Nutritional composition and heavy metal content in *Sardinella maderensis*, *Decapterus rhoncus*, *Sardinella aurita*, *Trachurus trecae* and *Sphyraena guachancho* off the coast of Angola

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1 ABSTRACT

In sub-Saharan Africa approximately 224 million people are undernourished (FAO, 2017a). Fish play an important role in enhancing the nutritional status and food security in Africa and is an important source of protein and micronutrients (FAO, 2017b). Completing nutrient profiles on different species is important to possibly aid in alleviating health concerns based on nutrient deficiencies. In addition, heavy metal content is important to investigate for all fish species which are to be consumed. Sampling of *Sardinella maderensis, Decapterus rhoncus, Sardinella aurita, Trachurus trecae* and *Sphyraena guachancho* was conducted off the coast of Angola in September-October 2017. The different analyses were completed at Institute of Marine Research laboratories. Phosphorus levels found in this thesis were around 250% higher than previously recorded results for *Sardinella aurita*; the phosphorus value was 344 mg/100 g (Table 4.2), while it was 98 mg/100 g from the previous study (Table 2.1). Calcium levels were higher for *Sardinella maderensis* and *Sardinella aurita* with values of 89.4 and 71.6 mg/100 g respectively, while the other three species studied ranged between 20.4 and 25.8 mg/100 g. More research is needed to accurately interpret the real contribution of these nutrients based on availability, access, and consumption, as well as more research on the potential health concern of heavy metal consumption of fish species in Angola.

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1 PREFACE

I want to thank my supervisors Marian Kjellevold and Jeppe Kolding, IMR, the crew aboard the Dr. Fridtjof Nansen, University of Bergen, my fiancé Henning Kvaløy, friends and family for all support and help during the writing of this thesis.

2 INTRODUCTION

2.1 FOOD SECURITY IN ANGOLA

Food security is a considerable concern since globally there are about 821 million people suffering from starvation and under- and malnutrition (FAO, 2018a). In sub-Saharan Africa approximately 224 million people are undernourished (FAO, 2017a). However, food security does not only concern the proper food intake or availability, but also the sufficient nutritional value of the food ingested (Sasson, 2012). Food security is defined by the Food and Agriculture Organization (2001) as "A situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life.". Approximately 700 000 people in Angola suffer from malnutrition and approximately 408 100 of them are children (UNICEF, 2018). Malnutrition could have severe social, physical, and economic consequences for the local communities (FAO, 2003).

FAO reported that per capita fish consumption in Africa was 9.9 kg in 2015 (2018b), however FAO (n.d.a) reported that fish consumption in 2013 in Angola was 18.6 kg per capita. Fish play an important role in enhancing the nutritional status and food security in Africa and is an important source of protein and micronutrients, while also being an affordable option for poor households (FAO, 2017b). In many parts of the sub-Sahara subsistence fishing is not only important for the families of full-time fishermen that bring home some of their daily catch, but also for poor families where fishing is done by the children after school or collected by wives and daughters during collective fishing festivals (Béné and Heck, 2005). Food security and good nutrition can come from these few hundred grams of subsistence catches (Béné and Heck, 2005). Most of the diet in low-income countries in Africa consist of wheat, rice, cassava, and maize, and a large portion of the nutrients and energy required is supplied by these (Gegios et al., 2010; Miller and Welch, 2013). However, these products do not contain, or only in small doses, some important nutrients such as iron, iodine, zinc, and calcium (Gegios et al., 2010; Miller and Welch, 2013). These nutrients must come from other foods, and fish can be a very important supplier (Béné and Heck, 2005; Mohanty et al., 2017). Although fish is an important

source of essential nutrients and protein, consumption in sub-Saharan Africa is declining (Gordon et al., 2013). This is the consequence of population growth and the capture fish industry leveling off. Between 2005 and 2015, capture fisheries and aquaculture had to increase fish production by 27.7% to meet the growing population (population growth set at 1.9% per year) (Béné and Heck, 2005).

2.2 IMPORTANCE OF MICRONUTRIENTS

The two essential elements found predominantly in marine species are selenium and iodine (Rehbein and Oehlenschläger, 2009). The iodine content of marine species varies between 0.05 and 0.8 mg/100 grams depending on species, while freshwater fish contain on average 0.005 mg/100 g (Haldimann et al., 2005). Fish skin of marine species contain an especially large amount of iodine (Rehbein and Oehlenschläger, 2009; Haldimann et al., 2005; Eckhoff and Maage, 1997). Iodine intake is especially important during pregnancy and early childhood, as deficiency during this time may cause damage to the developing brain and could be the largest cause of preventable brain damage in the world (Zimmermann and Boelaert, 2015). Insufficient iodine intake during pregnancy can lead to additional health complications, such as fetal loss, fetal goiter, and neonatal hypothyroidism (WHO, 2013). According to WHO (2013) the recommended amount of urinary iodine concentrations during pregnancy and lactation increase from 100-199 micrograms to 150-249 micrograms per day, which is a result of the iodine requirements of the fetus, increased thyroid hormone production, and enhanced renal iodine loss.

Iron is another important nutrient fish contain, it is responsible for binding oxygen to blood cells and is indispensable in the respiratory process (Berg, Tymoczko and Stryer, 2002). This nutrient is also vital for increasing the production of blood during pregnancy for mother and baby (FAO, n.d.c). In addition, iron plays an important role in synthesizing dopamine and serotonin, and other neurotransmitters, as well as providing oxygen for the brain parenchyma (Li et al., 2018). The diet in Angola consists mostly of grains and cereals (Gegios et al., 2010). Whole grains contain large amounts of iron but also contains phytic acid that decreases the

bioavailability of iron (Hunt, 2003). Even though fish, meat and poultry contain lower amounts of iron, it is in a highly bioavailable form (Hunt, 2003).

Adequate zinc intake is necessary for normal pregnancy outcome and good health and physical growth for children (WHO, 2007c). Inadequate zinc intake can lead to a syndrome of anemia, dwarfism, and hypogonadism (Roohani et al., 2013). Like iron, the bioavailability of zinc from cereals and grains are not as high as for animal food products (Roohani et al., 2013; Hunt, 2003).

2.3 FISHERIES ECONOMIC VALUE

The Angolan economy is dependent on fisheries, and even though oil and mining are more important, about 1.7 percent of the gross domestic product (GDP) of Angola comes from the fisheries sector (FAO, 2014). Approximately 310 000 tons of fish were produced in 2014 (Angola Country Commercial Guide, 2018), the small pelagic fish represented about half and are extremely important in domestic food supply (FAO, 2014). The small pelagic fishes caught consists mostly of the sardinella species and horse mackerel, and about 90 percent of the catch is sold domestically (FAO, 2014). About 63 percent of fish in 2013 were caught by industrial and semi-industrial sectors (Angola Country Commercial Guide, 2018), while artisanal fisheries caught the rest (FAO, 2014). The fisheries are also important when it comes to employment, and around 100 000 people, although probably underestimated numbers, get their income from fisheries indirectly or directly (FAO, 2014).

2.4 FISH STOCKS OF ANGOLA

According to FAO (2014) Angola's fish stocks seem to mostly be overexploited, the most significant exceptions are the sardinella stocks which are slightly underexploited. *Trachurus trecae* has been the main food fish in Angola, but the stock was in 2014 the most overexploited and is the reason policies to reduce capture of this species has been implemented by the Angolan Government (FAO, 2014). The slightly underexploited Sardinella stocks consists of *Sardinella*

maderensis and *Sardinella aurita* (FAO, 2014). Since these already are commercial species it is important to understand the nutrient composition of these fishes, since approximately 35% of the population in Angola is undernourished (Tomlinson et al., 2010).

2.5 CURRENT NUTRITIONAL PROFILES

The available data on nutritional value of the different species in Angola, nutrient intake, and fish production is limited. Thus, in addition to data from Angola, data from the region of sub-Saharan and West African regions will be incorporated into data gathered for this project. Previous data on nutrient composition in fish, as seen in Table 2.1 from The Food Composition Table For Use In Africa (Wu Leung, Busson and Jardin, 1968) from FAO and data from FAO (2012), do not reflect the large diversity of species available for consumption and have only focused on a few select nutrients rather than comprehensive nutrient profiles.

Table 2.1- Data on the nutritional content of Sphyraena spp., Trachurus spp., Sardinella aurita and Sardinella spp. Unit for calcium, phosphorus and iron is milligrams per 100 grams of edible sample, while for protein and fat it is grams per 100 grams of edible sample. For species Sardinella aurita and Trachurus spp. (Wu Leung, Busson and Jardin, 1968) and for Sphyraena spp. And Sardinella spp. (FAO, 2012).

	Sphyraena spp.	*Sardinella spp.*	*Sardinella aurita*	*Trachurus spp.*
Calcium	26	71	710	-
Phosphorus	175	281	98	-
Iron	0.9	1.8	1.3	-
Protein	19.0	19.4	18.3	25.0
Fat	0.7	2.9	3.6	4.0
Magnesium	31	35	-	-
Potassium	252	499	-	-
Sodium	89	77	-	-
Zinc	0.51	1.57	-	-

2.6 PUBLIC HEALTH CONCERNS IN ANGOLA

There are several serious public health concerns in Angola, and hidden hunger is one of them (UNICEF, 2018). Hidden hunger is defined as the lack of required micronutrients (vitamin and minerals) from the food ingested, and the term refers to the possible lack of visible signs of deficiency (FAO, 2014). 161 children out of 1000 live births die before they reach five years of age, this means about 116 000 children die each year (UNICEF, 2011). Approximately one third of these mortalities are caused by child and maternal undernutrition (UNICEF, 2009). Iron deficiency anemia (IDA) is one of the nutrient related health concerns in Angola (UNICEF, 2009). Approximately 68 percent of children in Africa within pre-school age suffer from IDA, while about 42 percent of the total population in Africa are iodine deficient (UNICEF, 2009). A study conducted in Angola tested 826 children for urinary iodine intake, and all sampled schoolchildren were, some to a greater and some to a lesser extent, iodine deficient (Tomlinson et al., 2010). Pregnant women and young children are at greatest risk of IDA, and continuous intake of iron is needed to reduce risk of birthing before term, low birth weight, and maternal mortality due to hemorrhage (McDonald, Hyder and Cossa, 2011). Pellagra is another health concern generated by nutrient deficiencies (Golden, 2002). Outbreaks are caused by a diet consisting of mostly maize, and thereby not consuming enough niacin (Golden, 2002). These outbreaks are widespread in certain regions of Angola (Golden, 2002).

