value = 0.5995 (4dp) for total mercury).

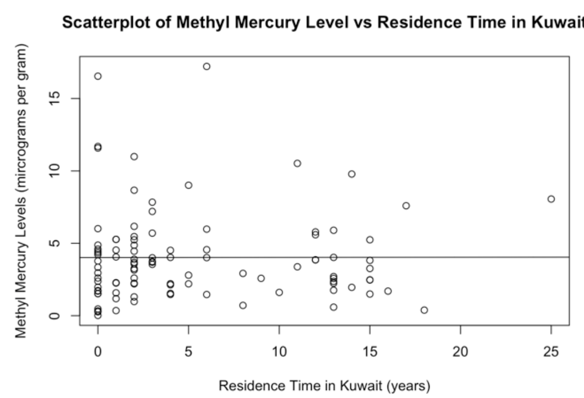


Figure 4: Statistical Relationship Between Methyl Mercury and Residence Time in Kuwait

3.3 Weight

Weight was approximately normally distributed in the sample. It had a minimum value of 59.0 kg, and a maximum of 92.0 kg. It had median a value of 72.5 kg and mean of 72.8 ± 7.01 kg.

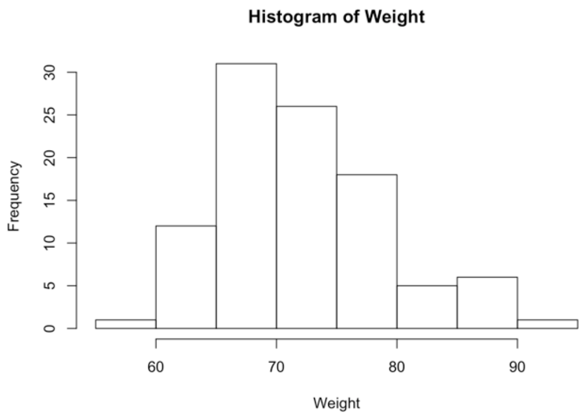


Figure 5: Distribution of Weight

Given the normal distribution of weight (as visible in figure 5), I was able to find a correlation and test the statistical significance to examine whether a relationship exists between weight and methyl mercury. The test yielded a correlation coefficient of $\rho = 0.4010$, meaning that 40.10% of variation in methyl mercury levels could be explained by the weight of the individual. This was found to be significant at the 1% significance level ($p < 0.0001$).

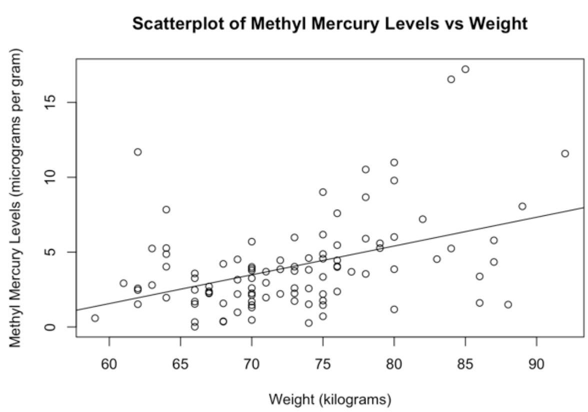


Figure 6: Statistical Relationship Between Methyl Mercury and Weight

3.4 Parts of Fish Eaten

In the data provided there were three possible options for what parts of the fish the consumer had eaten: muscle only, muscle only and sometimes the whole fish, and the whole fish. Of the 100 fishermen examined, 19 of them ate the muscle only, 72 ate muscle only and sometimes the whole fish, and 9 ate the whole fish, as seen in figure 7.

