MORPHOLOGICAL CHARACTERISTICS AND PROXIMATE ANALYSIS OF THE SARDINE (Sardina pilchardus Walbaum, 1792) IN THE NORTHERN AEGEAN SEA (GULF OF KALLONI, LESVOS)

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Abstract

The unique hydrological and biological characteristics of Kalloni Bay (Panayotidis and Klaoudatos, 1997) could affect the morphological and chemical characteristics of economically important pelagic species, such as *S. pilchardus*. Aim of this preliminary study was to investigate the morphometric and proximate characteristics of *S. pilchardus* speciments caught in Kalloni Bay and to compare them with speciments from open sea areas around Lesvos Island during a five-month period (mid-May to mid-October 2017). There was a significant difference on both the length and the weight of the individuals and the homogeneity of the samples between the Kalloni Gulf and the open sea areas. Variations for both areas were evident among different morphological characteristics of the speciments and the constituents of the proximate analysis that were determined during the sampling season. The morphology of *S. pilchardus* in the Kalloni Gulf along with the condition indices that were calculated and the proximate characteristics that were found during the laboratory analysis indicate that the individuals might be of the same population but at a different maturity state. Further research of this species in both the Kalloni gulf and the open Aegean Sea is substantial due to the economic importance of *S. pilchardus*, especially in areas having a nursery role, and will provide further useful information on the possible effects of hydrological and biological characteristics in the Kalloni Gulf.

Keywords: European sardine; North Aegean Sea; Morphological characteristics; Length-to-weight relationship; Hepatosomatic Index; Proximate analysis; Water content; Ash content; Bligh and Dyer method; Kjeldahl method;

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1. Introduction

The European sardine, *Sardina pilchardus* (Walbaum, 1792) is one of the most abundant and commercially important fish species in the Mediterranean Sea (Antonakakis et all, 2011). It is an omnivorous species, essentially filter feeder, with a diet mainly composed by mesozooplanktonic organisms (Nunes et all, 2015). Despite the fact that the Aegean Sea is generally characterized by oligotrophic waters, its northern sector presents high levels of productivity due to the outflow of the Black Sea Waters, which enter the Aegean Sea through the Dardanelles Strait as a surface current. This increases local productivity and induces high hydrological and biological complexity, characterized by high concentrations of mesozooplankton, and therefore high food availability for small pelagic fish (Antonakakis et all, 2011). The widely different environmental conditions of temperature, salinity, pressure, availability of food etc., profoundly influence the biochemical composition of the fish. There may be species specific or even group specific differences in the biochemical composition and morphology of *S. pilchardus* (Balock et all, 2017).

Lesbos Island is located in the northeastern Aegean Sea. Its Southwest side is intruded by the gulf of Kalloni which is the biggest natural gulf of the island. It is generally shallow with many of the most significant river outflows of the island at its Northeast side. Temperature and salinity are different in the gulf compared to the open sea and show a gradient according to the distance from the opening. The gulf shows characteristics of a higher eutrophic level than that of the rest oligotrophic North Aegean Sea. This is the result of its topographic characteristics and also anthropogenic factors such as tourism and agriculture (Panayotidis and Klaoudatos, 1997).

Taking into account the unique hydrological and biological characteristics of the gulf along with the importance of small pelagic species there is an increasing interest on the effects of those environmental conditions on the morphology and chemical characteristics of the fish compared to those of the open sea. The aim of this study was to establish morphometric and proximate characteristics of sardine *S. pilchardus* and identify any difference on morphology, growth and chemical composition of sardine populations from two neighboring sites, the gulf of Kalloni and the North Aegean Sea waters around the south coast of Lesvos, examining at the same time the effect of seasonality during the summer months.

2. Materials and Methods

2.1. Sample collection and preparation

The study samples were obtained from fishing communities located at the North Aegean Sea around the island of Lesvos and the Gulf of Kalloni. Batches of 100 fish per month and per location were collected between May and October 2017, a total of seven samples (400 individuals from the open sea and 300 individuals from the Gulf of Kalloni).

Samples were transported to the laboratory in coolers with ice where all fish were weighted and analyzed for morphometric characteristics (LT, LS, LF, LA, LH, LP, BD, OK and ED as shown in Fig. 1). All lengths were measured to the nearest mm and weighted to the nearest g (±0.0004) in wet mass.

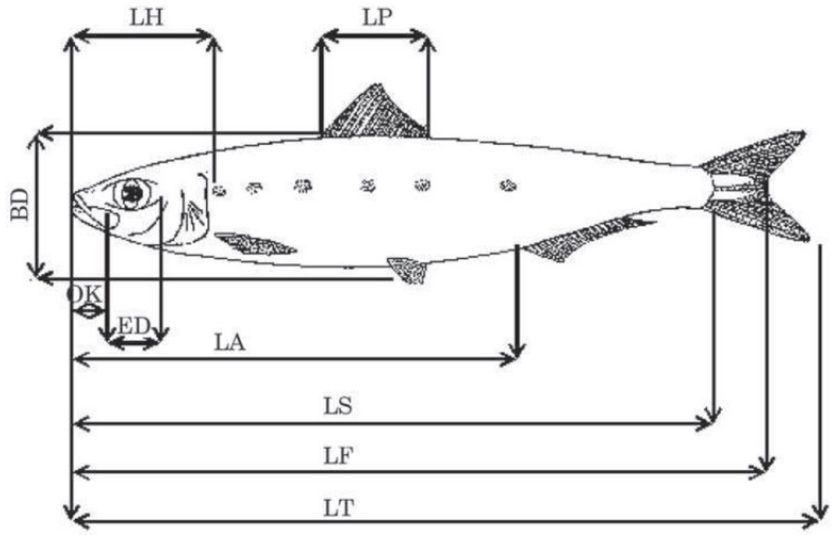

Figure 1 Morphometric characteristics that were measured on speciments of S. pilchardus in the Kalloni gulf and the open North Aegean Sea

For each patch, heads and guts were removed, and fillets were ground in a food processor until formation of a homogeneous mass, which was then used for further analyses. Two replicates R1 and R2 were created of the homogenous mass. For each parameter determined, 3 sub-replicates were created (R1.1-R1.3 and R2.1-R2.3) and were used for the corresponding proximate analysis.

2.2. Morphometric analysis

2.2.1. Length - Weight Relationship

Length–weight relationships for fish were originally used to provide information on whether somatic growth was isometric or allometric. In fisheries biology, length–weight relationships are useful in determining weight and biomass when only length measurements are available, as indications of condition and to allow for comparisons of species growth between different regions (Koutraksi et all, 2003).

Length-to-weight relationship for total body weight was calculated using the equation

$$W = a \times TL^b$$

where W is the total weight (expressed in grams), TL is the total length (expressed in mm), “a” is a coefficient related to body form and “b” is an exponent indicating isometric growth when equal to 3. The parameters “a” and “b” were estimated by linear regression on the transformed equation:

$$\log(W) = \log(a) + b\log(TL)$$

When b=3, increase in weight is isometric (length increases in equal proportions with body weight), while in any other case weight increase is allometric (positive or negative).

2.2.2. Physiological state

The Relative condition factor K was calculated for the overall individual (K), and for specific organs (Kliver) (Nunes et all, 2015). These metrics were calculated according to the following equations:

$$K = \frac{Wtotal}{Total\ Length^3} * 1000$$

$$K_{liver} = \frac{W_{liver}}{Total\ Length^3} * 1000$$

All metrics used total instead of eviscerated weight due to lack of the corresponding data.

2.2.3. Hepatosomatic Index

To evaluate the hepatic condition of the fish the Hepatosomatic index (HSI) was calculated (Nunes et all, 2011). HIS was obtained as the ratio of liver weight to total instead of gutted weight due to lack of the corresponding data :

$$HSI\ = \frac{W_{liver}}{W_{total}} * 100$$

2.2.4. Head Body ration

On account of the morphometric analysis of sardine samples from the open sea and the gulf, the ratio of head length to body length was used in order to find possible differences between the populations of two regions.

Head to body ratio was calculated using the equation:

$$HB = \frac{LH}{LS}$$

Where LH is the head length in mm and LS the body length in mm.

2.3. Proximate analysis

Proximate analysis of edible portion of the fish was carried out. More precisely, the following major constituents were determined; water content (WC), ash content (inorganics), total fat (TF) and proteins (Prts), according to the methodology described below.