As evidence of maternal malnutrition, across Africa between 5 and 20 percent of women are chronically hungry and as a result have low body mass index (BMI) (Lartey, 2008). Women, especially lactating or pregnant, have an elevated need for intake of micronutrients (Lartey, 2008). Across the continent of Africa 21 to 80 percent suffer from anemia, and zinc deficiency numbers are approximately equally high (Lartey, 2008). An estimated 15 000-20 000 women between 41 and 55 years of age die each year because of anemia caused by iron deficiency (Béné and Heck, 2005).

2.7 RECOMMENDED INTAKE OF NUTRIENTS

Table 2.2- The recommended intake of magnesium, iodine, calcium, potassium, iron, and selenium (Linus Pauling Institute, 2018c-h). All units are mg/day.

Recommended daily intake (RDI)	Magnesium	Iodine	Calcium	Potassium	Iron	Selenium
Men:	400-420	0.152	1000-1200	4700[(1)]	8[(2)]	0.055
Women:	310-320	0.152	1000-1200	5100[(3)]	18[(4)] 27[(5)]	

[(1)] This value is for all adults.

[(2)] This value is for postmenopausal women.

[(3)] This value is for breastfeeding women.

[(4)] This value is for premenopausal women.

[(5)] This value is for pregnant women.

Table 2.3- The tolerable intake amount of heavy metals; lead, mercury, cadmium, and arsenic. Reference for lead, cadmium, mercury, and arsenic (WHO, 2010b; WHO, 2003; WHO, 2007b; WHO, 2010a). Unit for cadmium is mg/day, while it is mg/kg body weight (b.w.) per day for the other three elements.

Tolerable intake of:	Lead	Mercury	Cadmium	Arsenic
For adults:	0.025[(6)]	0.002	0.01-0.035	0.015[(7)]

[(6)] Research conducted by the Joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) Expert Committee on Food Additives (JECFA) dictates that the previously provisional weekly intake (PTWI) of lead of 0.025 mg/kg b. w. per week could no longer be deemed health protective and was withdrawn. The Committee could not determine a new PTWI as the dose-response analysis did not give sign of the amount where the harmful effects of lead initiate.

[(7)] Research conducted by the FAO/WHO/JECFA dictates that the previously provisional weekly intake (PTWI) of 0.015 mg/kg b. w. per week for inorganic arsenic could no longer be deemed health protective and was withdrawn. The Committee could not determine a new PTWI.

Table 2.4- The recommended dietary allowance of fat, sodium, protein, zinc, and phosphorus. Reference for; fat, sodium, zinc, protein, phosphorus (Cleveland clinic, 2014; Linus Pauling Institute, 2018a; Linus Pauling Institute, 2018b; Pendick, 2018; EFSA, 2015). Units for sodium, zinc and phosphorus is mg/day, for fat it is g/day and for protein it is g/kg b.w.

Recommended dietary allowance:	Fat	Sodium	Protein	Zinc	Phosphorus
Men:	44-77[8]	1200-1500	0.8	11	550
Women:	44-77[8]	1200-1500	0.8	8	550

[8] The recommended dietary allowance based on 2,000 calories a day intake.

2.8 THE ROLE OF FISH IN DIET

Gathering knowledge about nutrient composition in food is very important and can be utilized to fully understand the links between nutrient access and intake and food production (Belton and Thilsted, 2014). This knowledge can be used to create programs and policies to optimize food supply that can fulfil nutrient requirements of the population. Fish play an important role in the Angolan diet, about 29 percent of total animal protein comes from fish (FAO, n.d.a). To evaluate the food security in Angola, all species of fish that can be commercially interesting should have a complete nutrient profile. Different species of fish contain an extremely variable nutrient composition and therefore, completing nutrient profiles should be considered a high priority (FAO, n.d.b). Meats such as beef, pork, mutton, and lamb are regarded as highly distinct kinds of meat, while herring, halibut and salmon contain a much greater difference in composition, but are usually lumped together (FAO, n.d.b). Recommended intake of fish is approximately 300-450 grams per week (Helsedirektoratet, n.d.), and the mean consumption in Angola in 2013 was approximately 357.7 grams per week (Nesheim, Oria and Yih, 2015; FAO, n.d.a). Although mean consumption is within the recommended intake of fish, many people in the population may still be consuming lower amounts than recommended.

The primary objective of this thesis was to report extensive nutrient and heavy metal composition profiles of commercially and potential commercially interesting species in Angola. The second objective was to evaluate different fish species in relation to recommended nutrient intakes and the health problems in Angola. Specific nutrients considered are iron, iodine and zinc, which are of known public health concern in Angola and the sub-Saharan region (Tomlinson et al., 2010; Béné and Heck, 2005).

3 MATERIALS AND METHOD

Before sampling started, a list was made by the Institute of Marine Research (IMR) with all species that were deemed commercially significant for Angola. Species were chosen both to add on to existing nutritional profiles (Wu Leung, Busson and Jardin, 1968) and to gather knowledge of nutrient and heavy metal composition of already commercially viable species lacking nutrient composition profiles.

Sampling of the species *Sardinella maderensis, Decapterus rhoncus, Sardinella aurita, Trachurus trecae* and *Sphyraena guachancho* was conducted on board the vessel Dr Fridtjof Nansen off the coast of Angola between 21st of September and 12th of October 2017. The methods described in section 2.1-2.5 were all developed by IMR. Below are photos of the trawl used for sampling, the five species that were sampled, and the fillets used for nutrient and heavy metal analysis.

Figure 3.1- A trawl catch (photo by Sofie Myhre Christiansen, 2017).

Figure 3.2- The catch released from the trawl onto the deck. (photo by Sofie Myhre Christiansen, 2017).

Figure 3.4- Decapterus rhonchus (Arias, 2010).

Figure 3.5- Sardinella aurita (Fishbase, 2013).

Figure 3.6- Trachurus trecae (Luna and Bailly, n.d.).

Figure 3.7- Sardinella maderensis (Heessen, 2006.).

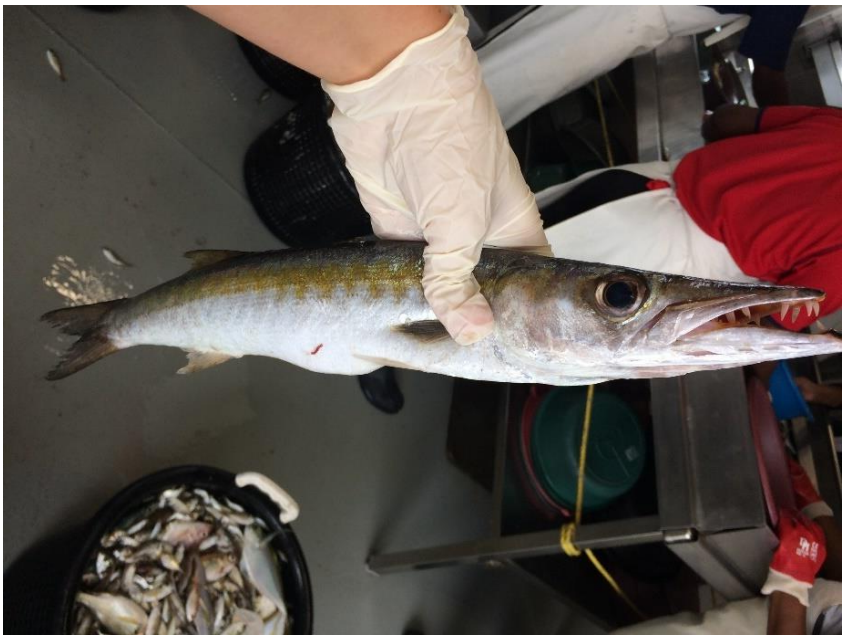

Figure 3.8 - Sphyraena guachancho (photo by Sofie Myhre Christiansen, 2017).

The total catch from the trawl, showed in *Figure 3.2,* was first sorted into baskets and a sub-sample was taken out while the other baskets were disposed of. The number of baskets in the sub-sample depended on the amount of catch, and then the sub-sample was divided into the different species. If 25 fish measuring more than 25 cm from one of the species on the list were in the sub-sample, these were collected, weighed, homogenized, and freeze dried. The samples, shown in Table 3.1, were collected at different trawl stations and at several different depths. Both a small four-panel 'Åkrahamn pelagic trawl' and a 'Gisund super bottom trawl' were utilized. The pelagic trawl, as shown in *Figure 3.19,* is a net that has a larger opening in the front held open by trawl doors. Mesh spacing decreases towards the terminating codend. The bottom trawl, as shown in *Figure 3.11,* has generally a smaller opening than the pelagic trawl. Mesh spacing is typically narrower than the pelagic trawl in the front and either decreases towards the posterior or remains constant. *Figure 3.12* shows the survey transect and all the trawling stations where sampling for this thesis were conducted. The units of the results have been changed so they correspond to the units in the RDI and tolerable intake values from Table 2.1, 2.2 and 2.3. Table 4.1 and 4.2 show the values from software program Laboratory information management system (LIMS Labware 7 PROD), while Table 4.3 shows the ratio between species from the same nutrient and heavy metal. All values were divided by the species with the lowest result from each element, to be able to easily compare the results between the species. I have decided to use LoD/2 (or LoD/$\sqrt{2}$) for the results that came back as under the limit of detection for the instrument used to analyze them. Other methods used are either to set the value as LoD or as zero. All three of these methods will cause bias when used in statistics, but since the values will be somewhere between LoD and zero the method used in this thesis was half LoD (Croghan, 1987).

