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MASTER THESIS

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Nutrient composition of 19 marine fish species from Sri Lanka and their potential contribution to food and nutrition security

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Abstract

Background: Sri Lanka is a country with several pressing nutritional issues, such as high wasting rates, micronutrient deficiencies, and non-communicable diseases. Fish is an important part of the Sri Lankan diet, and represent a rich source of protein, essential fatty acids, and several micronutrients. Accordingly, fish and seafood are essential for food and nutrition security (FNS) in Sri Lanka. However, existing data on the nutrient composition of fish in Sri Lanka is highly outdated and very limited and does not reflect the large diversity of fish available. Thus, comprehensive knowledge of the nutrient content in commonly consumed fish species is of great importance to further improve the FNS in the country.
Objective: Present comprehensive analytical data on the nutritional composition of some the most commonly consumed marine fish species sampled from the coast of Sri Lanka.
Methods: Species of fish were sampled during a survey from the north-western coast to the far north coast of Sri Lanka with the research vessel Dr. Fridtjof Nansen from the 24th of June to the 15th of July 2018. Species were categorized as either small (n = 12) or large (n = 7), and three composite samples were analyzed from each species. The composite samples of small species consisted of 25 small fish that were homogenized with their heads, bones, and viscera intact, in accordance to local eating customs. The composite samples of large fish consisted of five individual samples of fish fillet that were filleted and homogenized. The samples were then analyzed for macro- and micronutrients using accredited methods at the Institute of Marine Research in Bergen, Norway.
Results: A total of 19 marine species were sampled and analyzed. The results of this thesis showed that small species, commonly consumed whole, contained substantially higher concentrations of micronutrients than larger species. Calcium values ranged from 7.9 to 2000 mg/100g, iron from 0.21 to 10 mg/100g, zinc from 0.27 to 3.0 mg/100g, vitamin A from 2.7 to 2000 µg/100g, vitamin B_{12} from 0.64 to 20 µg/100g, vitamin D from undetected to 7.3 µg/100g, and eicosapentaenoic acid and docosahexaenoic acid from 11 to 250 mg/100g and 41 to 467 mg/100g, respectively. Several species were identified to contribute with ≥25% of the recommended nutrient intakes for women of reproductive age for several micronutrients.
Conclusions: Small species are a significant source of micronutrients, and even small amounts of fish in the diet can diversify diets otherwise dominated by staple foods. The analytical data presented here may represent an important contribution to the future development of the Sri Lankan food composition database.

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Abbreviations

DHA	Docosahexaenoic acid
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid
FAO	Food and Agriculture Organization of the United Nations
FBDG	Food-based dietary guidelines
FCD	Food Composition Data
FCD	Food Composition Table
FCDB	Food Composition Databases
FNS	Food and Nutrition Security
HIES	Household Income and Expenditure Survey
IMR	Institute of Marine Research
INFOODS	International Network of Food Data Systems
LMICs	Low- and middle-income countries
MUFA	Mono-unsaturated fatty acids
n-3 LCPUFA	Long-chain omega-3 polyunsaturated fatty acid
NARA	National Aquatic Resources Research and Development Agency
NCD	Non-communicable diseases
NORAD	Norwegian Agency for Development Cooperation
PUFA	Poly-unsaturated fatty acids
RNI	Recommended nutrient intake
SD	Standard deviation
SDG	Sustainable Development Goal
SFA	Saturated fatty acids
SIS	Small indigenous species
UN	The United Nations
USDA	The United States Department of Agriculture
WFP	The World Food Programme
WHO	The World Health Organization

1 Introduction

1.1 Food and nutrition security

In 2015, the 193 Member States of the United Nations (UN) embraced the new 2030 Agenda for Sustainable Development, replacing the former Millennium Development Goals from year 2000. The 2030 Agenda offers a vision for food and nutrition as key in achieving sustainable development (1), as prominently evidenced by sustainable development goal 2 (SDG2): "End hunger, achieve food security and improve nutrition, and promote sustainable agriculture" (2). The explicit emphasis on nutrition is correspondingly evident through target 2.1: "Ensuring access to safe, nutritious and sufficient food for all", and target 2.2; "Eliminating all forms of malnutrition" (2, 3). Because the success of the SGDs largely depend on effective and continuous monitoring and assessment, a framework of global indicators have been established (4). A total of 230 indicators exists for all SDGs combined, and for measuring SDG2 specifically, there are two key indicators for which the 14 specific indicators can be categorized accordingly: "food security" and "nutrition security" (2).

The term "food security" is a concept that has evolved over time, and was previously defined by the Food and Agriculture Organization of the United Nations (FAO) in 1996 as existing when "*…all people, at all time, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life*" (5; p. 1). The term is based on the four dimensions of food available in sufficient quantities, access to nutritious foods, utilization of food through an adequate diet, and the stability to be food secure at all times (5). Conversely, "nutrition security" is not a technical term but a generic term used by the FAO to report on the prevalence of undernourishment as defined by the proportion of the population whose habitual consumption of food is insufficient to provide the adequate energy, protein, and micronutrient intake required to maintain a healthy life (6-8). Thus, incorporated in to the definition is the aspect of an adequate nutritional status, which is determined by the interaction between nutrient intakes and health status, where the two are interchangeably connected through several additional factors such as a sanitary environment, adequate health services, sociological aspects, and biological aspects (3, 7, 8). Additionally, the term underlines other nutritional indicators such as the prevalence of wasting (weight-for-height), stunting (height/length-for-age), and underweight (weight-for-age) in children as defined by the World Health Organization

(WHO, (9)), in addition to anemia, overweight, and obesity in a population (2, 8). Therefore, the term "food and nutrition security" (FNS), which encompasses both the sufficiency, availability, and stability of food supply in addition to accentuating the dietary adequacy aspect and the health status of a person, will continuously be used throughout this thesis.

1.1.1 The importance of food and nutrition security

When the criteria of FNS is not met, a state of food insecurity arises (7). The United States Department of Agriculture (USDA) has described this as a situation that occurs when a person or a household has "*limited or uncertain availability of nutritionally adequate and safe foods or limited and uncertain ability to acquire acceptable foods in socially acceptable ways*" (10; p. 6), a definition supported by the FAO (7; p. 53). Food insecurity may be a result of many factors: some may be transitory, such as climate variability and/or extremes and warfare; others may be more chronic, such as inadequate purchasing power and decreased utilization of food due to poor health (3, 7, 11, 12). However, food and nutrition insecurity always represent a deprivation of a basic human need and right, and is a possible precursor to several health- and nutritional issues as well as developmental issues for the entire country (10).

1.1.2 Consequences of food and nutrition insecurity

Depending on the severity of the food and nutrition insecurity, various forms of malnutrition, which includes both undernutrition, overnutrition, and micronutrient deficiencies, may develop (3, 7; p. 53). Severe food and nutrition insecurity will predictably result in an inability to cover dietary needs due to both the reduction of the quantity and the quality of foods consumed, but also due to reduced health services, unsafe drinking water, poor hygiene, and the subsequent manifestations of various diseases (3, 13). Inadequate food intake over a long period is termed "chronic hunger", and may result in several conditions of undernourishment such as wasting and stunting in children, anemia in women of reproductive age, and various forms of micronutrient deficiencies (3, 14). The latter is frequently referred to as "hidden hunger" due to no visible, physical signs of deficiencies being characteristic (3, 15), and is estimated to affect approximately 2 billion people worldwide (16). In terms of global public health; iron-, vitamin A-, iodine-, folate-, and zinc deficiencies are the most common and widespread micronutrient deficiencies in the world, and are all associated with an increased risk of morbidity and mortality (17, 18). The FAO has estimated a rise in food insecurity for the third year in a row, approximating that nearly 821 million people, or one in

nine of the global population, were undernourished in 2017. The number is expected to continue rising (3).