The moisture content of the fish was determined before freeze-drying the samples. Moisture was determined gravimetrically after drying the material in an oven at 105^0 C for 24hrs according to the AOAC method (2002). Total lipids were determined using the Bligh and Dyer(1959) method, and protein content was determined according to the Kjeldahl method described in ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. The determination of mineral residues was measured gravimetrically after combustion of the sample in a muffle furnace at 550^0 C (AOAC). All analyses were performed in triplicate.

2.3.1. Quality assurance

For proximate analysis procedures, the coefficient of variation (CV) was calculated to express the precision and repeatability. (CVash=0.13, CVwc=0.01)

2.4. Statistical analysis

The data were subjected to t-tests and analysis of variance (Anova) in R software in order to undertake comparisons between the two study areas and to examine the effect of seasonality. Also, linear regression models were fitted on the raw data using R software.

3. Results

3.1. Morphometric analysis

3.1.1. Length – Weight Relationship

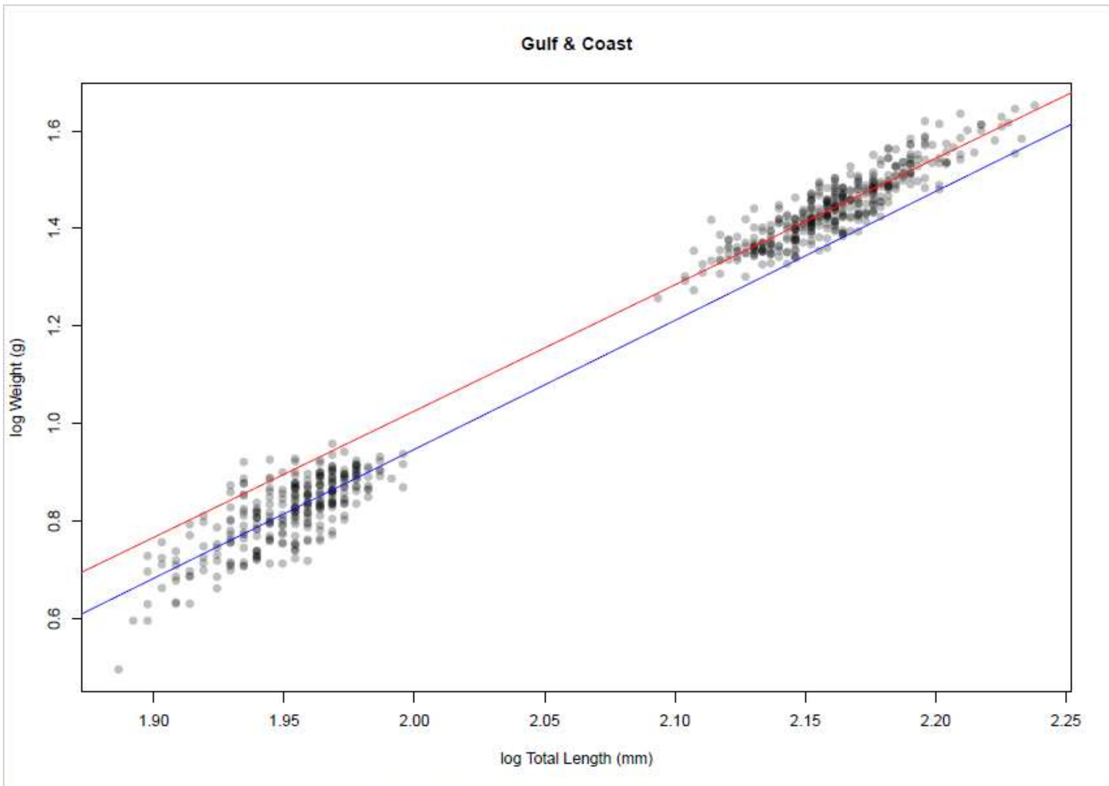

Figure 2 Linear regression models fitted on the raw data of TL and TWW of sardine in the Gulf of Kalloni (blue line) and the open North Aegean Sea around the coast of Lesvos Island (red line).

When linear regression models for the two regions were fitted on the raw data of TL and TWW from the gulf and the open sea, they were parallel. The similarity of the slope, which didn't change for the two regions was evident in the "a" and "b" values that were extracted from the corresponding equations. Growth of sardine from both regions was found to be negatively allometric.

Table 1 "a" and "b" values of the Length-Weight relationship of sardine calculated for the Gulf of Kalloni and the open North Aegean Sea

	Gulf	Open Sea
a	-4.42 (-4.84,-3.84)	-4.15 (-4.42,-3.88)
b	2.64 (2.38,2.90)	2.58 (2.46,2.71)

3.1.2. Comparison of the two study areas

Table 2 Statistics for TL and TWW data from the Kalloni Gulf and the Open Sea (Lesvos Island)

TLcoast	TWWcoast	TLgulf	TWWgulf
Min. : 95.0	Min. : 6.13	Min. : 77.00	Min. :3.130
1st Qu.:140.0	1st Qu.:24.80	1st Qu.: 87.00	1st Qu.:5.907
Median :145.0	Median :27.54	Median : 90.00	Median :6.830
Mean :145.3	Mean :28.21	Mean : 89.69	Mean :6.702
3rd Qu.:150.0	3rd Qu.:30.79	3rd Qu.: 93.00	3rd Qu.:7.540
Max. :173.0	Max. :44.91	Max. :107.00	Max. :9.090

Values of TL taken along the coastline varied between 95.0mm – 173.00mm whereas values from inside the gulf of Kalloni varied between 77.00mm – 107.00mm. Respectively, values of TWW varied between 6.13g – 44.91g and 3.13g – 9.09g.

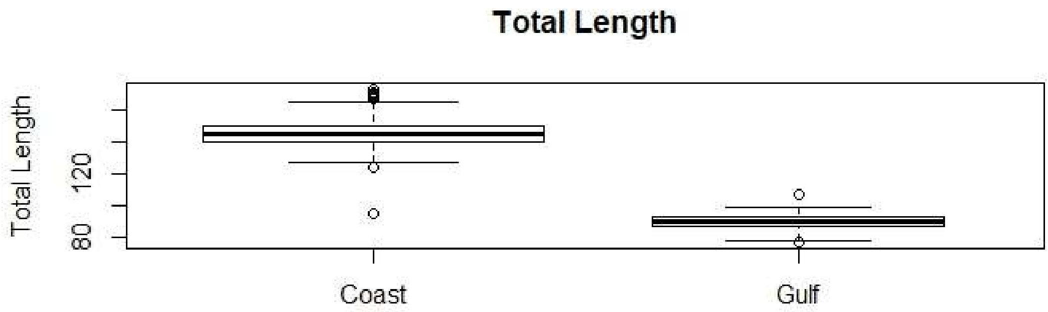

Figure 3 Boxplot of Total Length (mm) measured from speciments of S.pilchardus taken from the Gulf of Kalloni (TLgulf) and from the open sea around the south coast of Lesvos island (North Aegean Sea) (TLcoast).

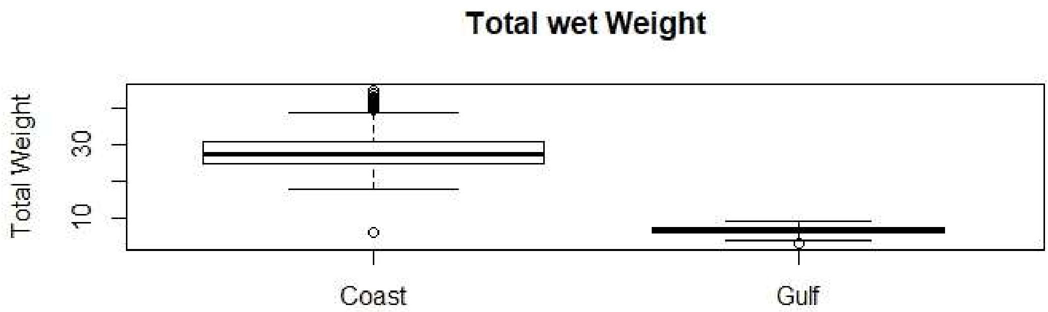

Figure 4 Boxplot of Total Wet Weight (g) measured from speciments of S.pilchardus taken from the Gulf of Kalloni (TWWgulf) and from the open sea around the south coast of Lesvos island (North Aegean Sea) (TWWcoast).