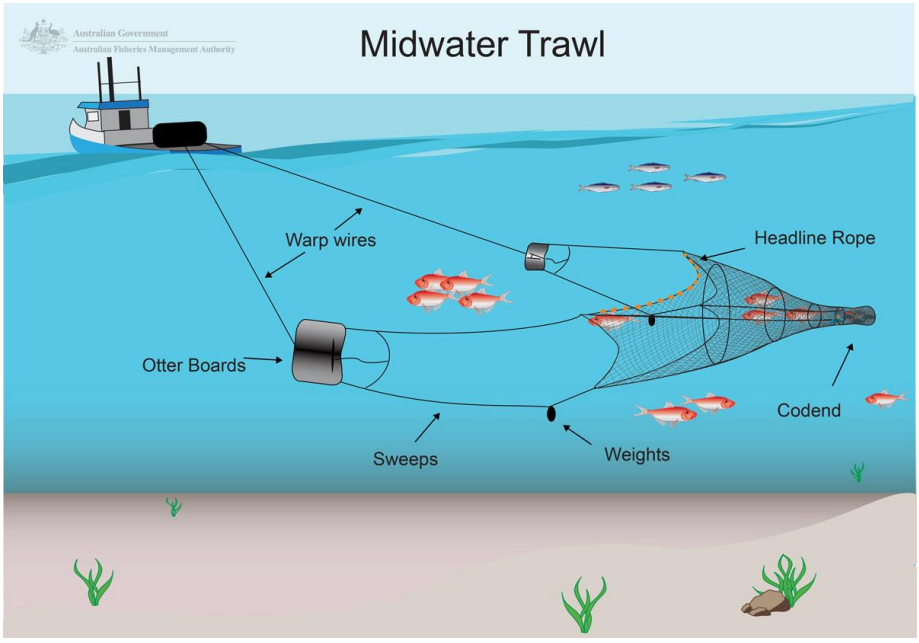

Figure 3.10- Animation of a general pelagic trawl (Australian Fisheries Management Authority, 2017).

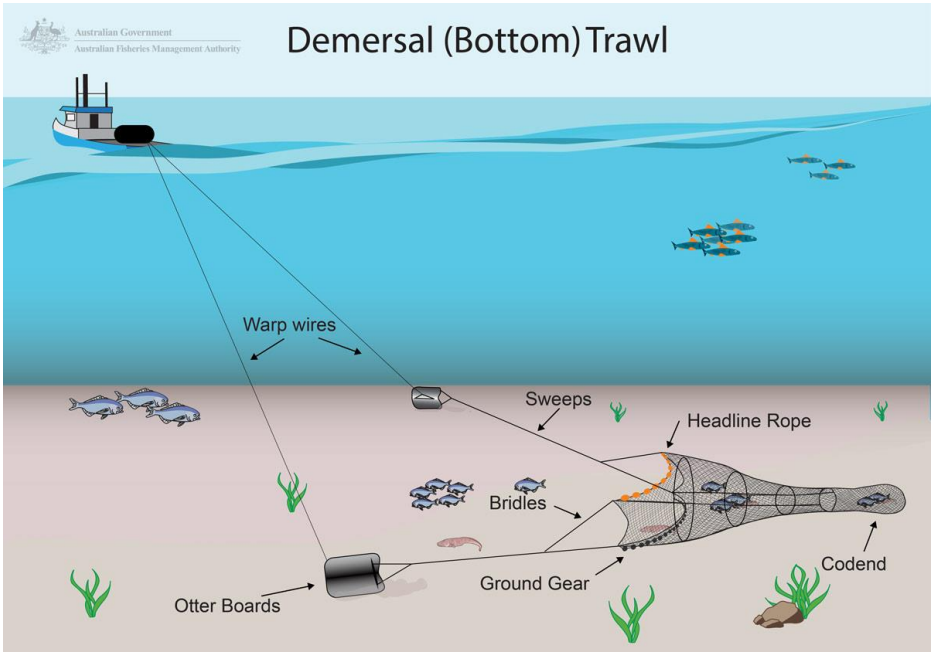

Figure 3.11- Animation of a general bottom trawl (Australian Fisheries Management Authority, n.d.).

Figure 3.12: Map showing transect (route for the ship) of trawl stations where sampling was conducted (Map Maker, 2018).

Table 3.1- Fish sampled off the coast of Angola.Unit for 'Big fish sampled' is amount in individuals, for 'Trawling depth' and 'Bottom depth' it is meters under the surface.

Date of capture	Species	Big fish sampled	Trawl station	Trawling depth start	Trawling depth stop	Bottom depth
03.10.2017	*Sardinella maderensis*	25	5	23	23	23
03.10.2017	*Decapterus Rhoncus*	25	5	23	23	23
06.10.2017	*Trachurus trecae*	25	13	0	0	98
06.10.2017	*Sardinella aurita*	25	14	10	10	56
07.10.2017	*Sphyraena guachancho*	25	21	58	59	59

3.1 PROCEDURE FOR FISH OVER 25 CM

Sampling was conducted when more than 25 fish of the same species that measured over 25 cm were caught in the trawl. The fish were weighed and measured individually. The liver was sampled from the first 15 fish and collected in 12.5 mL Nunc trays, and fillets were sampled individually from all 25 fish. Feces was collected from the first 15 fish in 12.5 mL Nunc trays, pooled samples of feces from five fish were made. Gonads were studied to determine sex for all 25 fish. Fillets from each fish was homogenized in a big food processor or in a small hand blender, scooped into 50 mL tubes for wet samples and salad trays for freeze-drying. Liver samples and wet samples from fillets were put in bags and vacuum packed with a note containing date, species, journal number, station number and the number of samples.

Salad trays were weighed with lids and placed in the freezer for at least 12 hours at -20 degrees Celsius (°C) or lower. These samples were then freeze dried for 72 hours without lids. For the first 12 hours the freeze-dryer was set to +25°C, then temperatures were decreased to -25°C. After 72 hours the samples were controlled to see if they were dry enough, this was done by breaking them in half and if they crumbled like biscuits they were finished. The new lids were attached, and the samples were weighed once more. The samples were homogenized again in the hand blender and funneled into 50 mL tubes. The tools used for homogenization were dusted off with a brush between samples. The samples were placed in bags and vacuum packed with a note with date, species, journal number, station number and the number of samples.

The gonads, feces and liver were not used in this thesis and will therefore not be discussed further.

3.2 LABORATORY ANALYSIS OF TOTAL FAT

This method is accredited for analyzing total fat content in food, feed, tissue and tissue fluids from animals and fish. The analysis was conducted by the author at IMR laboratories.

Before analysis of total fat could commence, the dry samples of individual fish were pooled together, with five fish in each sample. The five samples of individual fish were thoroughly

homogenized. The analysis of total fat by ethyl-acetate (grams/100 grams) was conducted at IMR laboratory. Freeze-dried samples were weighed in between 3-5 grams into an Erlenmeyer flask. The scale and computer were connected in such a way that the measured weight was directly transferred to the computer, and into LIMS. After the samples were weighed 30 mL of ethyl-acetate was added to each of the dry samples. The lid was placed tightly on the flask and shaken for a few seconds before being placed in the shaking machine for at least two hours. Due to circumstantial time constraints, some of the samples were instead shaken for ten minutes one day and ten minutes next day. The samples were left to rest so that the dry material could fall to the bottom. The samples were then funneled through a filter (S&S 597 1/2, Ø 150mm) in a fume hood, and 5 mL (10 mL if the fish was not very fatty) was pipetted of this new sample into an already zeroed out fuming bowl. After sitting in the fume hood for about an hour, or until all the ethyl-acetate were vaporized, the samples were placed in a heating cabinet at 70℃ for 2 hours. The samples were left to cool to room temperature in a desiccator and weighed.

3.2.1 Quality control of fat analysis

Quality control for this method is determined by:

1. Difference between replicates.
2. Analysis of control material and a control card ledger. All control material was analyzed within the limit set for this method.
3. Participation in SLP (Interlaboratory comparison). Average accuracy from a mean value of SLP from 2001-2004 is 98%.

3.3 ANALYSIS OF ALKALINE METALS WITH INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY, ICP-MS

This method is used to determine the amount of calcium (Ca), potassium (K), sodium (Na), magnesium (Mg) and phosphorus (P) in food, feed, tissue and tissue fluids from animals and fish. The same method is used to determine the content of silver, iron, cobalt, manganese, chromium, nickel, vanadium, molybdenum, and yttrium, but different internal standards are used. This method was completed by IMR laboratories.

First the sample is weighed, then added concentrated nitric acid (HNO_3 Suprapur or own distilled acid from p. a. quality) to it and heated in a microwave to decompose all organic material completely. The sample is atomized before placed into heated argon gas (plasma at about 7000 degrees Celsius). The solvent evaporates, and the elements are ionized. The ions are then led to a mass-sensitive detector where certain masses are detected as hits/second. An external curve calculates the concentration of elements. Scandium (Sc) is used as an internal standard for correction of operations for Ca, K, Na, Mg and P. Rhodium (Rd), Germanium (Ge), Indium (In) and Thulium (Tm) can be used as an internal standard for correction of operations. Gold (Au) is used to stabilize the mercury ions and is added with the internal standard and in the solutions for the standard curve.

3.3.1 Quality control

Quality control of the methods reliability is performed by:

- Keeping a log book.
- Parallels.
- Keeping a control card with control material.
- Blind samples.
- Participation in SLP.

Certified reference material (CRM) used for the alkaline method is skimmed milk powder BD150 from Environmental Resources Management (ERM) and Bovine liver 1577c from National Institute of Standards and Technology (NIST). Standard reference material used for determining silver, iron, cobalt, manganese, chromium, nickel, vanadium, molybdenum, and yttrium is oyster tissue, CRM 1566 from NIST, lobster hepatopancreas, TORT 3 from National Research Council Canada (NRC) and bovine liver 1577 from NIST. For the complete method see appendix.