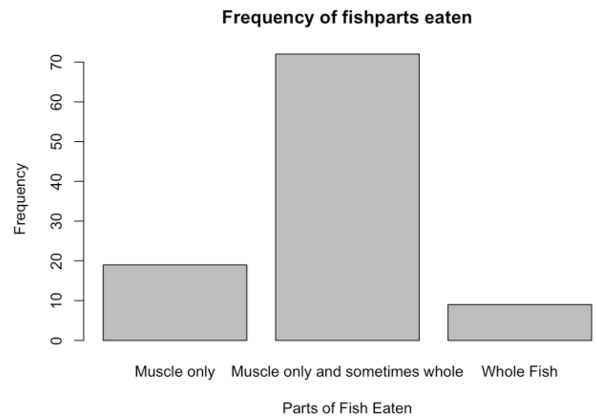


Figure 7: Histogram Showing Frequency of Fishparts Eaten

The group who ate muscle only and sometimes the whole fish had the lowest median and mean levels of both total (3.406 μg/g and 3.671 ± 2.41 μg/g) and methyl (3.256 μg/g and 3.542 ± 2.36 μg/g) mercury levels respectively. The group with the second-highest levels of total and methyl mercury was the group that routinely consumes the whole fish. This group showed median and mean values of methyl mercury levels of 3.820 μg/g and 4.889 ± 4.576 μg/g respectively. For total mercury these levels were 3.901 μg/g and 5.076 ± 4.73 μg/g. The group showing the highest levels of total and methyl mercury were the group who consume muscle only. These people had median and mean levels of methyl mercury of 4.026 μg/g and 5.446 ± 4.373 μg/g respectively. In terms of total mercury they had a median of 4.208 μg/g and a mean of 5.689 ± 4.51 μg/g.

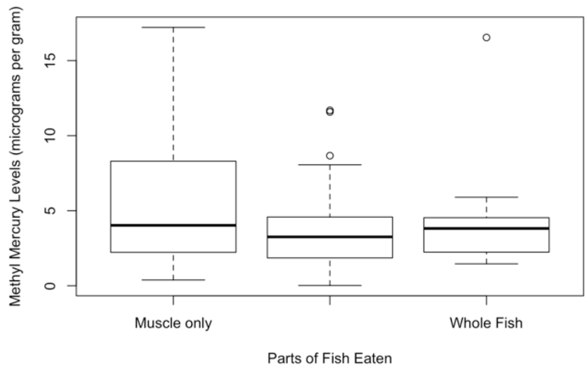


Figure 8: Boxplots Showing Different Distributions of Methyl and Total Mercury by Parts of Fish Eaten

Using this data I was able to conduct a Mood's median test to compare whether there was a significant difference in medians of samples. I used this method rather than an ANOVA test due to the fact that ANOVA assumes a normal distribution among samples, and I could not assume this due to some sample sizes being less than 30 (e.g number of people consuming the whole fish). The medians also look to have a more distinct difference than means in this sample. Mood's median test showed no significant difference between medians of Fishermen consuming different parts of the fish (p-value $= 0.668 > 0.05$) for both methyl and total mercury levels.

3.5 Number of Fish Meals Eaten per Week

In the data provided there were five frequencies in which people identified themselves in. Out of the total sample of 100 fishermen, 2 claimed to eat 3 fish meals per week, 12 claimed to eat 4 per week, 70 claimed to eat 7 per week, 5 claimed to eat 14 per week, and 11 claimed to eat 21 per week. Figure 9 illustrates the data transformation undertaken before analysis.

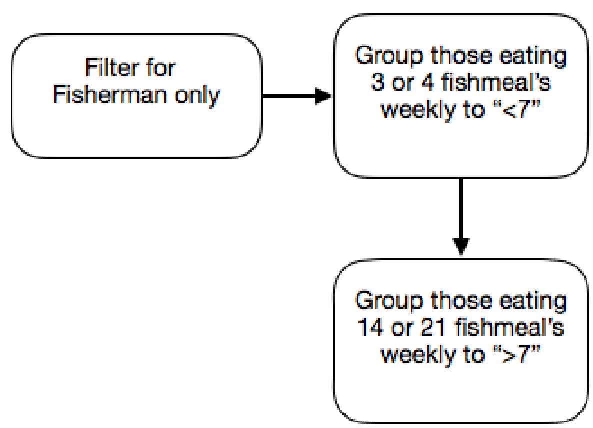


Figure 9: Flowchart Showing Data Transformation of Fish Meals Per Week Variable

The two outlier values of methyl and total mercury values may have skewed the data a fair amount considering that they are both present in the groups that had the lowest amount of observations (< 7 and > 7 fish meals per week). For this reason I have chosen to look at the median values of groups, as this measure is more robust to outliers.