The boxplot of TL from the two sights (Fig.3) shows difference on both the length of the individuals and the homogeneity of the samples. Fish from the gulf are much smaller in size and the samples taken show higher homogeneity. The same trend is shown at the boxplot of TWW (Fig.4). T-tests undertaken for both TL and TWW in order to examine the similarity of the two sights showed p values of 2.2e-16 in both cases and therefore a statistically significant difference (see Appendix).

3.1.3. Study of the seasonality

In order to examine the variation of the measurements among the four months of the sampling procedure, the two sights are studied separately.

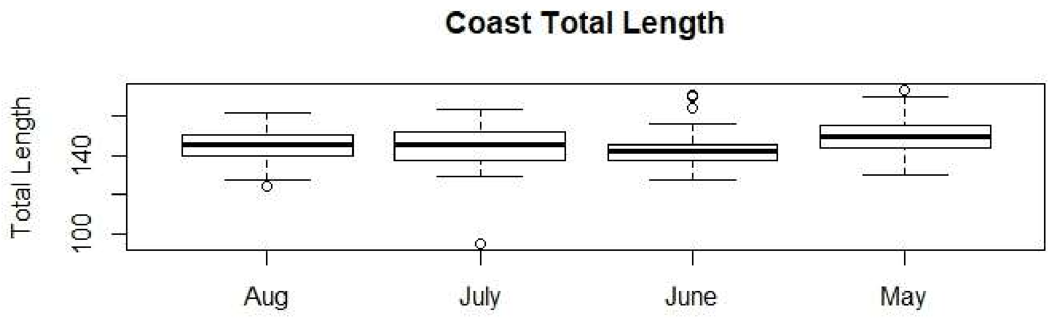

Figure 5 Boxplot of Total Length (mm) measured from speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) during a four months period (May-August).

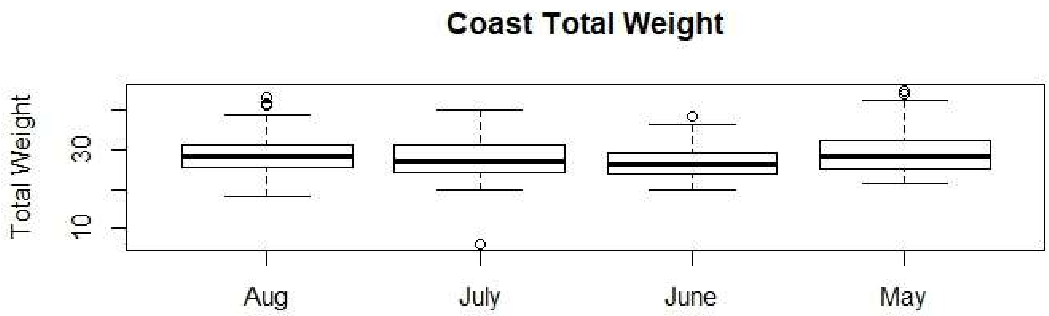

Figure 6 Boxplot of Total Wet Weight (g) measured from speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) during a four months period (May-August).

TL and TWW for samples taken along the coastline show corresponding trends and no significant variation between the four months. Sample taken in May, appears slightly higher in both TL and TWW values (Fig.5, 6).

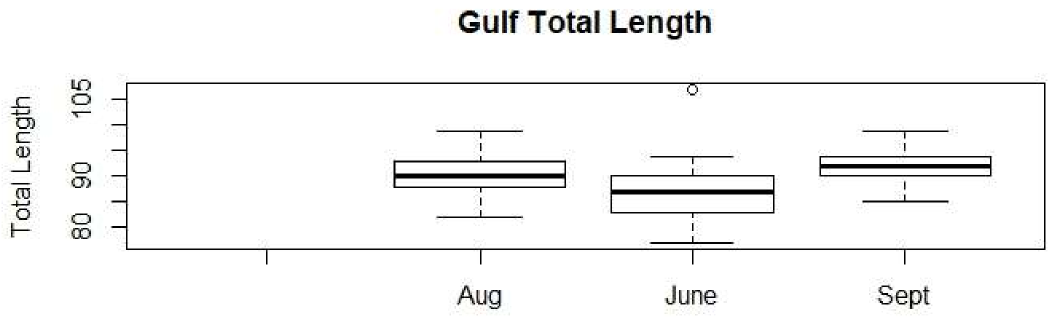

Figure 7 Boxplot of Total Length (mm) measured from speciments of S.pilchardus taken from the gulf of Kalloni (Lesvos Island) during a three months period (June- September).

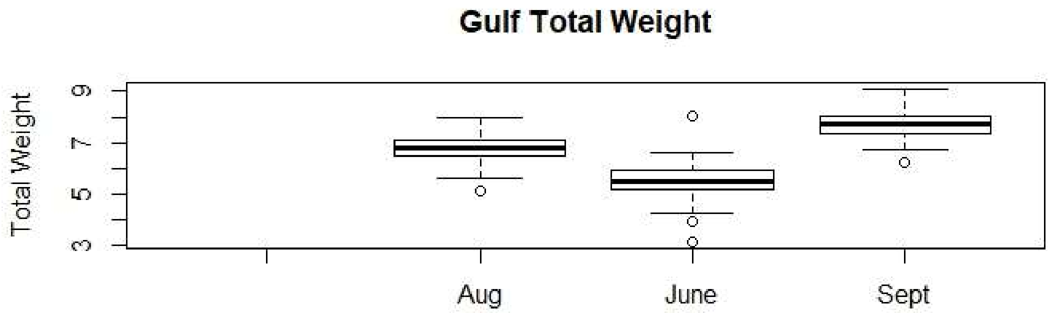

Figure 8 Boxplot of Total Wet Weight (g) measured from speciments of S.pilchardus taken from the gulf of Kalloni (Lesvos Island) during a three months period (June- September).

TL and TWW for samples taken from the gulf also show corresponding trends and a slight variation between the four months. From June to September, values of TL and TWW increase slightly each month (Fig.7, 8). Results of Analysis of Variance, showed statistically significant influence of month on values of both TL and TWW with p-values<0.05 (Appendix).

3.1.4. Condition Indices

Head-Body ratio

As shown in figure 9, the ration of head length and standard (body) length was the same for individuals from both sights.

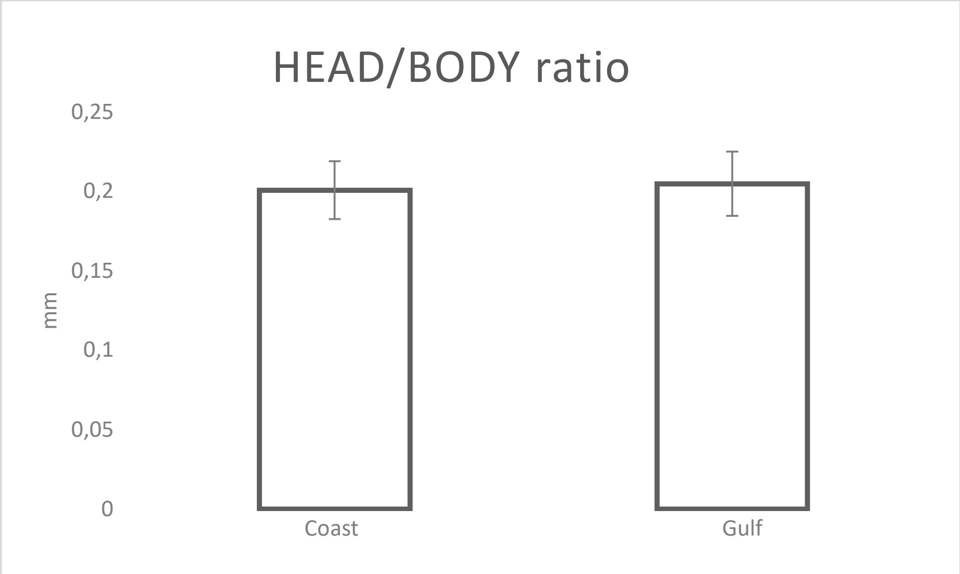

Figure 9 Head length to standard (body) length ratio of speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) (left) and the gulf of Kalloni (Lesvos island) (right).