3.4 RAW PROTEIN ANALYSIS WITH NITROGEN-ANALYZER

This method is accredited for analyzing nitrogen content in food, feed, tissue samples, feces, and other nitrogenous matrices. The analysis was completed by IMR laboratories. The limit of detection (LoD) for this instrument is 0.1-16.0 g N/100 g.

During the combustion of the sample, homogenous sample material is weighed in aluminum foil or a capsule and is placed inside the test carousel. The sample goes through a chamber where atmospheric gases are removed before it falls into a combustion pipe where the temperature is at 950°C. The sample combusts completely in the presence of O_2. Nitrogen, carbon, and hydrogen oxidizes to NOx, CO_2, and H_2O. The combustion gases are led to another combustion pipe by O_2 for more oxidation and removing of particles at 850°C.

The combustion gases then pass through a pre-cooler for removal of water vapor. The gases are then gathered in a ballast tank for homogenization/equilibration. They are then led through an aliquot loop by helium and passes through a reduction pipe filled with copper reagent and has a temperature of 700°C. NOx is reduced to N_2 and excess oxygen is captured her. The gas is the led through a pipe filled with Lecosorb and Anhydrone to remove CO_2 and H_2O.

The amount of N_2 is detected in a thermal conductivity cell (TC), and the result is given as % of total nitrogen. For the complete method see appendix.

3.4.1 Quality control

- Parallels.
- Keeping a control card for sulfanilamide.
- Keeping a control card for SMRD 2000.
- Participation in SLP.

3.5 IODINE CONCENTRATION DETERMINED WITH ICP-MS AFTER EXTRACTION WITH A BASE

This method is accredited for food, feed, tissue, and tissue fluids, but cannot be used on samples containing plant starch (more than 5% carbohydrates) and brown algae. The samples are weighed, and water and TMAH (tetramethylammonium hydroxide) is added and placed in a heating cabinet at 90 ± 3 °C. Iodine content is found by using ICP-MS and calculated by using a standard addition curve made in a matrix equivalent of the test samples. Tellurium (standard solution from NIST H_6TeO_6 1000mg/l in HNO_3 0,5 mol/l) is applied as an internal standard to correct the baseline for operations.

3.5.1 Quality control

1. Keeping a log book form.
2. Parallels.
3. Keeping a control card on the control material.
4. Blind samples.
5. Participation in SLP.

Standard reference materials used are skimmed milk powder ERM-BD 150 and fish muscle ERM-BB422. For the complete method see appendix.

3.6 STATISTICS

The following equations were used in this thesis. Equations 2 and 3 were used with the LIMS software. Excel version 1803 was used to calculate equations 1 and 4.

$$SD = \sqrt{\frac{\sum|x-\mu|\text{^}2}{N}}$$

Equation 1: For calculating standard deviation (Khan Academy, 2018).

$$\%\ fat\ in\ freeze-dried\ sample = \frac{A\ x\ B\ x\ 100}{C\ x\ (D-(1.1\ x\ B))}$$

where:

A = milliliters of ethyl acetate added to sample

B = grams of fat in the evaporating bowl

C = grams of sample weighed in (dry material)

D = milliliters of filtrate pipetted

Equation 2 – For finding the percentage of fat in a freeze-dried sample (From the method sheet from IMR).

$$\%\ \text{fat in "wet" sample} = \frac{\%\ fat\ in\ \text{dry}\ sample\ x\ \%\ dry\ material}{100}$$

Equation 3 – For calculating the percentage of fat in a wet sample (From the method sheet from IMR).

$$\%\ \text{Dry material} = \frac{(c-b)\ x\ 100}{(a-b)}$$

where:
a = Weight of the sample + container before freeze-drying (gram)
b = Weight of container (gram)
c = Weight of sample + container after it is freeze-dried (gram)

Equation 4 – For finding the percentage of fat in a freeze-dried sample (From the method sheet from IMR).

Small concentrations cannot be measured precisely by chemical analysis procedures because of their limitations and are therefore defined as under the LoD. To be able to use these values in statistical analysis they are usually substituted with a constant value, half the LoD, LoD divided by the square root of two, or zero are usually used. All these methods create bias, however the

size is dependent on the percentage of values below the LoD. The extrapolation method and the maximum likelihood method introduce less bias but need more statistical expertise (Croghan, 1987). The method used in this thesis is the LoD/2 for the results lower than the LoD.

4 RESULTS AND DISCUSSION

The main findings in this study was the difference in phosphorus levels between this study and a previous study (Wu Leung, Busson and Jardin, 1968); the previous phosphorus data reported 98 mg/100 g (Table 2.1) in *Sardinella aurita*, while the amount of phosphorus in *Sardinella aurita* analyzed in this paper was 344 mg/100 g (Table 4.2); this represents a dramatic increase that may be a byproduct of technological advances in data collection and analysis. Fat content in *Trachurus trecae* (Table 4.1) was 7.0 g/100 g, while for *Trachurus spp.* (Table 2.1) it was 4 g/100 g. Fat content for *Sphyraena guachancho* (Table 4.1) was 2.1 g/100 g, while for *Sphyraena spp.* (Table 2.1) it was 0.7 g/100 g. The previously recorded zinc content of *Sardinella spp.* (Table 2.1) was 1.57 mg/100 g, while for *Sardinella maderensis* and *Sardinella aurita* it was 0.56 and 0.52 mg/100 g respectively. Iron content of previously recorded data for *Sphyraena spp.* was 0.9 (Table 2.1), while for *Sphyraena guachancho* it was 0.4 mg/100 g (Table 4.2). 50 gram portion of *Sardinella maderensis* and *Sardinella aurita* provide 9.5 and 11.5% of the daily recommended iron intake, respectively (Table 4.3). Iodine content varied between the five species; range of 0.022-0.047 mg/100 g (Table 4.2). *Sphyraena guachancho* contained the highest amount of iodine, 0.047 mg/100 g (Table 4.2), while previous data reported a range between 0.018 to 1.21 mg/100 g (Ive et al., 2018) for six different fish species. No data for iodine content for the species covered in this paper was found. *Sphyraena guachancho* contained higher amounts of iron; a 50 gram portion would provide 15.3% of the daily recommended iron intake. Calcium content varied greatly for the five species covered in this paper; the content in *Sardinella aurita* and *Sardinella maderensis* were 71.6 and 89.4 mg/100 g respectively, while the content for the other three species ranged between 20.4 and 25.8 mg/100 g (Table 4.2).

4.1 PROTEIN AND FAT

Table 4.1 shows the mean fat content in the five species, which varied from 2.2 to 7.0 g/100 g. Previous data, shown in Table 2.1, differ from the data in Table 4.1. For instance, fat content of *Sphyraena guachancho* was 2.1 g/100 g (Table 4.1), while it is 0.7 g/100 g for *Sphyraena*

spp. (Table 2.1). The value for *Trachurus spp* (4 g/100 g) (Table 2.1), was 3 g/100 g lower than for *Trachurus trecae* (7.0 g/100 g) analyzed in this thesis (Table 4.1).

Fat usually varies more than other proximate components in fish such as protein (FAO, n.d.b), as corroborated by Orban et al. (2011) and Bogard et al. (2015). The study by Orban et al. (2011) showed the change in lipid content in *Trachurus trachurus* from 2.10 g/100 g in September, to 1.42 g/100 g in March and 1.57 g/100 g in December. The study by Bogard et al. (2015) also shows the variation in fat content for 55 species of fish; ranged from 0.3 to 18.3 g/100 g. The results in Table 4.1 coincides with this statement. Variations in fat content between species could be a result of anatomical differences or environmental factors (FAO, n.d.b). Environmental factors could include seasonal variations that govern food variability, migration patterns or different stages in the lifecycle (FAO, n.d.b). *Sardinella maderensis, Decapterus rhoncus* and *Sphyraena guachancho* have the lowest fat content out of the five species sampled, and this could reflect the food availability at the depth at which these species reside since these species were caught at the sea floor. *Sardinella aurita* and *Trachurus trecae* contain more fat and were caught higher in the water column (Table 3.1), this could reflect the higher abundance of food closer to the ocean's surface.

Protein content, as seen in Table 4.1, varied slightly less with a range between 20.8 and 23 g/100 g wet weight (ww). According to FAO (n.d.b) fish contain the amino acids lysine and methionine which are essential to maintain good health, thus, it can be presumed that the protein content of *Sardinella maderensis, Decapterus rhoncus, Sardinella aurita, Trachurus trecae* and *Sphyraena guachancho* represents high dietary quality. Protein content from FAO (2012) and Wu Leung, Busson and Jardin, (1968) was broadly consistent with protein content analyzed in Table 4.1.

Table 4.1 - Shows the results from laboratory analysis of the nutrients fat and protein. The results are shown as mean values ± standard deviation (SD).

Substance	*Sardinella maderensis*	*Decapterus Rhonchus*	*Sardinella aurita*	*Trachurus trecae*	*Sphyraena guachancho*
Fat (g/100 grams)	2.3 ± 0.5	2.2 ± 0.3	3.3 ± 0.4	7.0 ± 1.0	2.1 ± 0.8
Protein (g/100 g ww)	20.8 ± 0.4	23.0 ± 0	21.6 ± 0.49	20.8 ± 0.4	20.8 ± 0.4

Figure 4.1 shows the ratio of the substance in relation to the mean value from all five species, showing that the protein content is quite equal in all species. While the ratio of fat shows that there are large differences in the content; the fat content of *Trachurus trecae* is over twice as high as the average value of fat in all five species. While *Sardinella maderensis, Decapterus rhonchus* and *Sphyraena guachancho* contain a bit over half of the average amount of fat.