Moderate food and nutrition insecurity is often associated with a highly energy-dense, yet micronutrient poor diet, and is an example of how food and nutrition insecurity may contribute to overweight and obesity, as well as micronutrient deficiencies (3). Multiple forms of malnutrition, such as these, are today collectively evident in many countries, communities, households, and even individuals throughout a lifetime. The coexistence of undernutrition along with overweight, obesity, and diet-related non-communicable diseases (NCDs) is termed "the double burden of malnutrition" (3, 19, 20), while a third dimension labelled "the triple burden of malnutrition" exists when the latter two occurs in addition to micronutrient deficiencies (21, 22). Thus, poor access to food, and particularly nutritious food, which in terms leads to food and nutrition insecurity, is a contributor to both undernourishment and overweight (3).

1.2 Fish and seafood

As in agreement with the European Food Safety Authority (EFSA), the term "seafood" includes both vertebrate and invertebrate aquatic animals of both marine and freshwater origin, with the exception of aquatic mammals, - reptiles, - plants, echinoderms, and jellyfish (23). The terms "fish and seafood" are sometimes used interchangeably in the literature, but as the focus of this thesis will be on fish exclusively, the term "seafood" will only be used whenever the referred literature also applies this exact term.

Seafood, and particularly fish, play a crucial role in global FNS as it represents an important and nutrient-dense animal source to especially many low- and middle-income countries (LMICs) (24, 25; p. 113-114). In high income countries, conversely, the focus has been directed more towards the growing recognition of the many nutritional and possibly health-promoting qualities of fish consumption (26). Several epidemiological studies have evaluated the positive effects of fish on a series of health outcomes, providing the most convincing evidence of the beneficial effects of fish consumption on cardiovascular disease in adults (27, 28). This effect is predominantly linked to the high levels of the marine long-chain omega-3 polyunsaturated fatty acids (n-3 LCPUFA) eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) (27; p. 86-96, 28). However, a recent systematic review suggested that increased intake of EPA and DHA had little or no effect on cardiovascular health and/or –mortality, as evidenced mainly by several supplement trials (29). Moreover,

improved foetal health including increased birth weight, reduced risk of preterm birth, and improved neurodevelopment, have also been associated with LCPUFA (30).

1.2.1 The importance of fish for food and nutrition security in LMICs

Poor dietary diversity is a leading cause of malnutrition (31), and fish represents a relatively cheap and easily available means of nutritional diversification for people in many LMICs that depend heavily on a narrow range of staple foods. Even small quantities of fish in the diet can provide a range of essential nutrients otherwise lacking in diets predominantly centered around grains and tuber crops (25, p. 69). Fish also serves as an important source of protein in regions where livestock is relatively scarce, and in 2015 fish alone accounted for approximately 17% of the animal protein consumed by the global population. However, total fish consumption varies significantly across countries and within the same country due to cultural, economic, and geographical factors, and in many small island states and Asian countries fish accounts for far more than 50% of total animal protein intake (25; p. 69-71).. Thus, the fisheries sector, as a valuable natural resource to many countries, plays a crucial role when it comes to meeting the 2030 Agenda (25; p. 113).

Quantitative information on the role of fish for FNS in LMICs is however scattered and/or generally lacking; making fish rather absent in food-based approaches directed to improve FNS (25; p. 118, 26). The FAO recognizes the need for sustainable management of the seafood sector to support a continuously growing population (32), and part of the strategy to improve FNS worldwide is to support research in addressing current knowledge gaps, e.g., in coordinating existing databases on the nutritional composition of fish, to improve global collaboration between countries, and to ensure that the global catch of fish for consumption might continue in a sustainable manner (25, p. 118). Seafood may also influence and improve FNS through various other more indirect channels such as through employment opportunities, income generation, and as fish feed for aquaculture and livestock, but in this thesis the focus will only be directed towards the direct human nutritional aspects of fish and FNS (33, 34).

1.2.2 Food and nutrition security in Sri Lanka

The Democratic Socialist Republic of Sri Lanka is an island country located in the Bay of Bengal completely surrounded by the Indian Ocean; facilitating plenty of fishery activities to be conducted (35). After recovering from a 27-year civil conflict that ended in 2009, Sri Lanka is in 2018 considered a lower-middle-income country by definition (36), but with decreasing poverty rates (37). However, malnutrition remains a major challenge; particularly

acute malnutrition and micronutrient deficiencies (38, 39). A survey conducted by the World Food Programme (WFP) in 2016 reports that 22% of the Sri Lankan population is undernourished, and that the prevalence of acute malnutrition, or wasting, in children between 0-59 months was estimated to be 15% (38, 40), which is considered one of the highest wasting rates in the world (39). The rates of both wasting and stunting have remained unchanged for the last 10 years, with 17% of children being stunted (40). Furthermore, nearly every fourth (22%) pregnant woman is underweight at the time of registration of pregnancy, and 1 in 5 (18%) of newborns are born with a low birth weight (<2500g) (41). Micronutrient deficiencies are also rather prevalent (41, 42), with 29.3% of children, and 23% of pregnant women suffering from vitamin A deficiencies (15, 43), regardless of nationwide vitamin A supplementation programmes (44). Available data suggest a high prevalence of additionally calcium, iron, folate, and zinc deficiencies, but limited data are available for other nutrients such as vitamin D, vitamin B_{12}, selenium and copper (15). Of the 22.44 million inhabitants (45), an estimated 33% cannot afford a nutritious diet (38), and the food prices are continually rising (46). Additionally, Sri Lanka as an island nation is highly vulnerable to climate change and following natural disasters and extreme weather, which have an adverse effect on socio-economic progress and FNS (3; part 2). This was witnessed by the 2004 tsunami that hit two thirds of the country's coastal line and left the fisheries sector 80% devastated (3, 47), and approximately 750 000 people in need of international food assistance (48).

Sri Lanka is also a country affected by the triple burden of malnutrition, where ongoing, rapid demographic and socioeconomic transitions have created significant health and nutrition challenges (39, 49, 50). The prevalence of overweight, obesity, and abdominal obesity among Sri Lankan adults were 25.2%, 9.2%, and 26.2%, respectively, according to a national study from 2010 using the anthropometric cut-offs for Asians as proposed by the WHO (51). This is a clear upwards trend compared to data from 30 years ago (52). Furthermore, a rise in NCDs such as diabetes, cardiovascular diseases, chronic respiratory diseases, and some cancers, have been estimated to account for 83% of all deaths (53, 54), and is currently emerging as the major diet-associated health issue in the country (52).

1.2.2.1 The characteristics of a Sri Lankan diet

The ongoing Sri Lankan nutrition transition is characterized by globalization and Westernization from a predominantly crop- and plant-based diet with little or no animal products (with the exception of seafood), towards a diet high in saturated fats, refined carbohydrates, added sugars, salt, animal-source foods, and low in fiber, fruits, and vegetables

(49). Similar to that of other South-Asian countries, rice is still considered the staple food in Sri Lanka. It has been estimated that rice accounts for almost half of the total dietary energy intake for an average Sri Lankan adult with a monthly consumption of approximately 9 kg per person, depending on the variety of the type of rice consumed (55). Furthermore, it has been estimated that a typical Sri Lankan meal consists of three-quarters rice with a small amount of vegetable curry (15 g) and a small amount of meat or fish (15 g) with a starchy curry (56). Due to a lack of comprehensive national dietary data and nutrition studies in Sri Lanka, it is difficult to provide more accurate information on the eating patterns and food customs in the country.

1.2.2.2 The role of fish in the Sri Lankan diet

Marine fisheries are allocated a key role in Sri Lanka's social and economic life. Due to the country's multi-ethnic and multi-religious society, prejudices and biases prevent the consumption of meat for many, but fish has traditionally been, and still is, generally accepted (47). According to the Household Income and Expenditure Survey (HIES) from 2010, marine species accounted for 81% of total fish consumption with skipjack tuna, goldstripe sardinella, and sprats as the most commonly consumed marine species. They also found that 71% of fish were consumed fresh, and the remaining as either dried or otherwise processed products (57). Of the 285 000 tonnes of fish arriving at offshore landing sites, approximately 90% is consumed nationally, while an additional 70 000 tonnes of dried and canned fish are imported annually (47). Fish and fish products alone are estimated to contribute to 55% of total animal protein intake per capita (25; p. 70, 57), and are therefore considered the most important sources of animal protein in the Sri Lankan diet (58, p. 43). The Sri Lankan Food Based Dietary Guidelines (FBDG) from 2011 recommend fish intake twice a week at a minimum, in addition to advising inhabitants to use the fish as a whole, explicitly mentioning not discarding of the head. Further recommendations also specifically refer to small fish species eaten with bones and heads intact as a great means of reaching adequate calcium intakes (59).