Physiological State

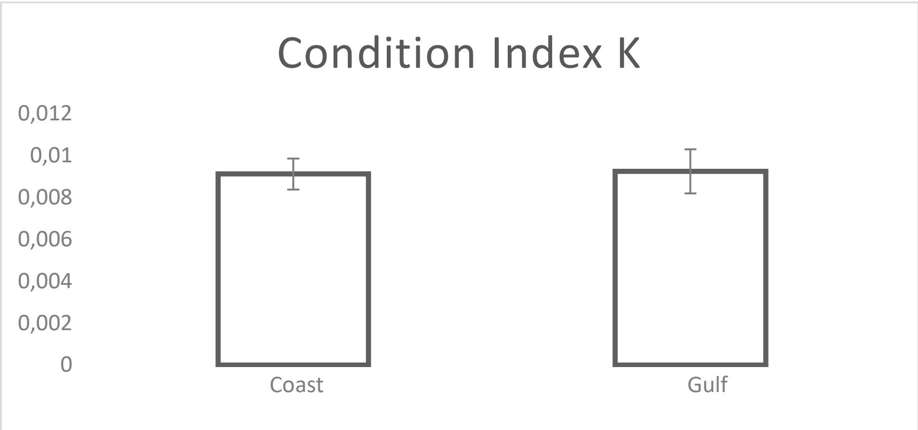

Figure 10 Condition Index K calculated for speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) (left) and the gulf of Kalloni (Lesvos Island) (right).

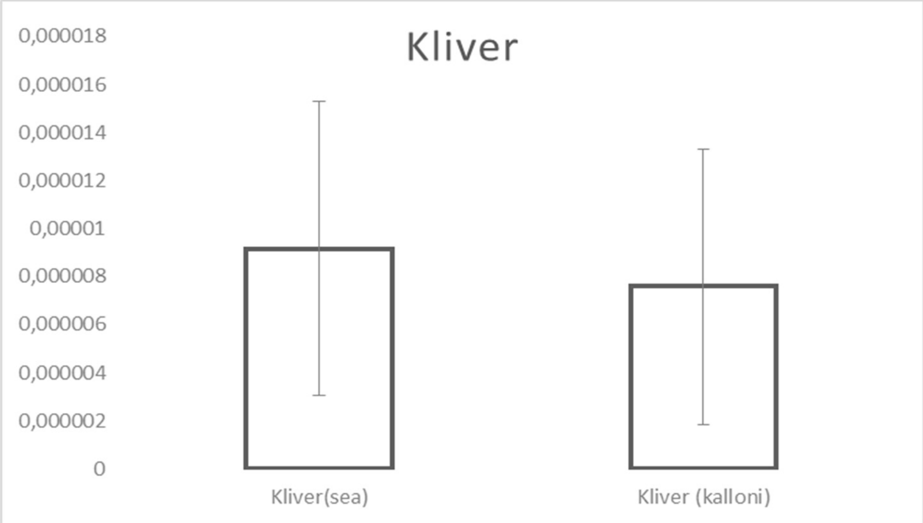

Figure 11 Condition Index K of the liver (Kliver) calculated for speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) (left) and the gulf of Kalloni (Lesvos Island) (right).

Condition factor K calculated for the whole individual (Fig.10) showed no difference between the two different sights, whereas condition factor of the liver (Fig.11) was lower for samples taken inside the gulf of Kalloni.

Hepatosomatic Index

The Hepatosomatic index, when plotted separately for the two sights, showed non-linear variations between months (Fig.12).

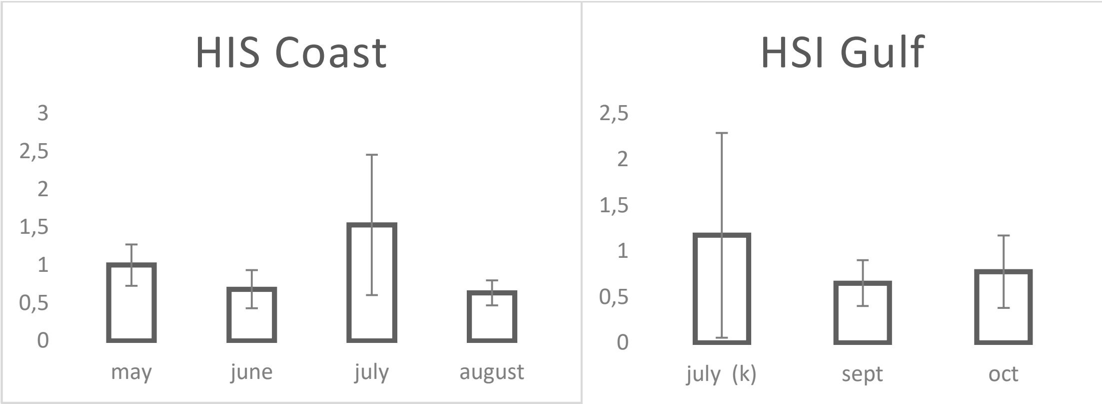

Figure 12 Hepatosomatic Index (HIS) calculated for speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) (left) and the gulf of Kalloni (Lesvos island) (right). For each sight the four and three month period of sampling is shown respectively in order to show any variations of the Index due to seasonality.

When the two sights were compared, HSI was lower for samples inside the gulf (Fig.13).

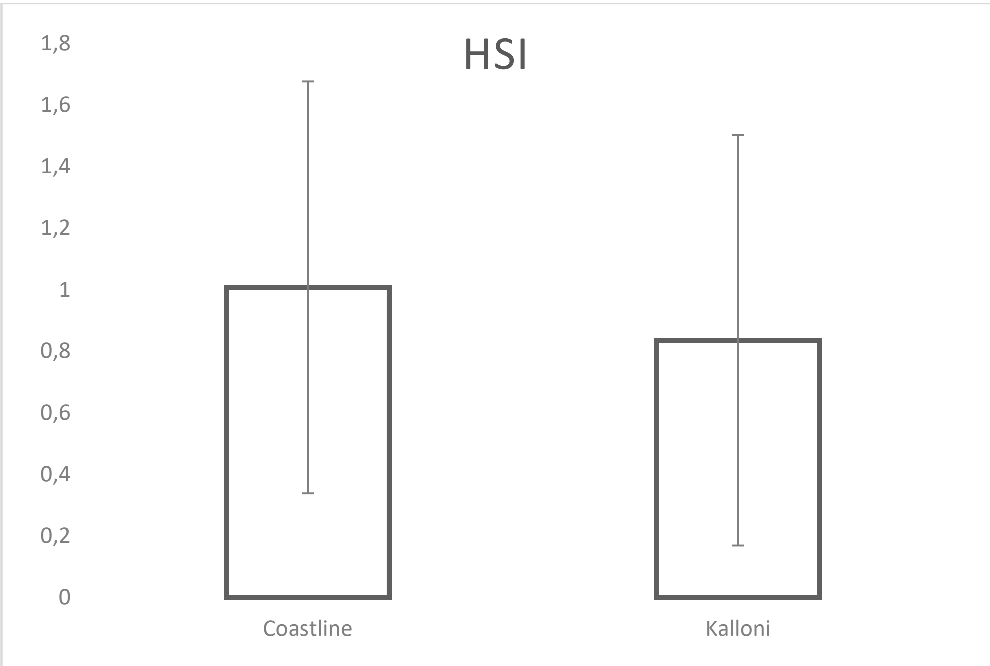

Figure 13 Hepatosomatic Index (HIS) calculated for speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) (left) and the gulf of Kalloni (Lesvos island) (right).

Visceral Fat

Samples taken from the coastline during July and August and samples taken from inside the gulf during September and October had the presence of visceral fat.

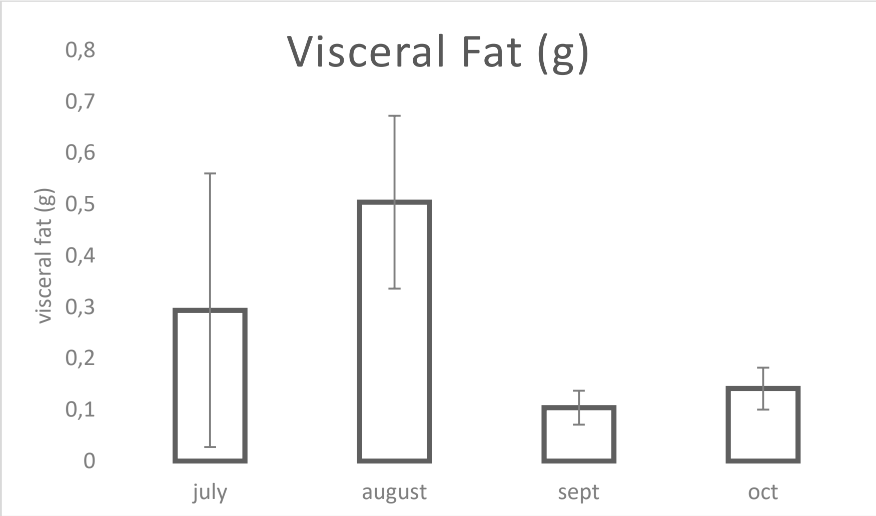

Figure 14 Visceral Fat measured for speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) for months July and August and the gulf of Kalloni (Lesvos Island) for months September and October when its presence was evident.