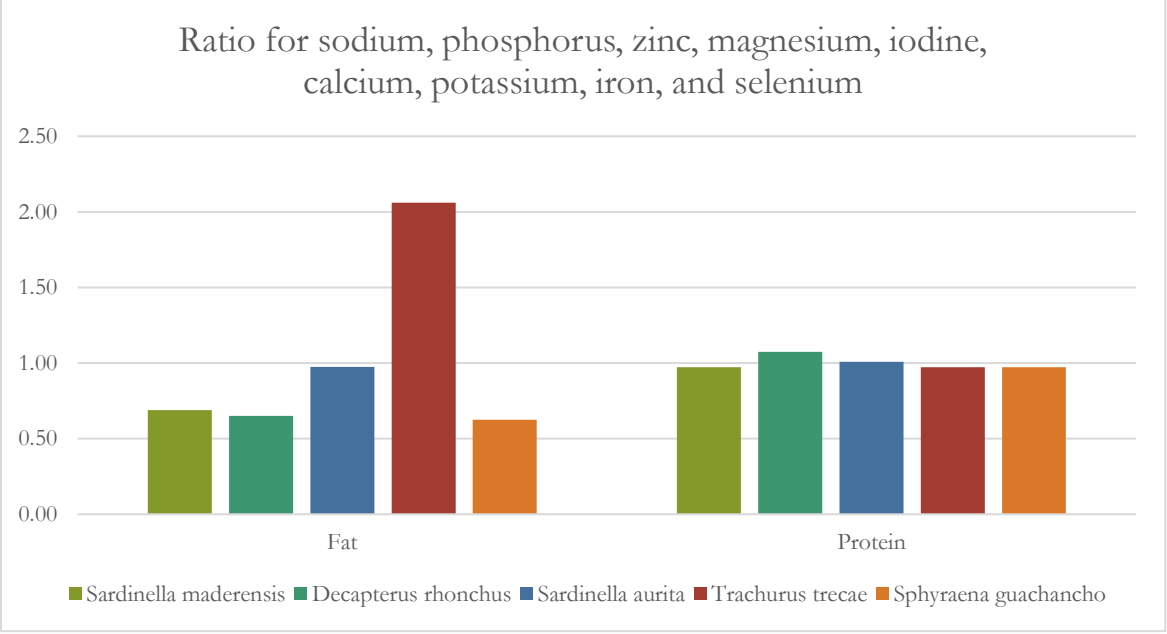

Figure 3.1- The table shows the ratio between the different species within the same substance; for fat and protein. All the results were divided in Excel by the mean value for each substance to make the results easier to compare and to easier graph them together.

4.2 MINERAL COMPOSITION

The sodium, phosphorus, zinc, magnesium, iodine, calcium, potassium, iron and selenium composition for all species are shown in Table 4.2.

Table 4.2 - Shows the results from laboratory analysis of the nutrients sodium, phosphorus, zinc, magnesium, iodine, calcium, potassium, iron, and selenium. The results are shown as mean values ± standard deviation (SD), the results that were below Limit of Detection (LoD) are shown as LoD/2.

Substance	*Sardinella maderensis*	*Decapterus Rhonchus*	*Sardinella aurita*	*Trachurus trecae*	*Sphyraena guachancho*
Sodium (mg/100 g)	46.6 ± 3.1	49.8 ± 0.8	45.2 ± 1.5	64.6 ± 1.0	44.8 ± 2.0
Phosphorus (mg/100 g)	338 ± 4.0	294 ± 8.0	344 ± 8.0	284 ± 9.9	310 ± 6.3
Zinc (mg/100 g ww)	0.56 ± 0.06	0.47 ± 0.03	0.52 ± 0.02	0.42 ± 0.02	0.33 ± 0.01
Magnesium (mg/100 g)	39.2 ± 0.8	37.4 ± 0.5	37.6 ± 0.5	35 ± 0.6	38.2 ± 0.8
Iodine (mg/100 g)	0.035 ± 0.004	0.022 ± 0.001	0.024 ± 0.002	0.027 ± 0.001	0.047 ± 0.003
Calcium (mg/100 g)	89.4 ± 8.7	25.8 ± 16.9	71.6 ± 8.0	24.6 ± 6.3	20.4 ± 3.3
Potassium (mg/100 g)	528 ± 12	480 ± 6	514 ± 5	456 ± 10	534 ± 10
Iron (mg/100 g ww)	1.52 ± 0.16	0.65 ± 0.01	1.84 ± 0.14	0.84 ± 0.01	0.40 ± 0.04
Selenium (mg/100 g ww)	0.05 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00

Figure 4.2 displays the ratios of mineral concentrations in the individual species compared to the average mineral concentrations across the five species; *Sardinella maderensis, Decapterus rhonchus, Sardinella aurita, Trachurus trecae* and *Sphyraena guachancho*.

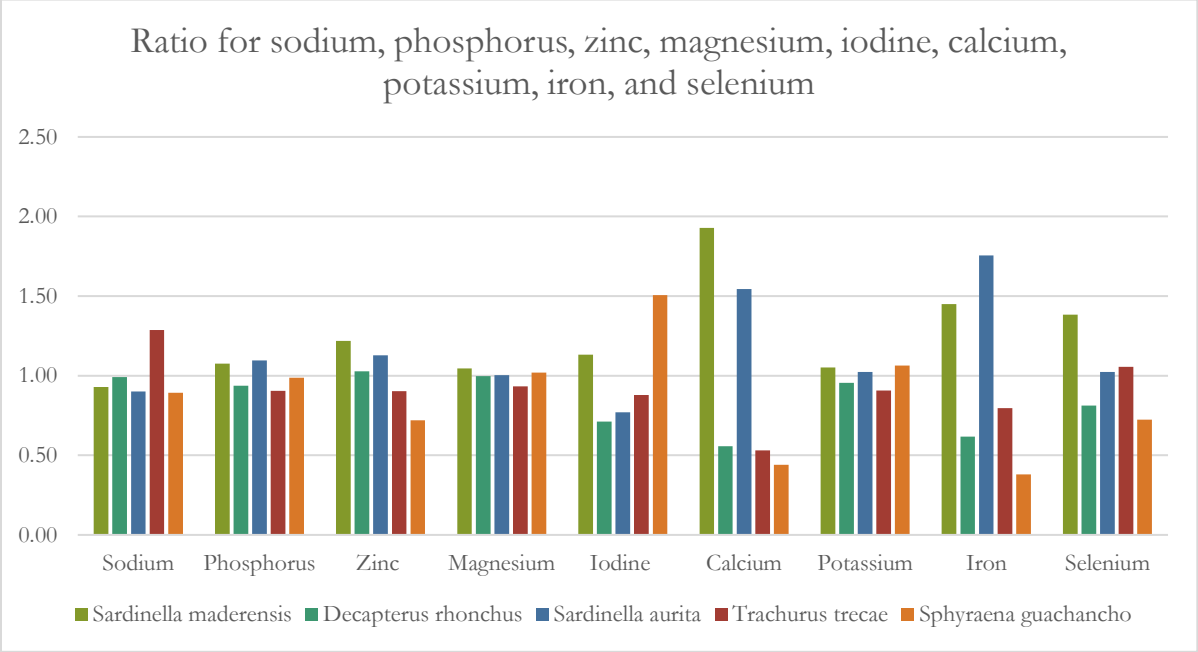

Figure 4.2 - The table shows the ratio between the different species within the same substance; for sodium, phosphorus, zinc, magnesium, iodine, calcium, potassium, iron, and selenium. All the results were divided in Excel by the mean value for each substance to make the results easier to compare and to easier graph them together.

Table 4.3 shows the percentage of recommended daily intake of various nutrients provided the consumption of a 50 gram serving of each species. Portion sizes lack a universally accepted standard; thus, a reasonable portion was required to be chosen. A 50 gram portion is cited in previous literature (Bogard et al., 2015), and represents a reasonable intake of fish, given a recommended 300-450 grams per week (Helsedirektoratet, n.d.).

Table 4.3- The percentage cover of daily recommended intake of zinc, iodine, and iron in Sardinella maderensis, Decapterus rhonchus, Sardinella aurita, Trachurus trecae and Sphyraena guachancho if 50 g of fish were consumed.

Substance	For	Sardinella maderensis	Decapterus Rhonchus	Sardinella aurita	Trachurus trecae	Sphyraena guachancho
Zinc (%RNI/50 g)	Men	2.6	2.2	2.4	1.9	1.5
	Women	3.5	3.0	3.3	2.6	2.1
Iodine (%RNI/50 g)	Adults	11.5	7.2	7.8	8.9	15.3
Iron (%RNI/50 g)	Postmenopausal women	9.5	4.1	11.5	5.2	2.5
	Premenopausal women	4.2	1.8	5.1	2.3	1.1
	Pregnant women	2.8	1.2	3.4	1.5	0.7

4.2.1 Zinc

Zinc concentrations varied slightly from 0.336 to 0.562 mg/100 g (Table 4.2). The zinc concentrations from Table 2.1 as reported by FAO (2012) for *Sphyraena spp.* was in this range with a value of 0.51 mg/100 g, although the value for *Sphyraena guachancho* was slightly lower at 0.332 mg/100 g (Table 4.2). The reported value for *Sardinella spp.,* with a value of 1.57 mg/100 g (Table 2.1), was almost three times higher than for both *Sardinella maderensis* and *Sardinella aurita* with values of 0.56 and 0.52 mg/100 g respectively (Table 4.2). This could reflect inter-species differences, seasonal variation, difference in sex or difference in life-cycle stage (Zubcov et al., 2012; Younis et al., 2015). Figure 4.2 shows that the zinc content in *Sardinella maderensis, Decapterus rhonchus, Sardinella aurita, Trachurus trecae* and

Sphyraena guachancho was quite similar. *Sphyraena guachancho* contained considerably lower amounts, with a ratio of 0.72 relative to the average value.