1.3 Food composition data

The aim of food composition data (FCD) is to describe the content of foods in regards to both nutrients, energy, and non-nutrient components such as water, additives, fiber, contaminants, etc. (60, p. 51-62). Data on the nutrient composition of foods are made publicly available

through food composition tables (FCT), which are printed booklets of selected foods and nutrients, and/or in recent years through food composition databases (FCDB), which are computerized and more extensive forms of the more traditional FCT (60; p. 5-6). The FAO and its group of food composition experts seated in The International Network of Food Data Systems (INFOODS) provide guidelines, standards of data quality, policy advice, technical assistance, and compilation tools made available for all countries in order to harmonize global FCD activities, and to facilitate the access, utilization, and interchange of FCD across borders. This synchronization between nations will also facilitate the linkage of agriculture and aquaculture, food systems, and health and nutrition in order to achieve improved FNS worldwide through adequate and reliable FCD (61).

1.3.1 Compilation of food composition data

FCT/FCDB are compiled from either direct nutritional analyses of foods, or from indirect methods of which the data are imputed, calculated or "borrowed" from additional literature and/or other FCT/FCDB. The latter is most commonly utilized when data and/or resources are limited, or the food supply largely consists of imported foods from other nations where FCD are available. Additionally, a third combination of methods where values in the FCD/FCDB consists of both direct analyses and indirect data exists and is today considered the most widespread method of compilation. Due to the different methods of which FCD are obtained, there is great variation in the quality of the data. Original chemical values from analytical laboratory reports are undeniably preferred, followed by imputed values, or calculated values, which are estimates derived from analytical values of a similar food item or processing method. Borrowed values include data acquired from other FCT/FCDB of which the reference to the original source may or may not be available or presented, therefore an evaluation of both data quality, reliability, and applicability of foods would be necessary before incorporating values in to a FCT/FCDB. By minimizing the amount of imputed and calculated values, the reliability and representativeness of the database increases (60; p. 6-9).

A reliable FCT/FCDB should be compiled according to international standards (i.e. INFOODS), contain comprehensive information and descriptions of important nutrients/components and food items (including cooked foods, mixed dishes, processed foods, fortified foods, and biodiverse foods) as well as be provided with good general documentation of the data source of each nutrient value (60; p. 14-15). Furthermore, it is necessary that the selected foods represent the national habitual food consumption and the majority of the most-consumed foods in the country, before including occasionally consumed foods. Data should

also include values on various styles of processing, commonly fortified foods, supplements, and values for food items "as consumed", and cover all of the nutrients and other components known to be important to human health (60; p. 33-39, 61).

1.3.2 Uses of food composition data

FCD is essential in providing the foundation for almost all aspects of nutrition and represent the basic tools to improve both nutrition, health, and FNS in all populations. Reliable and up-to-date FCD are of fundamental importance to a multitude of nutrition activities, e.g. health policies in establishing nutrient requirements, epidemiological research in estimating and evaluating the nutrient intake of individuals and/or populations, educational purposes, clinical practice, food regulation, the food manufacturing industry, government policy development and implementation, the consumers' demand for selecting foods consistent with a healthy diet, etc., as illustrated in figure 1 (60; p. 15-19, 61-64). Thus, various forms of FCT/FCDB, such as abbreviated, comprehensive or special-purpose tables, should be published to satisfy the requirements of different users in terms of coverage of food items, nutrients/constituents, and documentation (60; p. 11-12).

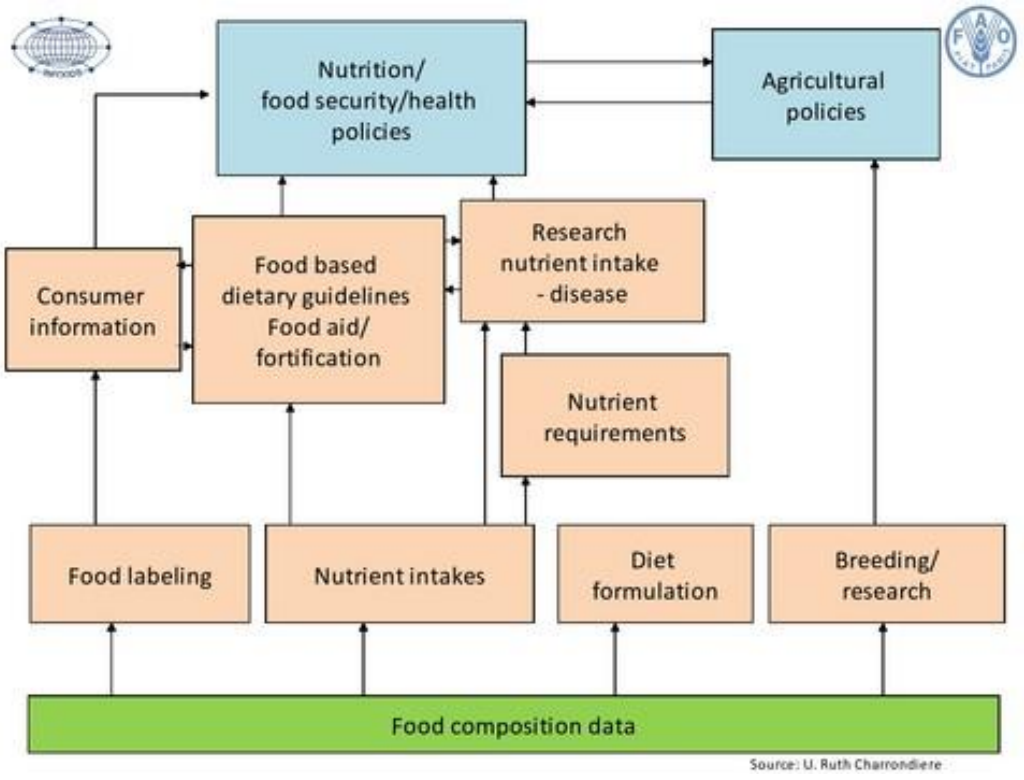

Figure 1: Food composition data (FCD) are the basis for several nutrition related activities (61).

1.3.3 Limitations and challenges of food composition data

One of the more obvious limitations of FCD is the natural variations of composition present in all biological matters such as food items, where no FCT/FCDB can accurately predict the exact composition of any given sample of food (60; p. 19-20). The nutrient contents in foods may vary significantly due to climatic conditions, seasonal variations, state of maturity of plants- and animal foods, food preparation/processing, and cultivar and breed variations (60; p. 19-20, 61). Thus, all FCD are essentially estimates. Furthermore, many FCT/FCDB, especially in developing countries, have partial or limited coverage of food items and/or nutrients/constituents. This is in many cases due to the FCT/FCDB being compiled by private initiatives as projects, and not within an institutional framework, as opposed to most developed countries where specific institutions with defined budgets and assigned staff are responsible for the compilation, development, and maintenance of the FCT/FCDB (60, 61; p. 188-195). Generally, raw foods are well covered in all FCT/FCDB, while processed foods, composite foods, supplements, and varieties/breeds of the same food are rarely included (61). Due to its time-consuming and expensive nature, compilers would by no means be able to cover all foods, recipes, and brand names consumed by an entire population, so FCD presented in FCT/FCDB will always be a subset of the total of foods available for consumption in a population at a given time. FCT/FCDB are never complete due to the continuous introduction of new foods presented, enhanced methods of analyses, and/or recently discovered food components associated with human nutrition, and should therefore continuously be updated (62). Consequently, many developing countries either have no FCT/FCDB or have one that is outdated and contains inadequate data (60; p. 190-195). As for nutrients and bioactive food components, decisions of inclusion must often be made on priorities, where the ones of greatest national interest, e.g. those with public health implications, should preferably be included over others when resources are scarce (60; p. 35). Furthermore, borrowing FCD from other countries also presents challenges due to differences in nutrient definition, food description, food group categorization, methods of analyses, source of data, and data expression (60; p. 163-170, 61).