When visceral fat presented in g for the two sights and four months (Fig.14) showed lower values for samples taken inside the gulf than those from the coast. For both sights, values of the second month were higher than those of the first month that the visceral fat was seen.

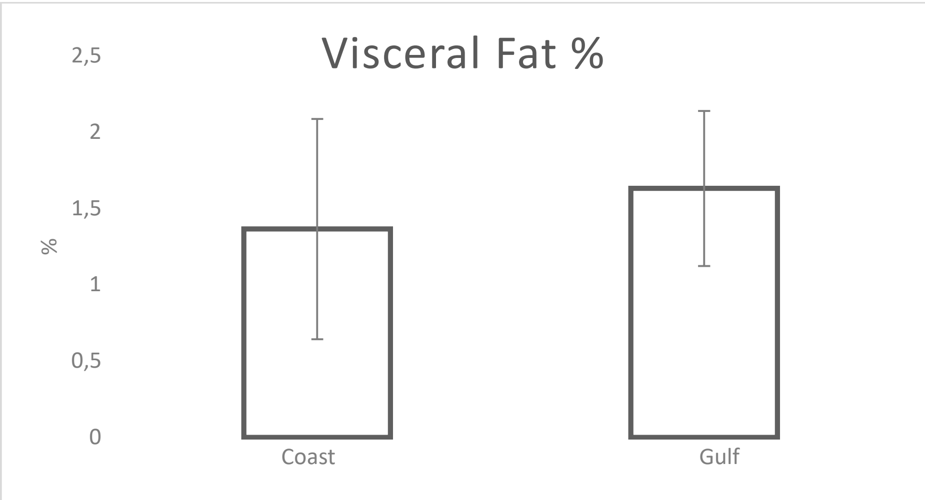

Figure 15 Visceral Fat measured for speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) and the gulf of Kalloni (Lesvos Island) presented in % according to the TWW of each individual.

As expected, when the visceral fat was presented in percentage %, according to the TWW of each individual (Fig.15), showed a significant difference between the two sights. Samples taken from inside the gulf of Kalloni showed much higher values of visceral fat (%) than those taken from the coastline.

3.2. Proximate analysis

3.2.1. Comparison of the two study areas

Table 3 Statistics for Water Content, Ash Content, Total Fat and Protein Content data from the Kalloni Gulf and the Open Sea (Lesvos Island)

WCcoast	Ashcoast	Fatcoast	Pcoast	Wcgulf	Ashgulf	Fatgulf	Pgulf
Min. :63.67	Min. :12.45	Min. :1.591	Min. :2.780	Min. :65.95	Min. : 9.468	Min. :0.1661	Min. :2.740
1st Qu.:65.05	1st Qu.:13.51	1st Qu.:3.328	1st Qu.:2.818	1st Qu.:67.03	1st Qu.:12.743	1st Qu.:0.7858	1st Qu.:2.850
Median :66.02	Median :17.79	Median :4.099	Median :2.920	Median :67.40	Median :19.857	Median :1.7197	Median :3.090
Mean :66.70	Mean :18.15	Mean :4.335	Mean :2.992	Mean :68.55	Mean :21.611	Mean :1.5538	Mean :3.090
3rd Qu.:67.13	3rd Qu.:22.32	3rd Qu.:5.279	3rd Qu.:3.122	3rd Qu.:71.20	3rd Qu.:21.748	3rd Qu.:2.1202	3rd Qu.:3.305
Max. :73.26	Max. :26.77	Max. :7.905	Max. :3.378	Max. :71.81	Max. :99.062	Max. :3.5289	Max. :3.490

Values of WC from samples taken along the coastline varied between 58.19% – 73.26% whereas values from inside the gulf of Kalloni varied between 56.21% - 71.81%. Respectively, values of Ash content varied between 12.45% - 26.77% and 9.468% - 99.062%. Values of Fat content varied between 1.591% - 7.905% and 0.1661 – 3.5289% whereas values of protein varied between 2.78% - 3.37% and 2.74% - 3.49% for the coastline and the gulf of Kalloni respectively.

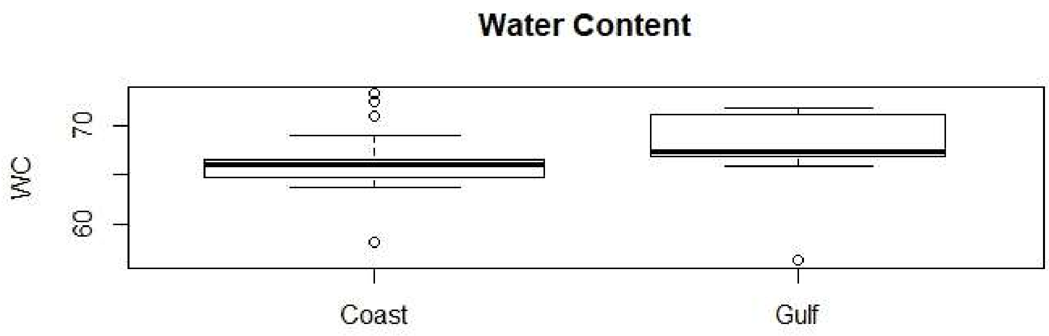

Figure 16 Boxplot of Water Content (WC) measured for speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) and the gulf of Kalloni (Lesvos Island)

WC appears to be slightly higher for the Gulf (Fig.16). T-test on the WC of the two regions showed (p value >0.05) that there is not statistically significant difference between the two.

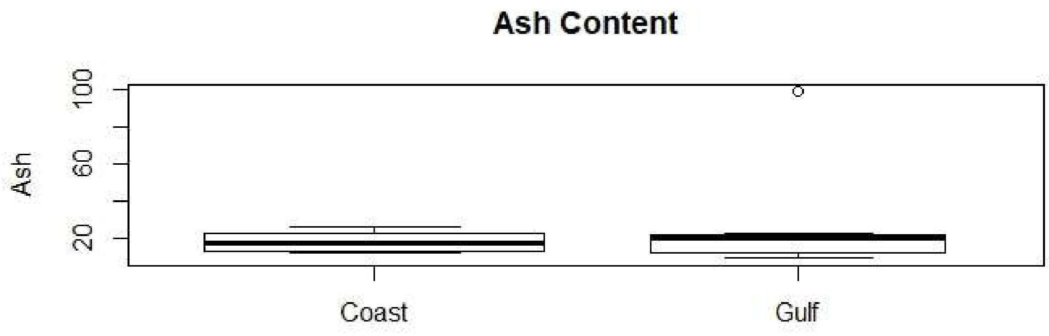

Figure 17 Boxplot of Ash Content measured for speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) and the gulf of Kalloni (Lesvos Island)

Ash content shows no difference between the two regions (Fig.17). T-test on the Ash content of the two regions showed (p value >0.05) that there is no statistically significant difference between the two.

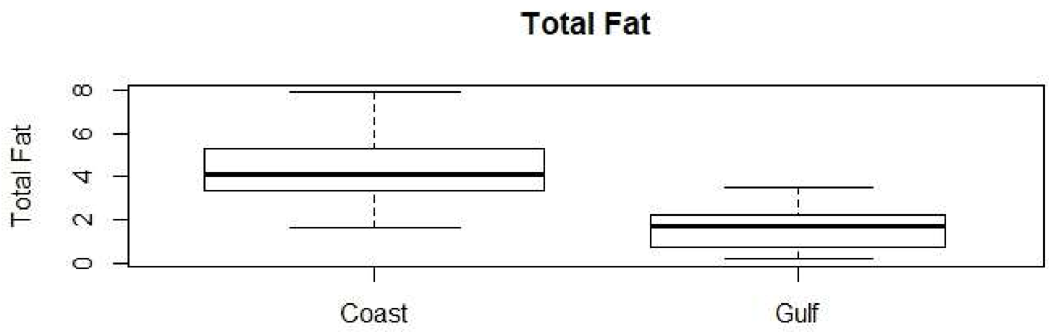

Figure 18 Boxplot of Total Fat Content (TF) measured for speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) and the gulf of Kalloni (Lesvos Island)

Total fat was higher for samples taken from the open sea (Fig. 18). T-test on the Total Fat of the two regions showed (p value <0.05) that there is statistically significant difference between the two.