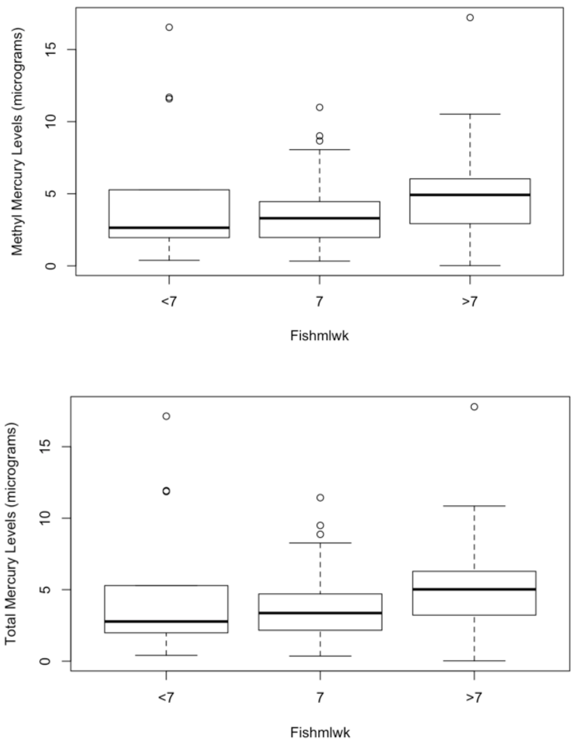


Figure 10: Methyl and Total Mercury Levels by Fish Meals Eaten per Week

It is clear from looking at figure 10, that those eating less fish per week tend to have lowest levels of mercury - those that consume less than 7 fish meals per week had median levels of total and methyl mercury of 2.643 μg/g and 2.773 μg/g respectively. Those consuming 7 fish meals per week have median levels of total and methyl mercury of 3.301 μg/g and 3.364 μg/g respectively. And finally those with the highest levels of total and methyl mercury were the group consuming more than 7 fish meals per week (medians of 4.921 μg/g and 5.022 μg/g respectively). I again conducted a Mood's median test to analyse differences of medians. I used this method for the same reasons as stated when analysing parts of fish eaten. The median test revealed an insignificant relationship between the number of fish meals eaten and methyl mercury levels (p-value $= 0.251 > 0.05$), but a significant relationship between number of fish meals and total mercury levels (p-value $= 0.091 < 0.10$).

3.6 Building a Model of Most Relevant Features

A final statistical method of analysis was to build a generalised linear model of the most informative independent variables used to predict the either methyl or total mercury. Figure 11 shows the steps taken to pre-process the data.