1.3.4 Food composition data in Sri Lanka

The Sri Lankan FCT was published in 1979 and was named “Tables of Food Composition for Use in Sri Lanka”. Since then, minor efforts have been made to update or further develop the FCT, which from the very beginning consisted of 90% borrowed values from the Indian FCT (65, 66). Although several working groups have been initiated to work on the further

development of the FCT, a systematic approach has not yet been implemented (67), and the FCT remains limited and outdated. However, a joint international initiative between Sri Lanka and three other countries (*The Biodiversity for Food and Nutrition Project*), recently made the FCD-values from 1979 electronically available, while also adding data from nutritional analyses of 52 varieties of 20 nutrient-rich indigenous agricultural species considered to be of priority for FNS (68). The original FCT[1] from 1979 does not provide any information on the source of documentation of the data, sampling procedures, nor any other aspects of data compilation or analyses (66). Common limitations such as a very limited coverage of foods, the lack of inclusion of processed, cooked, and mixed foods, a very narrow array of micronutrients, and no values for fiber, amino acids, and dietary fiber are also true for this FCT (66, 69). In compliance with information of the limited data on the nutritional composition of fish species consumed in several other LMICs (26), the Sri Lankan FCT does not include a comprehensive nutrient profile for any single species of fish (70)[2].

1.4 Nutrient composition of fish

It is recognized that the nutrient composition of freshwater, marine, farmed, and wild fish species varies (27, p. 84, 71). As for further contexts, the term "fish" always applies to wild and marine species, and not farmed or freshwater fish unless otherwise stated.

1.4.1 The principal nutrient composition of fish

Regardless of large variations in nutrient content among marine fish species, various species share a quantity of unified nutritional features, and are considered important sources of several essential nutrients in the human diet (23, 72, 73). The four major biological constituents of the muscle tissue (fillet) are water, protein, lipid, and ash, further being referenced to as the proximate composition of the fish (72, 74). Additionally, the bone fraction, which constitutes approximately 10-15% of the fish biomass, is rich in minerals such as calcium and phosphorus (25; p. 51, 75, 76). Although little is still known of the chemical composition of fish bones and their contribution to human nutrition, recent studies have

[1] This information is accurate for the electronic version available online (hence the reference to the electronic version of the FCT), but have not been verified for the hard-copy FCT only available in Sri Lanka.
[2] Due to the lack of studies available, information on this topic have also been confirmed through personal communications via my Sri Lankan collaborator, Thiruchenduran Somasundarampillai, M.Sc. Food Technology (2018).

shown that the bone mass also contains various concentrations of collagen associated amino acids and diverse lipids (75). Moreover, fish is also considered a good source of high-quality animal protein, marine n-3 LCPUFA, and several vitamins and minerals, including vitamin A, vitamin B_{12}, vitamin D, zinc, selenium, and iodine (23, 28, 77, 78). Similar to that of other animal sources, the quantitative amount of carbohydrate in marine fish is generally too small to be of any significance (<0.14%, (79)), and will therefore be considered negligible in this thesis (28, 72, 80).

1.4.1.1 Water content

Water is the main constituent of muscle tissue in fish with a reported mean value of 70%. However, individual specimens may at times contain anywhere between 30% and 90% water (72). The water content largely depends on the lipid content of the fish, and an inverse relationship exists between the two (78, 80, 81). When the fat content increases, the water content decreases, and vice versa; e.g., the sum of the percentages of the two components approximates 80% in a fatty fish (72, 80). Furthermore, as spawning approaches, the water content generally rises and the protein and lipid content decreases (78).

1.4.1.2 Protein content

The protein content is recognized as considerably more stable with values typically varying between 15% and 20% in muscle tissue (23, 72). However, values < 15% or > 28% are occasionally observed in some species (72). The protein composition in fish is declared to be of high-quality because of the balanced amino acid profile, which consists of significant amounts of all nine essential amino acids for human metabolism (23, 73, 77, 80). Additionally, protein found in fish and seafood is easily digestible due to small amounts of connective tissue (28, 82; p. 46).

1.4.1.3 Lipid content

The composition of lipids in fish is perhaps the overall most heterogeneous component. In addition to inter-species variability, the biochemical composition also differs according to geographical region, seasonal variations (72, 78, 80, 83), environment (water temperature, -salinity, and -pressure) (78, 83), diet/food supply (78, 80, 83, 84), and the maturity, sex, and reproduction stage of the fish (33, 80). Lipids are accumulated in the form of triacylglycerol, (78), and are heterogeneously accumulated throughout the fillet with increasing

concentrations from the tail region to the head, and decreasing concentrations from dorsal to ventral (72, 78, 85). Increased levels are also found in red muscle tissue and right below the skin of the fish (85).

1.4.1.3.1 Lipid categorization

Species are principally categorized as either lean, intermediate or fatty fish according to the percentage of lipid storage of total body weight in the muscle tissue of the fish (82; p. 43, 84). However, categorization may be rather arbitrary and differ among countries and authorities (78) as illustrated in Table 1, which summarizes the lipid percentages for each category presented by different authors, as compiled by EFSA in 2005 (78). The total lipid content may vary from approximately 1% in lean fish to 30% in fatty fish (33, 80). In fatty fish, lipid accumulation occurs in the muscle tissue and surrounding the viscera, while lean fish predominantly accumulate lipids in the liver (72, 78, 80, 84).

Table 1: Categorization of fish according to percentage of lipid content of total body weight. Modified from EFSA 2005 (78).

Author	Lean	Intermediate	Fatty
FSA, 2004	1-2 %	-	5-20 %
Steffens, 1979	< 1 %	1-5 %	> 5 %
Danish Fish Assessment, 2003	< 2 %	2-8 %	> 8 %
The Norwegian Directorate of Health, 2011	< 2 %	2-8 %	> 8 %

1.4.1.3.2 Fatty acid composition

The fatty acid composition of fish is generally characterized by various amounts of saturated fatty acids (SFA) and mono-unsaturated fatty acids (MUFA), and considerable amounts of poly-unsaturated fatty acids (PUFA) (78). The PUFA are distinguished by high concentrations of the marine n-3 LCPUFA, predominantly EPA (C20:5) and DHA (C22:6), and lesser amounts of n-6 PUFA (78, 80). The distinctive accumulation of n-3 fatty acids in fish is due to a natural occurring food chain consisting of n-3 fatty acid producing phytoplankton consumed by zooplankton, which in terms is part of the diet of the fish (82; p. 45). Furthermore, n-3 LCPUFA are more abundant in fatty fish and medium-fat fish, than in lean fish (33, 78, 82; p. 45). There are a wide range of various fatty acids present in fish, but for this thesis only the prominent n-3 LCPUFA EPA and DHA will be examined due to their potential health-promoting effects (78, 86).

1.4.2 Vitamin and mineral composition

As previously mentioned, fish is abundant in several vitamins and minerals, where vitamin A, vitamin B_{12}, vitamin D, iodine, selenium, and zinc are the most recognized (23, 28, 77, 78). In similarity to lipid content, concentrations of vitamins and minerals show considerable inter-species variability and fluctuations throughout the seasons (72, 80). Furthermore, there is a great degree of intra-species variability where concentrations are not uniformly distributed throughout the flesh of the fish (72, 80).