50 grams as a portion size was used to calculate how much each of the species studied in this thesis cover the recommended intake of zinc per day. 50 grams of *Sardinella maderensis, Decapterus rhonchus, Sardinella aurita, Trachurus trecae* or *Sphyraena guachancho* represents between 1.5 and 2.6 % of the recommended intake of zinc per day for men. For women these five species represent between 2.1 and 3.5 % of the recommended daily intake of zinc. Even though these species do not effectively supplement large amounts of zinc, bioavailability may be higher than other food groups, such as plants (Roohani et al., 2013; Hunt, 2003).

4.2.2 Iron

Iron content varied with a range from 0.40 to 1.84 mg/100 g. These values differ significantly from the previously reported values seen in Table 2.1, where the value for *Sphyraena spp.* is 0.9 mg/100 g and the results in Table 4.2 for *Sphyraena guachancho* is 0.40 mg/100 g. The reported value from FAO (2012) is over twice as high as the value found through analysis for this thesis. The variation in minerals can vary with season, as well as life-cycle stage and sex (Huss, 1995). These differences in values from earlier research, Table 2.1, and from this study, Table 4.2, could also be because of different sampling methods or differences in analysis methods. The value for *Sardinella aurita* in Table 2.1 is 1.3 mg/100 g, while in Table 4.2 it is 1.84 mg/100 g. The value for *Sardinella spp.* in Table 2.1 is 1.8 mg/100 g, which is close to the value of *Sardinella aurita.* However, the value for *Sardinella maderensis* is slightly lower at 1.52 mg/100 g. The differences between values from Table 4.2 and Table 2.1 should be further investigated.

Table 4.3 shows the coverage of recommended iron intake *Sardinella maderensis, Decapterus rhonchus, Sardinella aurita, Trachurus trecae* and *Sphyraena guachancho* gives in percentage per day. For postmenopausal women *Sardinella maderensis* and *Sardinella aurita* covers 9.5 and 11.5 % respectively, while the other three species only cover between 2.5 and 5.2 %. For premenopausal women these species cover between 1.1 and 5.1 %, while for pregnant women

these species cover between 0.7 and 3.4 % of the recommended iron intake per day. Although coverage of adequate daily iron intake from these five species is not very high (0.7-11.5%), the bioavailability of iron is high compared to iron from cereals and grains (Roohani et al., 2013; Hunt, 2003).

4.2.3 Iodine

Iodine content ranged from 0.022 to 0.047 mg/100 g (Table 4.2). Iodine content varies greatly with environment; marine species have a higher amount of iodine than freshwater fish and especially the skin contains a large amount of iodine (Eckhoff and Maage, 1997). Marine fish and seafood tend to be rich dietary sources with a range of 0.018 to 1.21 mg/100 g in marine fish reported elsewhere (Nerhus et al., 2018), the iodine content found in this study where in that range; 0.022-0.047 mg/100 g (Table 4.2). No previous data on iodine content in *Sardinella maderensis, Decapterus rhonchus, Trachurus trecae, Sardinella aurita* and *Sphyraena guachancho* were found.

The iodine content in *Sardinella maderensis, Decapterus rhonchus, Trachurus trecae, Sardinella aurita* and *Sphyraena guachancho* cover between 7.2 and 15.3 % of the recommended daily intake of zinc (Table 4.3). *Sphyraena guachancho* covers 15.3% of the daily intake, the highest coverage of these five species, but has given the lowest coverage of iron and zinc (Table 4.3). To access more iodine through fish the skin should be consumed as well, since the skin contains more iodine than the flesh (Rehbein and Oehlenschläger, 2009; Haldimann et al., 2005; Eckhoff and Maage, 1997).

4.2.4 Selenium, phosphorus, magnesium, sodium, calcium, and potassium

Selenium, phosphorus, magnesium, sodium, calcium, and potassium were analyzed for data completeness, but no research regarding public health concerns of these elements were found, and therefore, their nutritional significance is not discussed here.

Values for sodium ranged between 44.8 and 64.6 mg/100 g (Table 4.2). These values are lower than values reported elsewhere (FAO, 2012; Wu Leung, Busson and Jardin, 1968). Values from Table 2.1 shows values for sodium were 30-40 mg/100 g higher than the results from Table 4.2. The range in values for phosphorus was between 284 and 344 mg/100 g (Table 4.2). Phosphorus values for *Sardinella aurita* from Wu Leung, Busson and Jardin (1968) were 98 mg/100 g, while values from Table 4.2 shows a phosphorus content of 344 mg/100 g for the same species. This represents an increase of approximately 250%, calling into question the accuracy of these measurements. It may be possible that the development of more sophisticated measuring equipment could contribute to this large discrepancy. The values in phosphorus from FAO (2012) for *Sphyraena spp.* was 175 mg/100 g, while for *Sphyraena guachancho* phosphorus levels were 310 mg/100g in Table 4.2. Selenium content ranged between 0.03 and 0.05 mg/100 g (Table 4.2). No previous data for selenium content in the species researched in this study was found. The value reported by FAO (2012) for the potassium level of *Sphyraena spp.* is under half of the value found in this study (Table 4.2), however, the value for *Sardinella spp.* is broadly consistent with the values of *Sardinella maderensis* and *Sardinella* aurita (Table 4.2). Calcium and magnesium levels from Table 4.2 indicates that these values are broadly consistent with previous results from literature (FAO, 2012). The calcium results in Table 4.2 indicates that the previous calcium value for *Sardinella aurita* in Table 2.1 may no longer be representative; the value from Table 2.1 is approximately ten times higher than the result found in this study (Table 4.2).

4.3 HEAVY METALS

The cadmium, arsenic, lead and mercury composition for all species is shown in Table 4.4. Cadmium, arsenic, lead and mercury were analyzed for data completeness, and health implications of intake will not be discussed here. Figure 4.3 shows the ratio between species and the average value for each heavy metal.

Amount of cadmium ranged from 0.0001 to 0.0006 mg/100 g (Table 4.4), while arsenic levels were quite high, ranging from 0.056 to 0.226 mg/100 g. Figure 4.3 shows that arsenic levels in *Sphyraena guachancho* were very low compared to the other four species studied in this thesis,

under half of the average amount. Lead ranged between 0.00025 and 0.00096 mg/100 g (Table 4.4). Lead levels were over two and a half times higher in *Sardinella maderensis* compared to the other species, which contained quite similar amounts as seen in Figure 4.3. Mercury levels ranged from 0.0014 to 0.017 mg/100 g (Table 4.4). The mercury levels in *Sphyraena guachancho* were over three times higher than the average amount in all five species (Figure 4.3).

Table 4.4 - Shows the results from laboratory analysis of the heavy metals cadmium, arsenic, lead, mercury, and selenium. The results are shown as mean values ± standard deviation (SD), the results that were below Limit of Detection (LoD) are shown as LoD/2.

Substance	*Sardinella maderensis*	*Decapterus Rhonchus*	*Sardinella aurita*	*Trachurus trecae*	*Sphyraena guachancho*
Cadmium (mg/100 g ww)	0.0006 ± 0.0001	0.00044 ± 0.00015	0.00034 ± 0.00005	0.00028 ± 0.00008	0.0001
Arsenic (mg/100 g ww)	0.21 ± 0.02	0.154 ± 0.008	0.176 ± 0.016	0.226 ± 0.005	0.056 ± 0.01
Lead (mg/100 g ww)	0.00096 ± 0.00019	0.0003	0.0003	0.0003	0.00025
Mercury (mg/100 g ww)	0.0046 ± 0.0009	0.0022 ± 0.0002	0.0014 ± 0.0007	0.0026 ± 0.0006	0.017 ± 0.0052

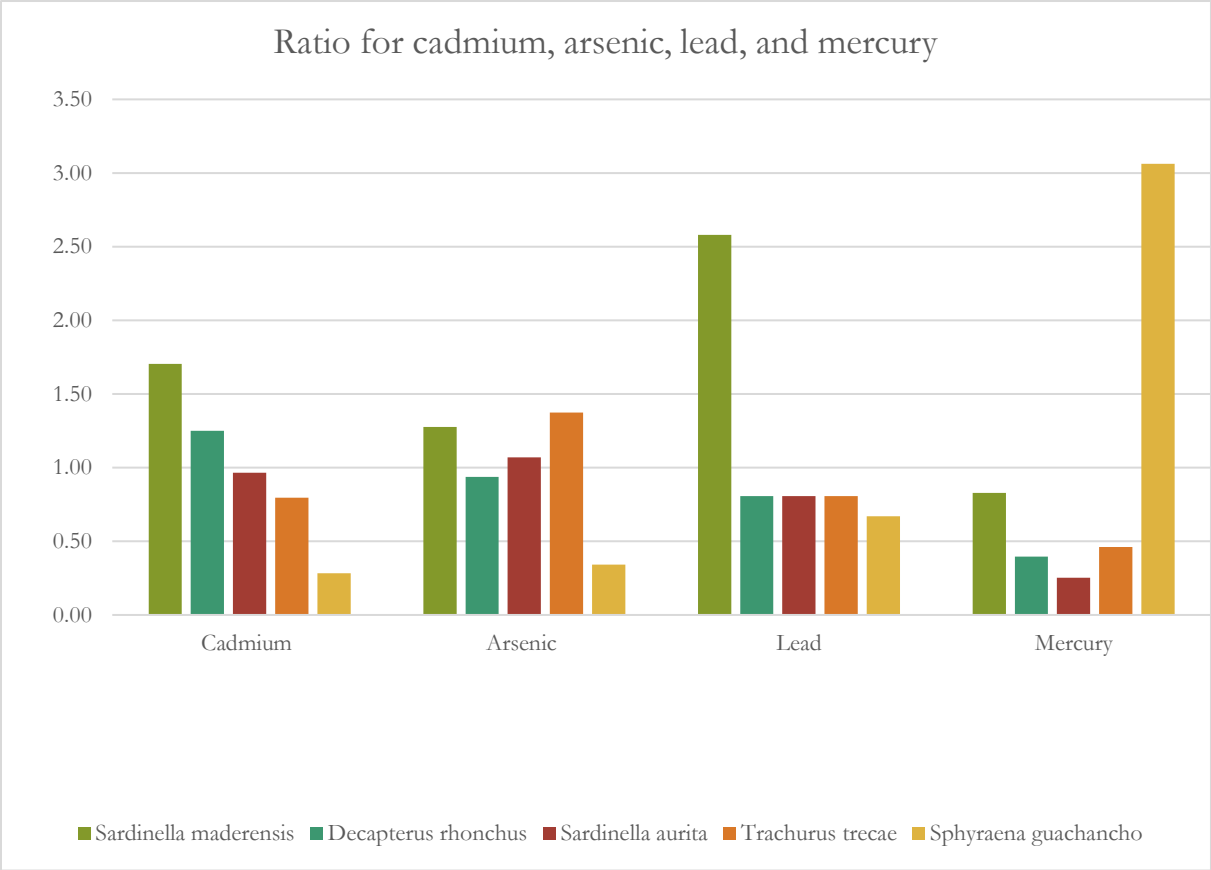

Figure 4.3 - The table shows the ratio between the different species within the same substance; for cadmium, arsenic, lead, and mercury. All the results were divided in Excel by the mean value for each substance to make the results easier to compare and to easier graph them together.