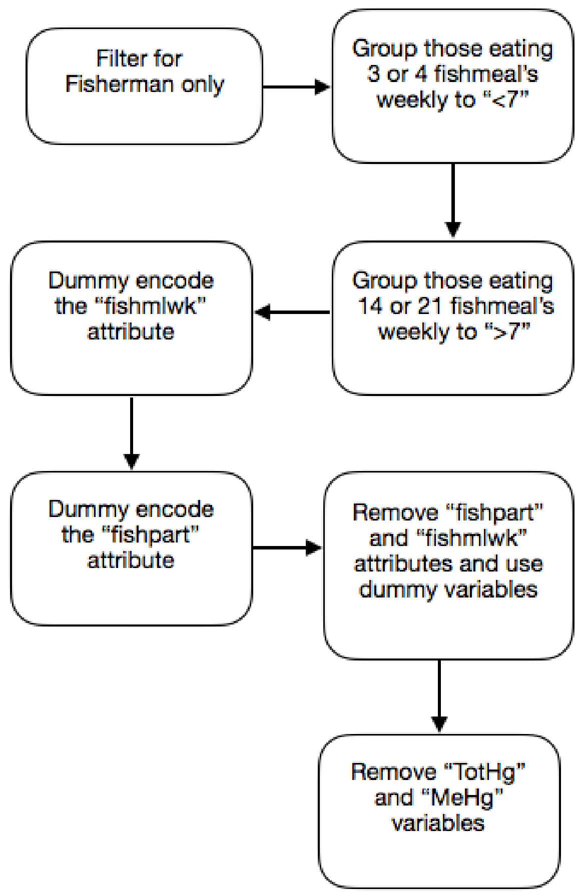


Figure 11: Flowchart of Data Transformation for Generalised Linear Model Use

This then was used in 2 generalised linear models, with methyl mercury and total mercury as the predictors respectively. I used AIC with backwards stepwise selection to find the optimal model, considering the family parameter to be from a gamma distribution. I chose to use gamma as the parameter due to the high positive skew of methyl and total mercury (Phillips, 2018). AIC was used as it considers the number of independent variables when selecting the optimal model (meaning that uninformative variables will be omitted). I consider this important, as the research question specifically wishes to identify a relationship between fish consumption and mercury levels, so it's inclusion or disclusion in the final model relative to other features is of interest. The generalised linear model that modelled methyl mercury found that the most informative variables were whether fishermen ate either more than or less than 7 fish meals a week, and the height and weight of the fishermen (AIC value 450.21 (2dp)) Of these variables, only weight (p-value <0.0001), < 7 meals per week (p-value = 0.064 < 0.10) and > 7 meals per week (p-value = 0.059 < 0.10) were found to be significant at the 10% significance level. The model considering total mercury found the same set of variables as optimal, but with a slightly higher AIC value (456.68 (2dp)) and p-values as < 0.0001, 0.063 and 0.055 respectively.

3.7 Collinearity of Features

Given that there are a collection of 6 difference independent variables, it is highly likely that there is some element of collinearity between some of these features. For example there is a very well-defined correlation between weight and food consumption (Gunnars, 2018) - so these variables are clearly not entirely independent of one another. Collinearity is important to consider, as it implies that the independent variables which we are most examining (consumption of fish) may be related to more than just the dependent variable(s). Below is a covariance matrix showing the collinearity of features (darker points mean a stronger correlation, and larger dots imply a more significant relationship).

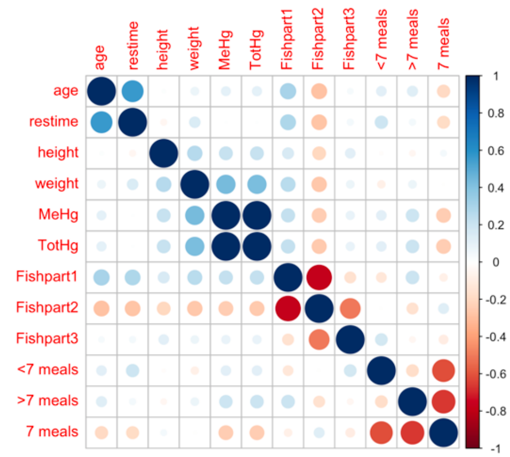


Figure 12: Correlation Matrix Showing Relationship Between Variables

Figure 13 shows the transformation of data undertaken to mould the data into a suitable format.

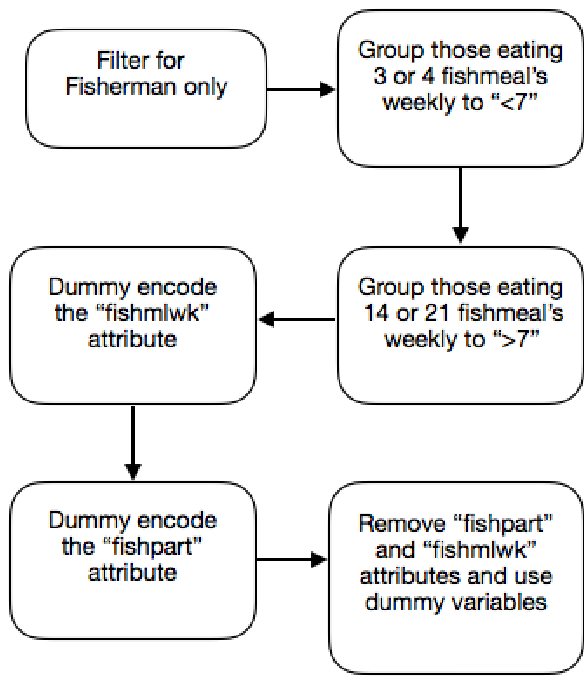


Figure 13: Flowchart Showing Data Transformations to Create Correlation Matrix

We can see that weight seems to have a fairly strong positive correlation with total and methyl mercury levels (as examined in section 3), as well as a positive relationship with eating more meals. It also had a positive (but weak) relationship with fishpart 2, meaning that those who weigh more are more likely to either eat the whole fish, or the muscle tissue only. This could lead us to infer that rather than weighing more being related to having more methyl mercury, it could rather be that people who weigh more tend to eat more fish, and therefore have higher levels of mercury. It is known that people who weigh more tend to eat more in general, and may be getting their mercury intake from foods other than just fish.