1.4.3 The EAF-Nansen Programme

The EAF-Nansen Programme (*Supporting of the Application of the Ecosystem Approach to fisheries Management considering Climate and pollution impacts*) is a joint initiative of Norway; financed by the Norwegian Agency for Development Cooperation (NORAD) and operated by the Food and Agriculture Organization of the United Nations (FAO) in close collaboration with the Institute of Marine Research (IMR) (87, 88). Since 1975, the programme has conducted regular surveys in developing regions all around the world aiming to support the countries in managing and developing their marine resources, enhancing knowledge, and promoting sustainable utilization and management of the marine environment (88). In May 2017 the newest research vessel in the series of Nansen vessels was launched, replacing the old vessel from 1993 with an identical name (89). The state-of-the-art features on this new vessel will make it easier to carry forward the old mission of the Programme, while also promoting the new EAF-Nansen Programme with an expanded emphasis on marine ecosystem research including oceanography, climate change impacts, plankton, biodiversity, fish stocks and distribution, bottom habitats, and now nutrition and food safety and -security (88). The latter was for the first time in 2017 included as part of the official science plan of the new EAF-Nansen Programme (90), which also focuses on knowledge-based decision-making while strengthening partnerships between countries in order to reduce poverty and improve FNS (87, 88).

2 Aim of thesis

This thesis is a part of the EAF-Nansen project, where each survey is a bilateral collaboration between IMR and the local host institution, which for this survey was the National Aquatic Resources Research and Development Agency (NARA) of Sri Lanka.

In general, there is a lack of scientific documentation concerning the fisheries sector and FNS, leaving fish for the most part relatively absent in the development of nutrition-based interventions to improve FNS in LMICs (25; p. 118, 26). Knowledge on the nutrient composition of important foods followed by comprehensive and well-documented FCT/FCDB are invaluable tools in enabling countries to better integrate fish into nutrition programmes and policies to achieve SDG2 by 2030 (25; p. 118). This is particularly crucial for fish due to the large biodiversity present with varieties of species, and therefore differences in nutritional composition (23, 72). Furthermore, building knowledge on available marine resources and how they may contribute to FNS worldwide is not only beneficial to governments, health organizations, and policy makers, but also to health practitioners, such as clinical dietitians, as well as for the awareness and understanding of the general population in all countries (60, p. 15-19, 61-63). However, existing FCD do not reflect the large diversity of species available for consumption in Sri Lanka[3] (67).

Based on the above, the overall objectives of this thesis were to generate and document comprehensive information on the nutritional composition of several marine species of fish caught off the coast of Sri Lanka. The overall aim was to fill current knowledge gaps by presenting analytical data on nutrient profiles of some of the most commonly consumed fish species found in the marine waters surrounding Sri Lanka.

The specific aims of the thesis were to:

- Describe the nutritional composition of fish caught off the coast of Sri Lanka and thereby document the importance of these fish species to FNS. The specific nutrients to be analyzed are the dry matter, total protein, total fat, the general fatty acid composition consisting of sum SFA, sum MUFA, sum PUFA, sum n-3, sum n-6, and

[3] Due to lack of studies available, information on this topic have also been confirmed through personal communications via my Sri Lankan collaborator, Thiruchenduran Somasundarampillai, M.Sc. Food Technology (2018).

the n-3 LCPUFA EPA and DHA, vitamin A, vitamin B_{12}, vitamin D, iodine, selenium, calcium, phosphorus, potassium, magnesium, sodium, iron, and zinc.

- Provide data to generate and populate databases with nutrient composition information.
- Compare findings to relevant existing data.
- Enhance the scope of the analyzed nutrients' utility in human nutrition by evaluation and demonstration of their potential contribution to the recommended nutrient intakes (RNI) of a selected population group in Sri Lanka.

3 Material and methods

3.1 Methodological considerations and limitations

3.1.1 Selection of nutrients

The context of this work was the estimation of nutrients in fish to populate FCT/FCDB. However, a selection of nutrients had to be made in order to delineate the thesis. The nutrients selected for analysis were those commonly known to be present in fish and of importance to human nutrition. Several of these micronutrients have public health implications in Sri Lanka, in which sustainable solutions have not yet been discovered (7, 15, 17, 18). The following nutrients were selected for analysis, and the results will subsequently be reported: total protein, total fat, total omega-3 and omega-6, the general fatty acid composition (SFA, MUFA, and PUFA), the n-3 fatty acids EPA and DHA, vitamin A, vitamin B_{12}, vitamin D, iodine, selenium, calcium, phosphorus, potassium, magnesium, sodium, iron, and zinc. Furthermore, several heavy metals, contaminants, and other nutrients such as cadmium, lead, mercury, chromium, manganese, cobalt, nickel, copper, arsenic, silver, molybdenum, and vanadium were also analyzed, but as the attention of this thesis was on FNS and not directly on food safety, in addition to the limited time-perspective of a master thesis, the results of these analyses will not be presented nor further discussed.

3.1.2 Exclusion and limitations

Originally, both individual samples, composite samples, and liver samples were sampled and prepared during the survey, as in accordance to protocol (see appendix I). For large fish, this included 25 individual samples of fish that were filleted, homogenized, and freeze-dried, liver samples of 15 of the 25 fish samples, and five composite samples made from the 25 individual samples; all prepared and freeze-dried. For small fish, 150 individuals were originally sampled and organized in to composite samples containing 25 individuals each (a total of 6 composite samples). Of these, 75 fish (3/6 composite samples) were individually filleted and had their head, tail, and general viscera removed, while the other 75 remained as whole fish with heads, bones, and viscera intact. However, the results of the samples mentioned above are not included in this thesis, as the selection of samples to be analysed was limited by time and the capacity of the laboratory to be able to complete the analyses, while also taking economic limitations in to consideration. Therefore, a total of three composite samples were selected for further laboratory analyses for each species of fish. For large fish,

the included composite samples consisted of five filleted fish only; while for small fish, the three composite samples selected were those consisting of 25 whole fish with heads, bones, and viscera kept intact throughout the process. The methodology of the selected three composite samples will be described in detail in this further section.

3.2 Preparatory work

In the weeks prior to the survey, I received first-hand preparatory training through field work at the IMR laboratories. This included proper instructions on how to fillet and handle fish of various sizes (see appendices II and III), how to determine the sex of the fish, and technical information on how to operate various instruments found on the ship (see appendix I). Part of the preparatory work was also a thorough walk-through of the various protocols (developed by IMR) that were followed on board during the survey.

3.3 Sampling procedures

Species of fish were sampled during the ecosystem survey with R/V *Dr. Fridtjof Nansen* from the north-western coast to the far northern coast of the island of Sri Lanka from June 24th to July 15th, 2018 (Figure 2). Pelagic and demersal trawls were continuously towed and placed on deck day and night throughout the survey, before the catch was subsequently sorted by the scientists on board, and then identified by taxonomists to the correct species. Fish were only selected for sampling if they were found in any of the lists of common commercial fish species in Sri Lanka (Appendices IV and V), and/or if the local scientists on shift all agreed that the particular species was a common food fish of importance to the local inhabitants of Sri Lanka. The group of local scientists on shift consisted of well-educated women and men in different areas of marine biology and fisheries management all allocated to NARA, and there were at least four-five scientists on each shift to consult with. The lists of common commercial marine species were obtained through the Department of Fisheries and Aquatic Resources of Sri Lanka (91) and Wikipedia (92), of which there was a great overlap and correspondence between the species found in each of the lists. Information concerning the date, the starting and ending position of the trawl (station number), the gear type used, the scientific name of the species, and the depth of the trawl were registered in the trawl forms (see appendices VI and VII) for each of the species that were sampled. Furthermore, samples were separated into two main categories for further processing: “small” fish species and

"large" fish species. As summarized in Figure 3, the following procedures were then carried out for each of the two categories.