4.4 QUALITY CONTROL

Table 4.4 shows that most of the parallels in the total fat analysis were quite equal values, between 0.06 and 3.55 % difference. The only exception occurred between the two parallels from the first pooled sample of *Spyraena guachancho* which had a difference of 13.27%, and according to the method makes this a medium level of measurement insecurity. The reasons for the differences in parallels could be because the samples were not homogenized well enough, the particles were too fine for the filtration paper or the samples did not get enough time to vaporize all the ethyl-acetate.

Table 4.4- Quality control run in analysis of fat. Shows the difference between the two parallels for each of the five pooled samples for each species.

Quality control of difference between parallels in fat in %					
Sample	*Sardinella maderensis*	*Decapterus rhoncus*	*Sardinella aurita*	*Trachurus trecae*	*Spyraena guachancho*
1	2.06	1.54	3.41	0.50	13.27
2	0.28	1.59	0.06	1.18	3.53
3	0.25	3.51	3.55	1.83	0.68
4	0.42	1.35	3.28	1.50	2.56
5	2.36	1.47	2.51	0.27	0.81

4.5 LIMITATIONS

Seasonal variation of nutrients in food, especially fish, has been documented (Ananthan et al., 2012; Nargis, 2006). The seasonal variation depends on life cycle stages, sex, food availability and environmental changes (Nargis, 2006; van der Heide et al., 2018). However, it was outside the scope of this study to attempt to account for these variations. Furthermore, a relatively small size of pooled samples was used and only two replicates for each pooled sample. The inconsistent time spent in the shaking machine during the total fat analysis could also contribute to differences in parallels. Even though these limitations of the study are recognized, given the incomplete and lacking nutrient profiles of fish species in Angola, the results are nevertheless significant and future analysis can be used for comparison.

5 CONCLUSION

This paper expands the current knowledge pertaining to the nutrient and heavy metal content of fish species in Angola. Several of the species studied in this paper can contribute to the recommended nutrient intake of multiple nutrients. A more diverse fish consumption especially with the large range in nutrient composition in *Sardinella maderensis, Decapterus rhonchus, Trachurus trecae, Sardinella aurita* and *Sphyraena guachancho* would likely promote a more comprehensive nutrient intake in Angola. A higher intake of certain nutrients, such as iodine and calcium, will be consumed if not only the flesh, but also skin and bones were to be included in the diet. Several of the species can contribute to RNI of multiple nutrients. *Sardinella maderensis* had the highest amount of zinc with a value of 0.56 mg/100 g, *Sphyraena guachancho* had the highest amount of iodine with a value of 0.047 mg/100 g (Table 4.2), and *Sardinella aurita* contained the most iron with a value of 1.8 mg/100 g (Table 4.2). This supports the compelling argument to devote more resources toward providing a more balanced diet to effectively target malnutrition. Although fish consumption in Angola was in 2013 almost as high as the world mean consumption (almost 20 kg per capita in 2013) (FAO, n.d.a), nutritional profiling could assist in finding species that are suited for areas where deficiencies of certain nutrients persist, and aid in reducing health concerns which are based on nutrient deficiencies. More research is needed to accurately interpret the real contribution of these nutrients based on availability, access, and consumption, as well as more research on the potential health concern of heavy metal consumption of fish species in Angola.

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7 APPENDIX

7.1 LAGOCEPHALUS LAEVIGATUS

The species sampled in this thesis were all on the list except for *Lagocephalus laevigatus*. This species was sampled at the recommendation of the Director of the National Fisheries Research Institute in Angola, Filomena Vaz-Velho. Since this species is consumed in Angola, but is not one that is of commercial interest, I decided to cut it from the scope of this thesis. However, the results may be of use and I am therefore reporting them here in the appendix.

Figure 7.1- Lagocephalus laevigatus (photo by Sofie Myhre Christiansen, 2017).

Table 7.1 Fish sampled off the coast of Angola.

Date of capture	Species	Big fish sampled (amount in individuals)	Trawl station	Trawling depth start (meters under the surface)	Trawling depth stop (meters under the surface)	Bottom depth (meters under the surface)
05.10.2017	*Lagocephalus Laevigatus*	25	10	60	58	60

Table 7.2 - Shows the results from laboratory analysis of the nutrients fat, protein, sodium, phosphorus, zinc, magnesium, iodine, calcium, potassium, iron, and selenium. The results are shown as mean values ± standard deviation (SD), the results that were below Limit of Detection (LoD) are shown as LoD/2.

	Mean value analyzed
Substance	*Lagocephalus laevigatus*
Fat (g/100 grams)	2.07 ± 0.17
Protein (g/kg ww)	1.9 ± 0
Sodium (mg/kg)	506 ± 10.198
Phosphorus (mg/kg)	3240 ± 80
Zinc (mg/kg ww)	5.96 ± 0.512

Magnesium (mg/kg)	308 ± 7.483
Iodine (mg/kg)	0.137 ± 0.027
Calcium (mg/kg)	37 ± 1.095
Potassium (mg/kg)	5960 ± 135.647
Iron (mg/kg ww)	2.32 ± 0.133
Selenium (mg/kg ww)	0.206 ± 0.0102

Table 7.3 - Shows the results from laboratory analysis of the heavy metals cadmium, arsenic, lead, mercury, and selenium. The values are the maximum amount to be consumed. The results are shown as mean values ± standard deviation (SD), the results that were below Limit of Detection (LoD) are shown as LoD/2.

	Mean value analyzed
Substance	*Lagocephalus laevigatus*
Cadmium (mg/kg ww)	0.00045

Arsenic (mg/kg ww)	1.82 ± 0.271
Lead (mg/kg ww)	0.0025
Mercury (mg/kg ww)	0.0738 ± 0.0241

Table 7.4 – This table shows the nutrients contained in Lagocephalus laevigatus if 100 grams of fillet were to be consumed since this is the preferred unit for food composition tables.

Substance	*Lagocephalus Laevigatus*
Fat (g/100 grams)	2.07
Protein (g/100 g ww)	0.19
Sodium (mg/100 g)	50.6
Phosphorus (mg/kg)	324
Zinc (mg/100 g ww)	0.596
Magnesium (mg/100 g)	30.8
Iodine (mg/100 g)	0.0137

Calcium (mg/100 g)	3.7
Potassium (mg/100 g)	596
Iron (mg/100 g ww)	0.232
Selenium (mg/100 g ww)	0.0206

Table 7.5 – This table shows the heavy metal content in Lagocephalus laevigatus if 100 grams of fillet were to be consumed since this is the preferred unit for food composition tables.

Substance	*Lagocephalus Laevigatus*
Cadmium (mg/100 g ww)	0.000045
Arsenic (mg/100 g ww)	0.182
Lead (mg/100 g ww)	0.00025
Mercury (mg/100 g ww)	0.00738

Table 7.6- Quality control run in analysis of fat. Shows the difference between the two parallels for each of the five pooled samples for each species.

Quality control of difference between parallels in fat in %
Lagocephalus laevigatus
1.92
3.03
1.17
4.04
3.01

7.2 ANALYSIS OF ALKALINE METALS WITH INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY, ICP-MS

This method is used to determine the amount of calcium, potassium, sodium, magnesium and phosphorus in food, feed, tissue and tissue fluids from animals and fish. It has been implemented by NIFES staff to my samples.

Certified reference material, CRM, used in for this method is Skimmed milk powder BD150, ERM and Bovine liver 1577c, NIST. Chemicals used in this method is:

- Concentrated nitric acid (HNO_3) Suprapur, or own distilled acid from p. a. quality.
- Hydrogenperoxide (Perhydrol) (H_2O_2) 30% p. a. ISO.
- Deionized and filtrated water.
- Scandium (Sc) Spectrascan 1006 ± 3 mg/l in 5% HNO_3.
- Spectrascan multi standard: Na (500 ± 3 mg/l), Mg (250 ± 1 mg/l), K (500 ± 3 mg/l), Ca (250 ± 1 mg/l) and P (500 ± 3 mg/l).

Equipment used in this method is:

- Analysis scale, to 4 decimal points.
- UltraClave (Milestone) or UltraWave (Milestone) for acid and microwaves with different sample containers.
- Water filtration facility: Millipore RiOs connected to Millipore Milli-Q Academic.
- 10-25-50-100 ml volumetric flasks in plastic (polyethylene) or glass.
- Centrifuge tubes from polyethylene with screw cap: 50 ml and 15 ml.
- Finn pipette with tips.
- ICP-MS iCap Q (Thermo Fisher) with collision cells and FAST SC-4DX autosampler. Software Qtegra iCap Q.
- Argon ultra-pure 5.0 (99.999%).
- Helium ultra plus 6.0.