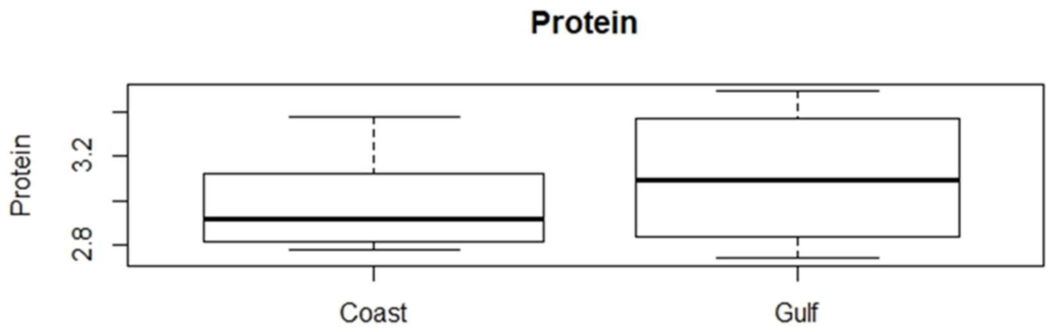

Figure 19 Boxplot of Protein Content (Prtns) measured for speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) and the gulf of Kalloni (Lesvos Island)

Protein content appears slightly higher for samples taken from the gulf (Fig. 19). T-test on the protein content of the two regions showed (p value >0.05) that there is no statistically significant difference between the two.

3.2.2. Study of seasonality

Water Content

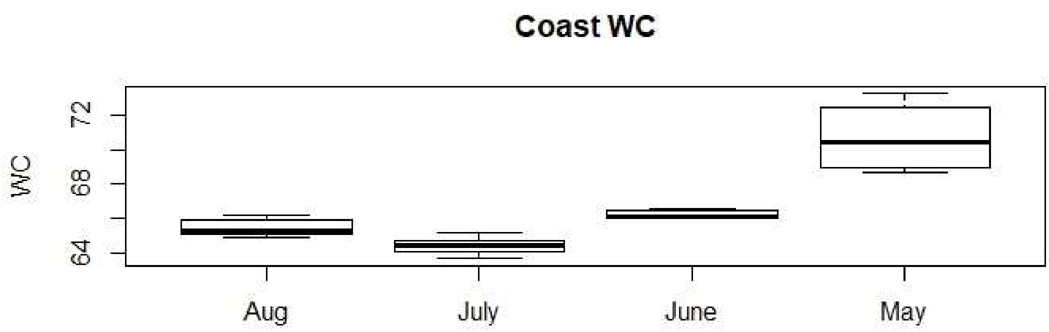

Figure 20 Boxplot of Water Content (WC) measured for speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) during a four month period (May-August).

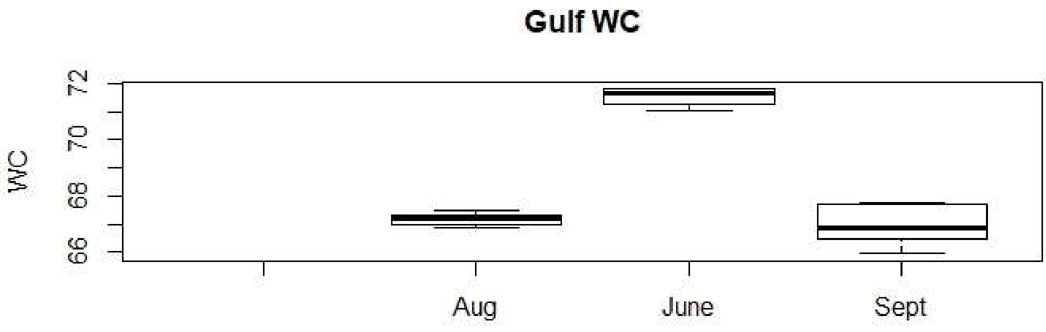

Figure 21 Boxplot of Water Content (WC) measured for speciments of S.pilchardus taken from the gulf of Kalloni (Lesvos Island) during a three month period (June - September).

WC for samples from the open sea was higher in May and decreased during June and July, while during August it increased again (Fig.20). WC from inside the gulf, was higher in June and also decreased during August and September (Fig.21). Analysis of Variance for coast and gulf samples separately showed statistically significant influence of month only on WC values of the gulf (Appendix).

Total Fat

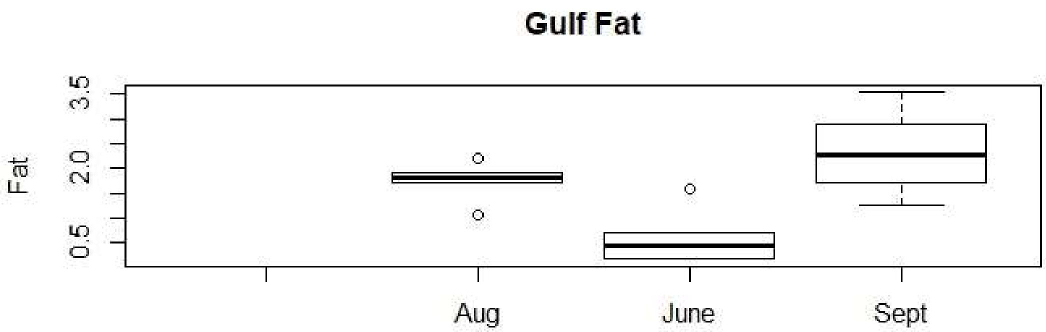

Figure 22 Boxplot of Total Fat Content (TF) measured for speciments of S.pilchardus taken from the gulf of Kalloni (Lesvos Island) during a three month period (June - September).

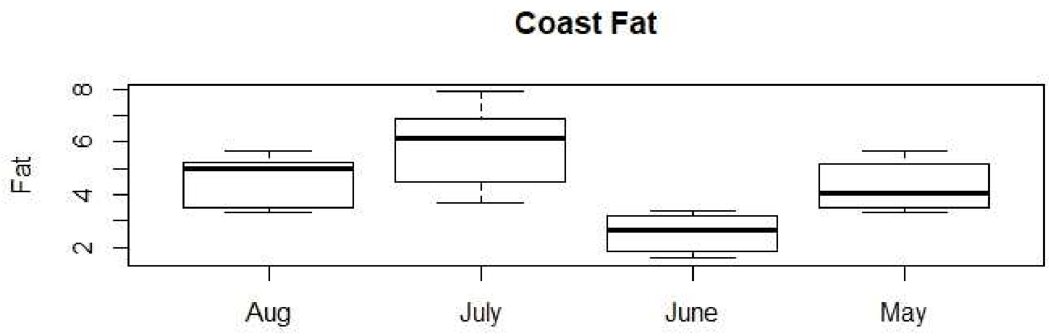

Figure 23 Boxplot of Total Fat Content (TF) measured for speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) during a four month period (May-August).

Total fat from inside the gulf increased from June to September (Fig.22).Total Fat from the open sea was higher for May and July, slightly lower in August and the lowest values were noted during June (Fig.23). Analysis of Variance for coast and gulf samples showed statistically significant influence of month in both sights (Appendix).

Ash Content

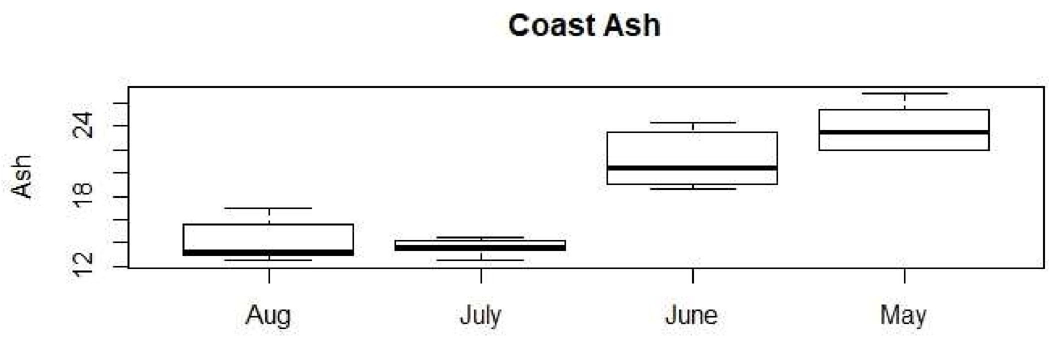

Figure 24 Boxplot of Ash Content measured for speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) during a four month period (May-August).