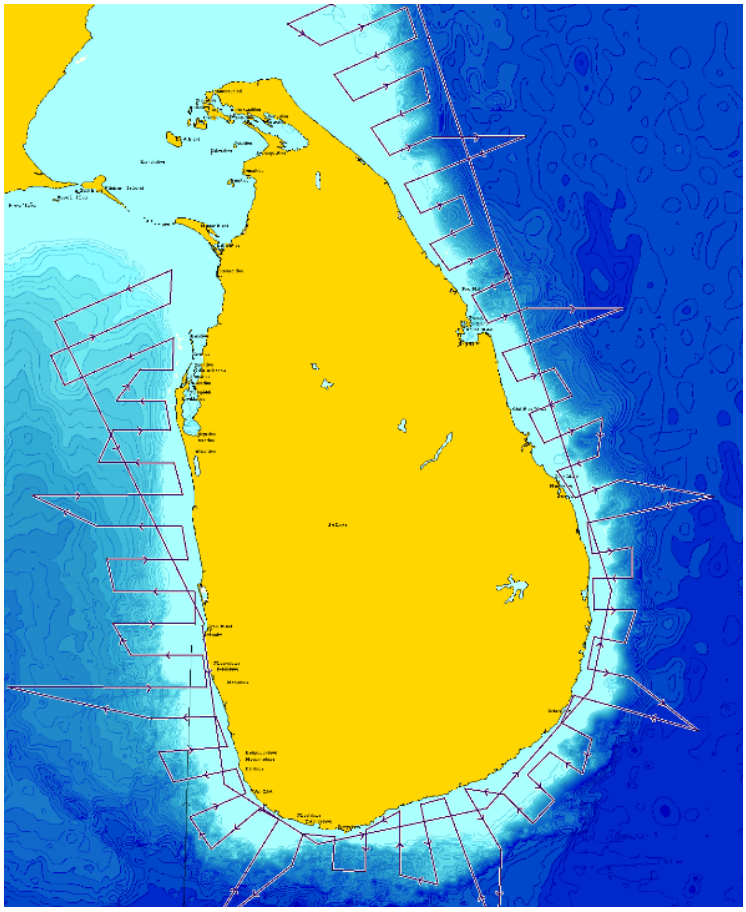

Figure 2: Graphic schematic of the planned Nansen cruise track of the 2018 survey around Sri Lanka.

3.3.2 General fish handling

The samples were collected randomly from the baskets of sorted trawl catch shortly after species determination and kept cool in a refrigerator (approximately -5°C) if handling could not begin immediately following sorting. If species were sampled and held-off by the scientists on shift during the night, they were kept frozen (approximately -20°C) until handling could begin the following morning. The samples were kept out of direct sunlight, and the study did not include any live animals.

3.3.3 Small fish

Fish < 25 cm were classified as small. This was the category for fish that are commonly and culturally eaten as a whole, with the head, tail, viscera, skin, and bones intact. From each location a minimum of 75 individuals, or a minimum of 120g of wet sample material, of small

fish were collected. These samples were further divided in to 3 composite samples containing 25 randomly selected fish each and were then labelled with a number ranging from one to three. Average weight (g) and length (cm) for each composite sample were then measured and recorded in the trawl form (Appendix VI). Length was measured on a marine measuring board for 25 fish from each species; the length was measured from the tip of the snout to the deepest fork of the caudal fin. The mean length of the 25 individuals where then calculated, representing the average length of the species in that catch. Lengths were measured to the nearest centimetre with one decimal, while weight measurements were recorded to the nearest gram with no decimals. Each composite sample was then homogenised in a common household food processor (*Braun Multiquick 7 K3000*) until the composition was identical throughout; this could take anywhere between 1 and 3 minutes depending on the sample material. Furthermore, 30g of the homogenous paste was then randomly selected from various locations in the grinding container, before being added to a 50 mL Nunc sample tube labelled with pre-printed labels marked with "wet sample", "whole fish", and the pre-assigned number of the composite sample. The samples were then put in the freezer on board at -20°C for storage. Subsequently, another 50-100g of the homogenous paste was randomly extracted from anywhere in the bowl using a spatula and added to a plastic tray. The wet weight of the sample was registered on a two-decimal scale and noted in the trawl form. The samples were then put in the freezer until freeze-drying would take place a couple of days later after thorough freezing of the sample had been ensured. This latter sample was labelled "dry sample".

3.3.4 Large fish

This was the category for fish where only the filet is commonly consumed, more specifically fish > 25 cm. A total of 15 individuals had to be collected of each species in one station. Fish were put on a large tray and labelled with numbers ranging from 1 to 15. Length and weight measurements were recorded in accordance to the protocol for small fish, with the exception of fish in rigor mortis that had to be flexed gently before measurements. For three large species, *Carangoides fulvoguttatus, Nemipterus bipunctatus,* and *Selar crumenophthalamus,* the average length was < 25 cm. Based on information from the Sri Lankan scientists on board on the eating practice of the species (where only the filet is eaten), a decision was made on-board to categorize the species as large fish although their length was below cut-off value. This implied that only the filet was used for further analyses. Furthermore, the sex (m/f) of each individual of all large fish were then recorded by studying the gonads of the fish. The

individuals were skinned and filleted using a cutting board, sharp scalpel, and a filleting knife according to protocol (Appendix III). In order to present a representative sample of the edible parts of the fish as a whole, both white muscle tissue and as much as possible of the red muscle tissue were obtained during fileting. The utilized equipment was thoroughly wiped off using hot water, soap, and a paper towel in between each sample of fish. The fileted sample from each of the 15 individual fish were then homogenised individually in the food processor (*Braun Multiquick 7 K3000)* and added to a plastic tray labelled with pre-printed labels containing a number corresponding to the previously assigned number of the fish. Furthermore, 3 composite samples were extracted from the 15 individual samples by collectively adding sample material from 5 x 3 samples of fish. This was accomplished by subtracting a 20g sample from each of the plastic trays containing the homogenized material of the individual fish samples and adding it to a new composite plastic tray. The new composite sample consisting of five individual samples was then homogenized once more, and labelled "Composite sample fish 1-5", while the others were labelled accordingly as 6-10 and 11-15. From this new plastic tray containing the 100g of composite sample, an aliquot of the sample of approximately 30 mL was randomly extracted from the homogenous paste, put in a 50 mL Nunc-tube, and labelled "wet sample". When all three composite samples were collected in the Nunc-tubes and labelled appropriately with journal number, species, station, date, and country of origin, they were put in the freezer on board (-20°C) for further storage. Subsequently, the remainder of sample material in the plastic trays was weighed, recorded in the trawl form, and put a lid on, before they were labelled "dry sample" and put in the freezer awaiting the process of freeze-drying.