A test series (samples analyzed same day and with the same method) must contain at least three blind samples (blanks) and two control samples (with standard reference material). Out of the three blind samples disregard the highest and lowest response and use the middle sample as a test blank. Start analyzing the test blank, then control samples. If the control samples are within the right limits the analyzing of the proper samples can begin.

Tabell 1: The method's measuring uncertainty and measuring range.

	MU (%)	Measuring range (mg/kg t.l.)
Na	15	110 – 6250
Mg	15	10 – 3125
K	15	50 – 13000
Ca	15	35 – 3125
P	15	3 – 6250

7.2.1 Quality control

Quality control of the methods reliability is performed by:

- Keeping a log book.
- Parallels.
- Keeping a control card with control material.
- Blind samples.
- Participation in SLP.

7.3 MULTI-ELEMENT ANALYSIS WITH ICP-MS AFTER PRESSURE DIGESTION IN A MICROWAVE

This method was used by technicians at NIFES laboratories on my samples. The method can be performed on food, feed, tissue and tissue fluids for silver, iron, cobalt, manganese, chromium, nickel, vanadium, molybdenum, and yttrium.

Standard reference material used for this method is oyster tissue, CRM 1566, NIST, lobster hepatopancreas, TORT 3, NRC, bovine liver 1577, NIST. Chemicals utilized in this method:

- Concentrated nitric acid (HNO_3) Suprapur or own distilled acid from p. a. quality.
- Hydrogen peroxide (Perhydrol) (H_2O_2) 30 % p.a. ISO.
- Deionized and filtrated water.
- Mercury (Hg) Spectrascan 1005±6 mg/l in 5 % HNO_3.
- Rhodium (Rh) Spectrascan 1012±2 mg/l in 5% HCl.
- Thulium (Tm) Merck SertiPUR 1000mg/l in 2-3% HNO3.
- Germanium (Ge) Spectrascan 1000±3 mg/l in H2O.
- Gold (Au) Spectrascan 999±5 mg/l in 2% HNO_3.
- Multielement standard from Spectrascan:
  1000 mg/l Al, Fe, Mg, Zn, 50mg/l As, Ba, Cu, Mn, Se, Sr and 10 mg/l Ag, Cd, Co, Cr, Mo, Ni, Pb, U, V.

Instruments and equipment used:

- Analysis scale, to 4 decimal points.
- Support units UltraWave and UltraClave from Milestone with accompanying test tubes (in Teflon or quarts) and a test tube stand.
- 10-25-50-100 ml volumetric flasks in plasteic (polyethylene) or glass.
- Centrifuge tubes made of polyethylene with screw cap: 50 ml and 15 ml.
- Finn pipettes with tips.
- ICPMS (Thermo iCapQ) with collision cell and FAST autosampler.
- Argon ultra-pure 5.0 (99.999%).
- Helium ultra plus 6.0.

At least three blind samples are to be analyzed first, pick the one with the middle value as a representative for the blind test. Analyze then the control samples and if these are within the set limits in the control card you can begin analyzing your samples.

7.4 RAW PROTEIN ANALYSIS WITH NITROGEN-ANALYZER

This method analyzes nitrogen content in food, feed, tissue samples, feces, and other nitrogenous matrixes. The limit of detection for this instrument, LoD, is 0.1-16.0 g N/100 g.

Chemicals used:

- Magnesium Perchlorate (anhydrone) art. nr. 501-171-HAZ
- Copper tunings art. nr. 502-656
- Lecosorb art. nr. 502-174-HAZ
- Copper sticks, art. nr. 502-705
- N-catalyst art. nr. 502-049
- Quarts wool strips, art. nr. 608-379
- Furnace Reagent, art nr. 501-605-HAZ
- Steel wool, art. nr. 502-310
- Vacuum grease art. nr. 501-241
- Sulfanilamide, art. nr. 05 001 726

- EDTA, art. nr. 502-092, supplier Leco Corporation Svenska AB
- SMRD 2000, supplier LGC Standards AB.
- O_2, 50-liter, quality 5.0.
- He, 50-liter, quality 5.0.

Instruments and equipment:

- Leco FP 628.
- Computer.
- Sartorius Analysis scale.
- Printer.
- Tinn Foil Cups for dry samples, art. nr. 502-186-200
- Tin Capsules for wet samples.
- Reduction tube art. nr. 619-154.
- Combustion tube, art. nr. 619-065.
- Ash crucible, art. nr.614-961-110.
- CO_2 absorber tube, art nr. 633-103-225.
- Scrubber tube.
- Sample Cup Holder art. nr. 604-398.
- Crucible extractor tool art. nr. 616-152.
- Regulators for oxygen-, helium gas and high-pressure air. Adjustable between 0-3 bar.

There are three parts of this process:

1. Combustion of the sample.
2. Reduction and separation.
3. Detection of the analyte.

During the combustion of the sample homogenous sample material is weighed in in aluminum foil or a capsule and is placed inside the test carousel. The sample goes through a chamber where atmospheric gases are removed before it falls into a combustion pipe where the temperature is at 950°C. The sample combusts completely in the presence of O_2. Nitrogen,

carbon, and hydrogen oxidizes to NOx, CO_2 and H_2O. The combustion gases are led to another combustion pipe by O_2 for more oxidation and removing of particles at 850°C.

The combustion gases then pass through a pre-cooler for removal of water vapor. The gases are then gathered in a ballast tank for homogenization/equilibration. They are then led through an aliquot loop by helium and passes through a reduction pipe filled with copper reagent and has a temperature of 700°C. NOx is reduced to N_2 and excess oxygen is captured her. The gas is the led through a pipe filled with Lecosorb and Anhydrone to remove CO_2 and H_2O.

The amount of N_2 is detected in a thermal conductivity cell (TC), and the result is given as % of total nitrogen.

7.4.1 Quality control

- Parallels.
- Keeping a control card for sulfanilamide.
- Keeping a control card for SMRD 2000.
- Participation in SLP.

1.1 IODINE CONCENTRATION DETERMINED WITH ICP-MS AFTER EXTRACTION WITH A BASE

This method is accredited for food, feed, tissue, and tissue fluids, but cannot be used on samples containing plant starch (more than 5% carbohydrates) and brown algae. Standard reference material used are skimmed milk powder ERM-BD 150 and fish muscle ERM-BB422.

Chemicals used:

- Iodine standard (I) Spectrascan 998 ±4 mg/L in H_2O Tekno lab (Art.nr SS-11I)

- TMAH – tetramethylammonium hydroxide ultra-pure Wako, CAS-nr:200-882-9.
- Tellurium standard solution from NIST H_6TeO_6 1000mg/l in HNO3 0,5 mol/l (Art.nr 1195140100)
- ICP-MS stock tuning solution (100 ml) part # 5188-6564 10 mg/l Ce, Co, Li, Ti and Y.
- α-amylase from Bacillus licheniformis from Sigma (LOT#SLBS5923).

Instruments and equipment:

- Analysis scale with 4 decimals
- Glass equipment: 25-100-250 - 1000 ml volumetric flasks og100 ml beakers.
- Centrifuge pipe of polyethylene with screw cap: 50 ml and 15 ml
- 10 ml syringe without cannula
- Syringe filters, 0,45µm
- Finn pipettes with tips. 0-200µl, 200-1000 µl and (1-5 ml).
- Heating cabinet
- ICPMS (Agilent 7500) with collision cells and CETAC autosampler, Agilent Mass hunter Workstation software.
- Mini shaker.
- Thermo Fisher centrifuge.
- Deionized and filtrated water from Millipore water treatment plant.
- Argon ultra-pure 5.0 (99.999%).
- Helium ultra pluss 6.0.
- Shaking instrument for water Grant OLS 200.

The samples are weighed and water and TMAH (tetramethylammonium hydroxide) is added and placed in a heating cabinet at 90 ± 3 °C. Iodine content is found by using ICP-MS and calculated by using a standard addition curve made in a matrix equivalent of the test samples. Tellurium is applied as an internal standard to correct the baseline for operations.

7.4.2 Quality control

6. Keeping a log book form.
7. Parallels.
8. Keeping a control card on the control material.
9. Blind samples.
10. Participation in SLP.

7.5 RESULT AND DISCUSSION

Table 7.1, 7.2 and 7.3 shows the ratio of the results from each nutrient compared to the values from the other species.

Table 7.1- Ratio between the value of the substance and the mean value of the substance of all five species.

Substance	*Sardinella maderensis*	*Decapterus rhoncus*	*Sardinella aurita*	*Trachurus trecae*	*Sphyraena guachancho*
Fat	0.69	0.65	0.97	2.06	0.63
Protein	0.97	1.07	1.01	0.97	0.97

Table 7.2- Ratio between the value of the substance and the mean value of the substance of all five species.

Substance	*Sardinella maderensis*	*Decapterus rhoncus*	*Sardinella aurita*	*Trachurus trecae*	*Sphyraena guachancho*
Fat	0.69	0.65	0.97	2.06	0.63
Protein	0.97	1.07	1.01	0.97	0.97
Sodium	0.93	0.99	0.90	1.29	0.89
Phosphorus	1.08	0.94	1.10	0.90	0.99

Zinc	1.22	1.03	1.13	0.90	0.72
Magnesium	1.05	1.00	1.00	0.93	1.02
Iodine	1.13	0.71	0.77	0.88	1.51
Calcium	1.93	0.56	1.54	0.53	0.44
Potassium	1.05	0.96	1.02	0.91	1.06
Iron	1.45	0.62	1.76	0.80	0.38
Selenium	1.70	1.25	0.97	0.80	0.28

Table 7.3- Ratio between the value of the substance and the average value of the substance.

Substance	*Sardinella maderensis*	*Decapterus rhoncus*	*Sardinella aurita*	*Trachurus trecae*	*Sphyraena guachancho*
Cadmium	1.99	1.46	1.13	0.93	0.33
Arsenic	1.25	0.92	1.05	1.35	0.34
Lead	2.73	0.85	0.85	0.85	0.71
Mercury	0.79	0.38	0.24	0.44	2.90