4 Discussion

4.1 Fishpart and Fish Meals Eaten per Week

Via analysis in section 3 I have deemed that the amount of fish meals eaten per week plays an important role in the methyl and total mercury levels in fishermen in Kuwait, with more fish eaten per week meaning higher levels of methyl and total mercury. Mood's median test found a relationship between only total mercury and fish meals per week, however the generalised linear model found a relationship between fish meals per week and both types of mercury. Given the weaker statistical power of Mood's median test, I do believe there exists a relationship between fish meals per week and both forms of mercury in fishermen. I haven't found a statistically significant relationship between parts of fish consumed and mercury levels in fishermen.

4.2 Comparison With Previous Findings

The findings in section 3 are consistent with previous scientific findings in the area (Giangrosso et al., 2016). It had been well established that methyl-mercury and total-mercury levels had been related to fish-consumption across studies such as those conducted in the Faroe Island and Seychelles. The study conducted in Sicily had also reported that fishermen eating more than 5 fish-meals per week had significantly higher hair-mercury concentrations than those who ate fish less than three times per week.

4.3 Limitations

Given that there is a diverse range of fish residing in Kuwait (Fishbase, 2012), this could mean that some fishermen are consuming very mercury heavy fish, while some are eating fish with small amounts of mercury. Although the fishermen stated that they had "no preference for fish", some fishermen may subconsciously avoid different types of fish, such as those low in mercury. This could mean fishermen could consume large amounts of fish, yet still have low levels of mercury.

There is also little information as to what constitutes a 'fish meal', as this could range from anything from a single oyster to a large portion of fish. Furthermore, there have been a number of other foods - such as fructose corn-syrup which have been found to have high concentrations of methyl-mercury (Dufault et al., 2009), which could be skewing the results, making the results of my analysis less accurate. Another limitation is that there is no reported duration on how long the fishermen had been following their reported levels of fish consumption - for example a fishermen that reported eating 21 fish meals per week may have only been doing so for 6 months, while another may have been consuming this level for 5 years. Other limitations include the small sample size, meaning that reliable mean-testing could not be considered due to the lack of application of the central limit theorem. A preferred test would have been an ANOVA test when considering mercury levels among different fish consumption levels. Another preferable test that could have been performed was a Wilcoxon signed-rank test, which does not need to assume a normal distribution of data. However this can only test for differences from two populations at a time, whereas both fishpart and fish meals per week were split into three categories, so this test was unfeasible. The alternative used in this paper - Mood's median test is known to be not as statistically powerful as other mean/median tests that could not be applied (Glen, 2016).

4.4 Further Research

To examine the exact question "Does the amount of fish consumed have an effect on mercury levels in the hair of fishermen", I would suggest to collect samples from a control group - fishermen who eat no fish at all in order to be able to conduct difference of means tests - such as a t-test - to examine whether those who did eat fish had higher mercury levels than those who did not eat fish. A larger amount of samples under the same original conditions could also be gathered in order to apply more efficient statistical testing techniques.

4.5 Conclusion

Through statistical techniques I find a significant relationship between the number of fish meals eaten per week and total mercury levels in the fishermen of Kuwait ($p < 0.10$). I did not find a statistically significant relationship between the parts of fish eaten and mercury levels, although I would have expected to see that those eating the whole fish would have higher mean and median values of methyl and total mercury levels. Through constructing a generalised linear model, I find that the most informative subset of features were the height and weight of the fishermen, and whether they ate more than or less than 7 fish meals per week. Through analysis of correlation using Spearman's rank-order test, I find that a significant correlation between residence time of fishermen in Kuwait and mercury levels does not exist ($p > 0.10$), so the dataset of fishermen in Kuwait should act as an accurate inference of fishermen in the general population. I also discovered a very statistically significant relationship between weight and methyl mercury ($p < 0.0001$) , and from figure 12 there appears to be a statistically significant positive correlation between weight and eating more than 7 fish meals per week. This led us to infer that weight and fish meals per week are collinear in predicting mercury levels.

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