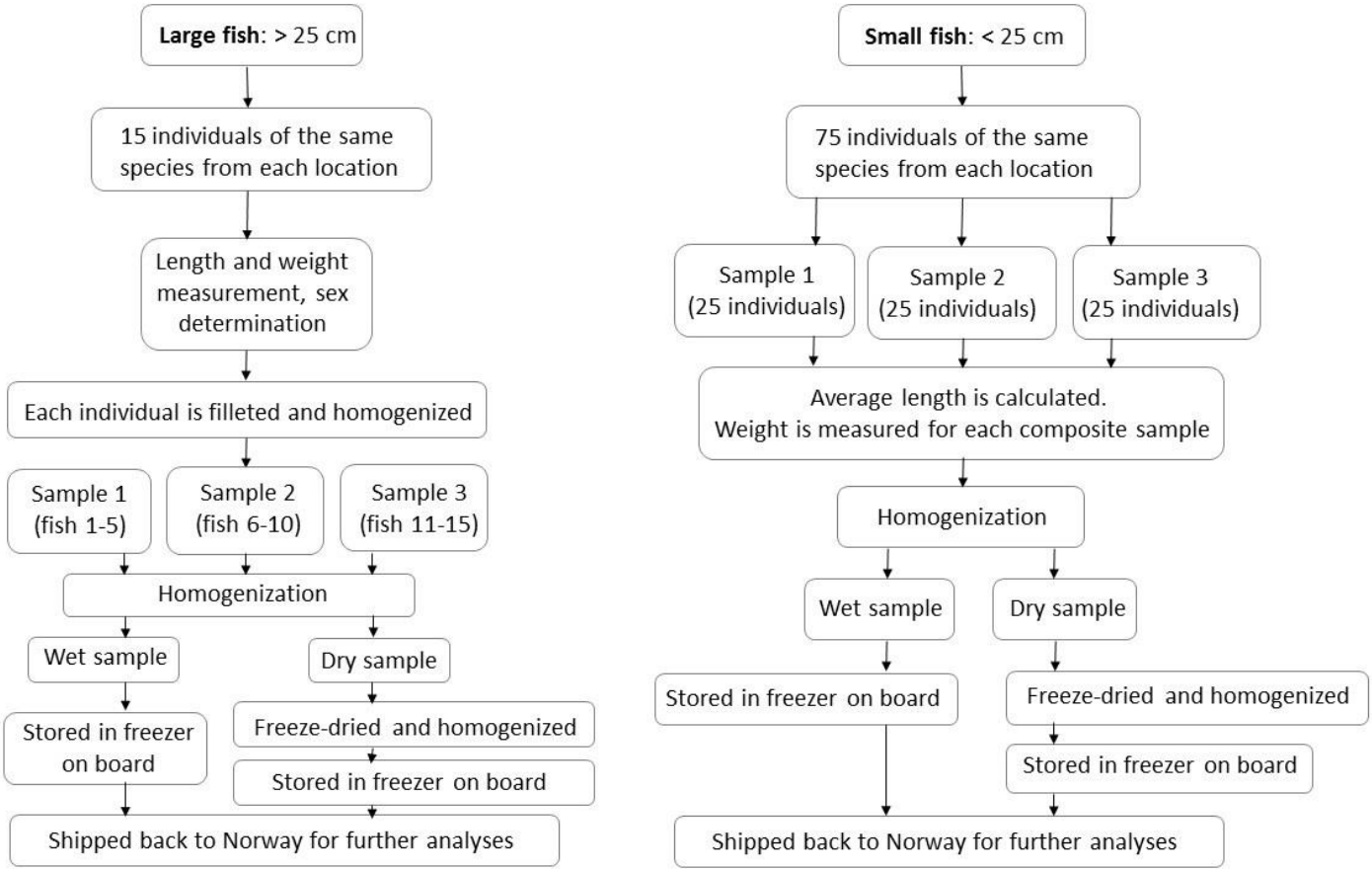

Figure 3: Summarizing flow chart of the various sampling procedures conducted during the Nansen survey for each fish species, categorized as either small or large fish.

3.3.5 Freeze-drying

The dry matter, and subsequently the water/moisture content, of the samples labelled "dry sample" were estimated using the freeze-dryer located onboard the ship (*Labconco Freezone 18 liters mod. 7750306*). This is a method that withdraws the water from the frozen samples by inducing a vacuum that alters the state of the water where the water evaporates from the state of solid ice already present in the sample. After at least 12 hours in the freezer at -20°C to ensure thorough freezing of the samples, the plastic trays were put in bulk in the freeze-dryer without the lids on for approximately 72 hours. This involved 24 hours at -50°C, immediately succeeded by 48 hours at +25°C to ensure that the moisture was completely drawn out of all samples. When exiting the freeze-dryer, a random selection of samples were cracked in two to confirm that all samples had been properly dried (using disposable plastic gloves). All samples were put in an exicator cabinet with new lids on to avoid drawing humidity from the air immediately following being removed from the freeze-dryer. The dry samples were then weighed with lids on a two-decimal scale once again, enabling the dry matter to be calculated based on the differences in weight of the sample between entering and

exiting the freeze-dryer (percentage of moisture withdrawn from the sample), where water is measured as the loss of weight (g) after drying the sample. Calculation of the concentration of dry mass was automatically completed in the LabWare LIMS (Laboratory Information Management System) 7 PROD software system by adding the weighed measurements manually, and then by using the following formula:

$$\%\ dry\ matter = \frac{(c - d)\ x\ 100\%}{(a - b)}$$

Where: a = weight of sample + container before drying (g)
b = weight of container (g)
c = weight of the sample + container after drying (g)

3.3.6 Homogenisation after freeze-drying

Following freeze-drying, the samples were then homogenized once more in an even more efficient knife mill (*Retch Grindomix GM 200*), enabling representative aliquots of finely pulverized samples to be taken from any location in the grinding container and transferred into smaller 50mL Nunc tubes for transportation back to the IMR laboratory in Bergen, Norway. This was accomplished by breaking the samples into the blender bowl and mixing for 15-20 seconds until the result was a homogenous powder. The powder was then added to a 50 mL Nunc tube pre-labelled with pre-printed labels stating the number of the sample, the country of origin, and state of the matter, i.e., "dry sample". The tools used for homogenization were thoroughly dusted off with a suitable brush between samples to ensure no residues of old or previous samples would interfere with the new ones, and disposable plastic gloves were utilized throughout the process.

3.3.7 Storage and shipment

Both wet samples (samples taken directly from the homogenized paste) and dry samples (freeze-dried material) were vacuum sealed and put in insulated boxes before being stored in the freezer on board at -20°C until shipment by air cargo to Bergen, Norway from Cape Town, Africa in November 2018. Careful labelling with a note indicating the journal number corresponding to the trawl form, number of samples, types of samples (dry or wet), and country of origin followed in each vacuum sealed bag. The samples arrived in Norway at the IMR laboratories in Bergen late-November of 2018 and were sorted and stored. Dry samples were stored in boxes at room temperature, while wet samples were stored in boxes in a -80 °C freezer until laboratory analyses would proceed in January 2019.

3.4 Laboratory Analyses

3.4.1 Sample preparation and methods of analyses

Common names and scientific names of each of the species were confirmed according to the global species database "FishBase" (93) prior to being entered in to LIMS for further analyses. Before analyses could begin, all samples had to be divided into smaller Nunc-tubes (12.5 mL and 20 mL) according to the amount required for each laboratory analysis, as summarized in Table 3. Wet samples had to be slightly thawed to get the sample out of the original Nunc tube, but were immediately put in an insulated box equipped with freeze blocks upon completion. Additionally, when working with photosensitive samples; all electric lights were turned off and window blinds were closed, leaving the room as dark as possible. Samples were then delivered to their respective labs, where all wet samples were stored in temperatures as illustrated in Table 2 until analyses would begin.

Table 2: Analytical methods used for the nutrient composition analyses of the samples of fish. The table includes list of analytes, laboratory method, amount- and state of samples.

Analyte(s)	Method performed	Amount of sample required	State of sample for analyzes	Storage temperature (°C)	Additional information
Dry matter	Freeze-drying	10g	Wet	-20	
Fat (total)	Ethyl acetate, gravimetric analyses (94)	10g	Wet	-20	-
Fatty acid composition	GLC (95)	1g	Wet	-20	
Crude protein	Nitrogen analyzer: Dumas-method (96)	5g	Freeze-dried	-20	-
Vitamin A_1 and vitamin A_2	HPLC (97, 98)	1g	Wet	-80	Photosensitive
Vitamin B_{12}	Microbiological assay (99)	0,5 - 5g	Wet	-20	Photosensitive
Vitamin D_3	HPLC (100)	1g	Wet	-80	Photosensitive
Calcium, sodium, potassium, magnesium, phosphorus	ICP-MS (101)	5g	Freeze-dried	-20	-
Iron, zinc, selenium	ICP-MS (102)	5g	Freeze-dried	-20	-
Iodine	ICP-MS (103)	5g	Freeze-dried	-20	-

Abbreviations: ICP-MS: Inductively coupled plasma/mass spectrometry; GLC: Gas-liquid chromatography; HPLC: High performance liquid chromatography; g: grams; °C: degrees Celsius.

3.4.2 Analysis quality

All analyses of both proximate components and vitamins and minerals were analysed in singular parallels conducted in accordance to respective protocols at the IMR laboratories in Bergen, Norway. The IMR laboratory is accredited to ISO 17025 standards from 2017, in accordance to the International Organization for Standardization (104). The laboratory also participates in national and international proficiency tests with approved results as an external means of assuring quality of their analyses on a regular basis. The methods performed in this thesis includes Certified Reference Materials in each of their nutrient analysis series to control the accuracy of the analytical method (105; p. 80), and all values were within accepted area of the analyses. The analytical methods performed for each nutrient component are listed in Table 2, and corresponding limits of quantification (LOQ) are summarized in Table 3.