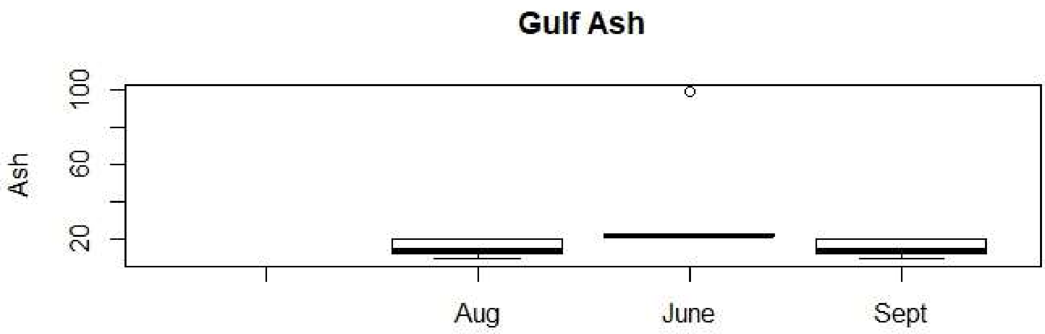

Figure 25 Boxplot of Ash Content measured for speciments of S.pilchardus taken from the gulf of Kalloni (Lesvos Island) during a three month period (June - September).

Ash content from inside the gulf show no variations between the three months (Fig.25) while ash content from the open sea was higher for May and June and lower in July and August (Fig.24). Analysis of Variance for coast and gulf samples showed statistically significant influence of month, only on coast Ash content values. For values from inside the gulf, analysis showed no statistically significant influence.

Protein Content

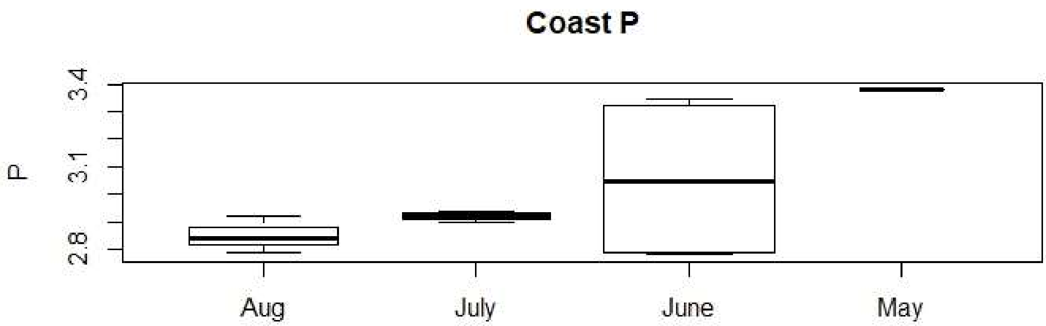

Figure 26 Boxplot of Protein Content (Prtns) measured for speciments of S.pilchardus taken from the open sea around the south coast of Lesvos island (North Aegean Sea) during a four month period (May-August).

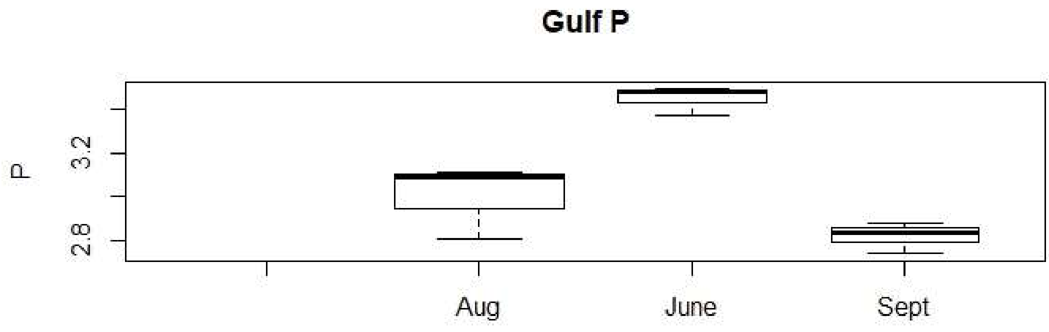

Figure 27 Boxplot of Protein Content (Prtns) measured for speciments of S.pilchardus taken from the gulf of Kalloni (Lesvos Island) during a three month period (June - September).

Protein content from inside the gulf (Fig.27) and also the open sea (Fig.26) show a decrease during the months of sampling as shown in the boxplots. When Analysis of Variance was performed for coast and gulf samples, results showed statistically significant influence of month, only on gulf protein content values. For values from the open sea, analysis showed no statistically significant influence.

4. Discussion

The two sights that were studied, show grate difference on the size (TL and TWW) of the individuals with Kalloni Gulf having smaller sized individuals than those of the open sea around the coast of Lesvos Island. Individuals in the Gulf of Kalloni were found to be of a smaller body size (77.00mm – 107.00mm) than those reported in previous literature, while values of the size observed along the coastline of Lesvos Island (95.0mm – 173.00mm) show small differences. Koutrakis and Tsikliras (2003), have reported that the sardine total length (TL) range between 100.0 — 184.0 mm in three estuarine systems of the North Aegean Sea.

T-tests showed statistically significant influence of seasonality during the summer months on both TL and TWW of the individuals for both sights. In addition, the size of the fish inside the gulf had a grate increase within the three month period while fish of the open sea increased at a slower rate. The size of the fish along with the calculated condition indices that are mentioned later on in this study, point towards the hypothesis that fish from the gulf are at a younger maturity state. In this case, the higher growth rate of the younger individuals that was observed in the gulf is expected.

Samples from the Gulf of Kalloni were also characterized by high homogeneity, whereas samples from the open North Aegean waters were not. The gulf is of a certain enclosed area whereas fisheries from the open sea were caught from various fishing grounds each month according to the respective fishing vessel. In addition, the fishing method used within the gulf is different from the ones of the open sea vessels. Fish of the open sea can come from different schools and different maturity states within the same fishing day. Therefore these differences mentioned above, could probably account for the difference in the homogeneity of the samples between the gulf and the open Aegean Sea.

After examining the Length – Weight relationship of sardine populations in the gulf of Kalloni and the open North Aegean Sea, the increase in body weight was found to be negatively allometric for both sights. The estimated L-W relationship parameters "a" and "b" approximately coincide with the values estimated from past studies concerning sardine in Greek waters and were found to be equivalent between the open North Aegean sea and the gulf of Kalloni (Antonakakis et all, 2011; Lamprakis et all, 2003). The parallel linear regression models that were fitted on the raw data of TL and TWW for the gulf and the open Aegean Sea might suggest that the two populations are the same but at a different maturity state.

Differences in shape among groups of fish may reveal different growth rates that are relevant for the definition of stocks and separate two populations (Silva, 2003). On the process of determining morphometric variations between sardine in the Atlantic and the Mediterranean waters, Silva (2003) used the head to body ratio to separate the two populations. There have also been cases where there was a latitudinal gradient found from small head and large body size of sardine to a large head and small body size (southern Portuguese waters, and the Gulf of Cadiz) (Silva, 2003). In this study head to body ratio was found to be the same between the two regions giving another reason to support the idea that the two populations are the same.

The Morphometric condition factor that was calculated for the overall individual (K) was found to be the same between the two regions, whereas physiological condition factor that was calculated specifically for the liver (Kliver) was higher for individuals of the open sea around the Island of Lesvos. Morphometric condition indices that are based on mass and length, such as the relative condition index (Kn) used in this study, vary with both fat and protein content and can obscure a seasonal pattern of fat reallocation between the different organs of the body. Therefore, the relative condition index (Kn) is not obsolete to track condition of small pelagic fish, but indicates a correlation with the lipid content outside of the reproductive period and might be sensitive to other parameters, such as fish protein content (Brosset et all, 2015). This may explain the lack of a relationship between Kn and Total Fat measurements between the two sights, since it has already been stated that the "two populations" are in a different maturity state. On the other hand, protein levels were found to be the same between the two sights, such as Kn factor, which supports the hypothesis that there is a strong relationship between total muscle protein and Kn. The difference in Kliver that was found between the two sights was also expected due to the difference in the maturity state of the populations.

The Hepatosomatic Index (HSI) showed nonlinear variations between the four months of the sampling procedure, and was higher for individuals of the open sea. Fish inside the gulf are of a smaller size and lower liver condition and HSI. Amenzoui et all. (2006) stated that larger fish have different maximum sexual activity than smaller fish (later in time and smaller) resulting in differences in HSI between the two populations, supporting the idea of a different maturity state of the fish inside the gulf. Also according to Amenzoui et all. (2006) small pelagic fish adopt a spawning strategy that aims at minimizing the losses by advection, in order to reproduce outside the season of maximum upwelling, which could account for the difference in maturity state between the two regions, since the gulf and the open north Aegean waters have different upwelling conditions and temperature regimes (Olivar et all, 2003).

During the analysis of the samples in the laboratory, visceral fat was found in fish of both

sights during the last two months of the sampling season. In July and August visceral fat was found in the open Aegean Sea samples, whereas in September and October it was found in individuals of the gulf. Although the amount of the extracted visceral fat in grams was higher in individuals from the open sea where the size was bigger too, when it was presented in percentage %, according to the TWW of each individual, fish from the gulf had a higher percentage of visceral fat. Although its presence can be the result of the reproductive cycle among other factors, its amount might be directly related to the quality and quantity of food, since it has already been stated that the gulf is of a slightly higher eutrophic level.
In both cases, the second out of the two months that the visceral fat was observed, was higher in amount suggesting that further sampling during the following months could reveal a further increase.

WC, Ash content and Protein content were the same for individuals from the two sights while Total Fat content was found to be higher for individuals from the open sea. The four proximate constituents that were measured were not affected by seasonality in the same way between the two sights. The effect of seasonality was found to be significant for WC, Total Fat and Protein content inside the gulf of Kalloni, whereas for individuals form the open Aegean Sea, seasonality affected the Total Fat and Ash content only. With regard to total fat, the low values found in sardines were comparable with those reported in the literature (Bouderoua et all, 2011). It is known that the lipid content of sardines varies according to season. This is due to the different availability of food and different temperature of the water during the year, as well as to the different rate of reproduction activity (Caponio et all, 2004). A seasonal difference of lipid content is most often in relation to the feeding activity of fish (intense during suitable season) and the seasonal variation of nutritional quality of ingested prey (Bouderoua et all, 2011). As for total protein content and ashes, values obtained were comparable with those reported in the literature for different fish species such as those in Di Matteo et al., 1990; Carnovale and Marletta, 1997; Alasalvar et al., 2002 (Caponio et all, 2004). The content of ash also decreased for both regions throughout the four months, but had no difference in amount between the gulf and the open sea. The high ash content is of significance in measuring the mineral content of the species as the amount of ash shows the richness of the food in terms of element composition (G. Nair et all, 2016). Therefore, the differences that were found in the proximate constituents of the fish during this study correspond with the significantly different hydrological and biological characteristics of the Kalloni Gulf compared to the open North Aegean Sea.

5. Conclusions

The morphometric characteristics of *S. pilchardus* of Kalloni Gulf when compared to speciments of the open Aegean Sea around the island of Lesvos indicate that the individuals might be of the same population but at a different maturity state, indicating that the Kalloni gulf could provide a suitable nursery ground for younger *S. pilchardus* individuals. For species of economic importance such as *S. pilchardus*, especially in areas having a nursery role, the ongoing research is substantial. Therefore further research on the proximate composition of this species in both the Kalloni gulf and the open sea could support these findings and provide further useful information on the effects of the unique hydrological and biological characteristics of the Kalloni Gulf on *S. pilchardus*.

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Appendix

- **Length – Weight Relationship (fitting of linear regression models)**

lm(formula = log10(TWW) ~ log10(TL), data = datacoast)

Residuals:

Min 1Q Median 3Q Max

-0.075136 -0.019729 -0.000598 0.019644 0.097117

Coefficients:

Estimate Std. Error t value Pr(>|t|)

(Intercept) -4.1543 0.1379 -30.11 <2e-16 ***

log10(TL) 2.5899 0.0638 40.59 <2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.0316 on 395 degrees of freedom

Multiple R-squared: 0.8066, Adjusted R-squared: 0.8061

F-statistic: 1648 on 1 and 395 DF, p-value: < 2.2e-16

lm(formula = log10(TWW) ~ log10(TL), data = datagulf)

Residuals:

Min 1Q Median 3Q Max

-0.151356 -0.029821 -0.000244 0.031545 0.147298

Coefficients:

Estimate Std. Error t value Pr(>|t|)

(Intercept) -4.3424 0.2549 -17.03 <2e-16 ***

log10(TL) 2.6447 0.1306 20.25 <2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.04756 on 289 degrees of freedom

Multiple R-squared: 0.5866, Adjusted R-squared: 0.5852

F-statistic: 410.1 on 1 and 289 DF, p-value: < 2.2e-16

- **Anova analysis on Date and Region for TL and TWW**

	TL	TWW
	Df Sum Sq Mean Sq F value Pr(>F)	Df Sum Sq Mean Sq F value Pr(>F)
Date	4 230126 57531 1254 <2e-16 ***	4 32359 8090 577.6 <2e-16 ***
Region	1 297492 297492 6483 <2e-16 ***	1 46272 46272 3303.5 <2e-16 ***
Residuals	686 31477 46	686 9609 14
Signif. codes:	0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1	0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

- **T-tests on TL and TWW data for the two regions**

	TL	TWW
	Welch Two Sample t-test	
	t = 109.1, df = 624.29, p-value < 2.2e-16	t = 84.163, df = 449.06, p-value < 2.2e-16
	alternative hypothesis: true difference in means is not equal to 0	
95 percent confidence interval:	54.64506 56.64822	21.00935 22.01397
mean of x mean of y	145.33500 89.68836	28.214025 6.702363

- **Anova analysis on Study of Seasonality for TL and TWW**

	TL	TWW
	Df Sum Sq Mean Sq F value Pr(>F)	Df Sum Sq Mean Sq F value Pr(>F)
Date	4 230126 57531 120.1 <2e-16 ***	4 32359 8090 99.46 <2e-16 ***
Residuals	687 328969 479	687 55881 81
Signif. codes:	0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1	0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

- **T-tests on proximate constituents (WC,Ash,Total Fat,Prtns) for the two regions**

	WC	Ash	Fat	P
	Welch Two Sample t-test			
	t = -1.6393, df = 32.868, p-value = 0.1107	t = -0.72321, df = 18.514, p-value = 0.4786	t = 7.0806, df = 38.038, p-value = 1.903e-08	t = -0.872, df = 17.628, p-value = 0.3949
alternative hypothesis:	true difference in means is not equal to 0			
95 percent confidence interval:	-3.9120510 0.4210946	-13.500432 6.575759	1.986308 3.576774	-0.3349008 0.1386488
mean of x	66.20700	18.14889	4.335343	2.991874
mean of y	67.95248	21.61123	1.553802	3.090000

- **Anova on Study of sesonality for proximate constituents**

	Coast	Gulf
	WC	
	Df Sum Sq Mean Sq F value Pr(>F)	Df Sum Sq Mean Sq F value Pr(>F)
Date	3 62.03 20.676 2.69 0.0738 .	2 122.9 61.46 8.831 0.00292 **
Residuals	20 153.70 7.685	15 104.4 6.96
Signif. codes:	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
	ASH	
Date	3 469.5 156.50 46.17 3.56e-09 ***	2 1584 792.0 2.315 0.133
Residuals	20 67.8 3.39	15 5132 342.1
	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	
	FAT	
Date	3 33.76 11.254 9.082 0.000536 ***	
Residuals	20 24.78 1.239	
Signif. codes:	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	
	Prtns	
Date	3 0.2414 0.08046 1.892 0.219	2 0.6172 0.30862 26.55 0.000539 ***
Residuals	7 0.2977 0.04253	7 0.0814 0.01162
Signif. codes:		0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Date and region

	WC	Ash	Fat	Prtns
	Df Sum Sq Mean Sq F value Pr(>F)			
Date	4 131.66 32.92 3.934 0.00948 **	4 1602 400.4 2.646 0.0492 *	4 86.49 21.62 24.78 6.5e-10 ***	4 0.5944 0.14860 5.319 0.00718 **
Region	1 41.48 41.48 4.957 0.03234 *	326 325.9 2.153 0.1510	1 35.00 35.00 40.11 2.5e-07 ***	1 0.2747 0.27466 9.831 0.00681 **
Residuals	36 301.25 8.37	36 5449 151.4	36 31.41 0.87	15 0.4191 0.02794
Signif. codes:	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1