Table 3: Limit of quantification of methods used for nutrient analyses of fish samples per 100g.

Analyte	Units	LOQ
Components		
Dry matter	g/100g	2
Fat (total)	g/100g	0.1
Protein	g/100g	0.1
Fatty acids	mg/100g	1
Fatty acids	%	0.1
Minerals		
Iron	mg/100g	0.01
Zinc	mg/100g	0.05
Calcium	mg/100g	3.5
Iodine	µg/100g	0.004
Selenium	µg/100g	0.001
Phosphorus	mg/100g	0.3
Potassium	mg/100g	5
Magnesium	mg/100g	1
Sodium	mg/100g	11
Vitamins		
Cobalamin	µg/100g	1
Vitamin A	µg/100g	0.5
Vitamin D	µg/100g	1

Abbreviations: LOQ: Limit of quantification; g: grams; mg: milligrams.

3.4.3 Statistical analysis and presentation of data

If not otherwise specified, the data are presented as means ± standard deviations (SD) per 100g of the three composite samples of each species of fish, reported to the same units of expression and rounding procedures as advised in the FAO guidelines "*Food composition data*" (60, p. 164-166). Data were exported from LIMS to Microsoft Excel 2013 version 15.0 for calculation of means and SD. The same number of significant figures generated in the original dataset as provided by the laboratory for each analysis, are reported for each nutrient. For samples with values < LOQ, values are presented as the unadjusted LOQ value as reported by the laboratory. When calculating the mean value of the nutrient where one or more samples presented values <LOQ, the LOQ was divided by 2 to obtain a number that could be included in the calculations. Analyzed values for all vitamins and minerals were reported in mg per kg sample material from the laboratory, but were converted to mg per

100g for ease of use as recommended by the FAO protocol for FCD (60, p. 165-166). The SFA, MUFA, PUFA, ∑n-3, and ∑n-6 content was reported in mg/g and as the percentage (%) of total fatty acids, but were also converted to g/100g for compliance with the presentation of FCD. The fatty acids EPA and DHA were converted from mg/g to mg/100g and were also reported as the percentage (%) of total fatty acids. Vitamin A components are presented as µg/100g of the vitamin A isomers retinol (the sum of 13-, 11-, 9-cis and all-trans retinol (A_1)) and 3.4 didehydro-all-trans retinol (A_2). Vitamin D is presented as the amount of vitamin D_3 (cholecalciferol) present in the sample, as the amount of vitamin D_2 (ergocalciferol) is considered negligible in fish (105; p. 85, 106).

3.5 Calculation of potential contribution to RNI

As in agreement with the FAO/WHO (107), the RNI is in this thesis defined as the estimated daily intake which meets the nutrient requirements of almost all (97.5%) healthy individuals in an age- and sex-specific population. The potential contribution of each species to daily RNI was calculated in reference to the recommendations for 19-50-year-old non-pregnant, non-lactating, healthy females of reproductive age.

3.5.1 Micronutrient selection and portion size of fish

The micronutrients selected for evaluation were those considered to be of greatest public health relevance in Sri Lanka, and are often considered problematic to obtain in cereal- and tuber-based diets such as the rice-dominant diet present in Sri Lanka (17, 18, 107, p. 325-327). Thus, the selected nutrients are calcium, iron, zinc, and vitamin A (7, 15). The various species' contribution to the RNI of these nutrients were calculated in reference to a standard Sri Lankan serving size of fish (30g), as in accordance to the Sri Lankan FBDG (59), and a 100g portion of fish for comparison. For simplicity, the values were calculated for raw fish, as this was the analyzed state of the samples.

3.5.2 Calculations

For both iron and zinc, the RNI vary according to estimated overall dietary bioavailability, which is dependent on the presence of other enhancers and inhibitors in the diet (107). Because of the lack of comprehensive national dietary data and nutrition studies in Sri Lanka, it is difficult to estimate an appropriate level of bioavailability. The typical Sri Lankan diet was here assumed to best fit the criteria of low (10%) bioavailability for iron, and low (15%)

bioavailability for zinc, due to the presence of large amounts of phytates and lesser amounts of animal protein. For vitamin A, the FAO/WHO adopted the term "Recommended safe intake" due to the lack of data to derive a mean requirement for any specific group. The term is set to prevent clinical signs of deficiency and allow normal growth, but prolonged periods of disease or stress are not taken into consideration. The recommended safe intake of vitamin A is expressed as µg retinol equivalents (RE), where 1 µg retinol = 1 RE (107). Values for vitamin A_1 were included in the calculations, while values for vitamin A_2 were excluded due the small amount present and the reduced biological activity of dehydroretinol isomers (108).

4 Results

4.1 Sample characteristics

This thesis included 19 fish species sampled during the 2018 Nansen survey around Sri Lanka. Two species, *Decapterus macrosoma* and *Photopectoralis bindus*, were collected twice on two separate locations, and will further be marked with corresponding numbers, 1 and 2, respectively. This is to differntiate the two identical species in accordance to the respective trawling coordinates of the sample location as presented in appendix VIII. Thus, a total of 17 different species are included in this thesis. A map of the cruise track and sampling locations are presented in Figure 3, and in appendix VIII more detailed information on the coordinates and station numbers of the various sampling locations are presented. The identification details of each species sampled, including scientific name, English name (common name), Sinhalese and Tamil names when available, and the natural habitat of each species, are presented in Table 4. In this thesis, samples will further be referred to by their scientific name.

Table 4: Identification details and overview of species collected during the 2018 Nansen survey around Sri Lanka.

Scientific name	English name[a]	Sinhalese name[b]	Tamil name[b]	Habitat[a]
Large fish				
Carangoides fulvoguttatus	Yellowspotted trevally	Thumba parawa[d]	Manjal parai	Reef-associated
Diagramma pictum	Painted sweetlips	Gobaya	Kallu kallewa	Reef-associated
Lethrinus olivaceus	Long-face emperor	Uru hota[d]	Thinan	Reef-associated
Lutjanus lutjanus	Bigeye snapper	Hunu ranna[d]	Nooleni	Demersal
Nemipterus bipunctatus	Delagoa threadfin bream	-[c]	Cundil	Demersal
Selar crumenophthalmus	Bigeye scade	Bolla[d]	Chooparai	Reef-associated
Sphyraena jello	Pickhandle barracuda	Silava[d]	Jeela	Reef-associated
Small fish				
Amblygaster sirm	Trenched sardinella	Hurulla[d]	Keerimeen saalai	Pelagic
Auxis thazard	Frigate tuna	Alagoduwa[d]	Urulan soorai	Pelagic
Decapterus macrosoma	Shortfin scad	Linna	Mundakan kilichchi	Pelagic
Encrasicholina devisi	Devis' anchovy	Halmessa	Neththili	Pelagic
Equulites elongatus	Slender ponyfish	Karalla	Karal	Demersal
Leiognathus dussumieri	Dussumier's ponyfish	Karalla[d]	Vari karai	Demersal
Photopectoralis bindus	Orangefin ponyfish	Karalla	Tatnam-kare	Demersal
Rastrelliger kanagurta	Indian mackerel	Kumbalava	Kanang keluththi	Pelagic
Stolephorus indicus	Indian anchovy	Halmassa[d]	Neththili	Pelagic
Sillago ingenuua	Bay whiting	-[c]	Kelangan	Demersal

[a] Information on English names and habitats were obtained through the global species database "FishBase" (93).

[b] Sinhalese and Tamil names were obtained through personal communications via my Sri Lankan collaborator, Thiruchenduran Somasundarampillai, M.Sc. Food Technology (2019).

[c] The Sinhalese names of all species were not available.

[d] Sinhalese names that were confirmed using the global species database "FishBase" (93).

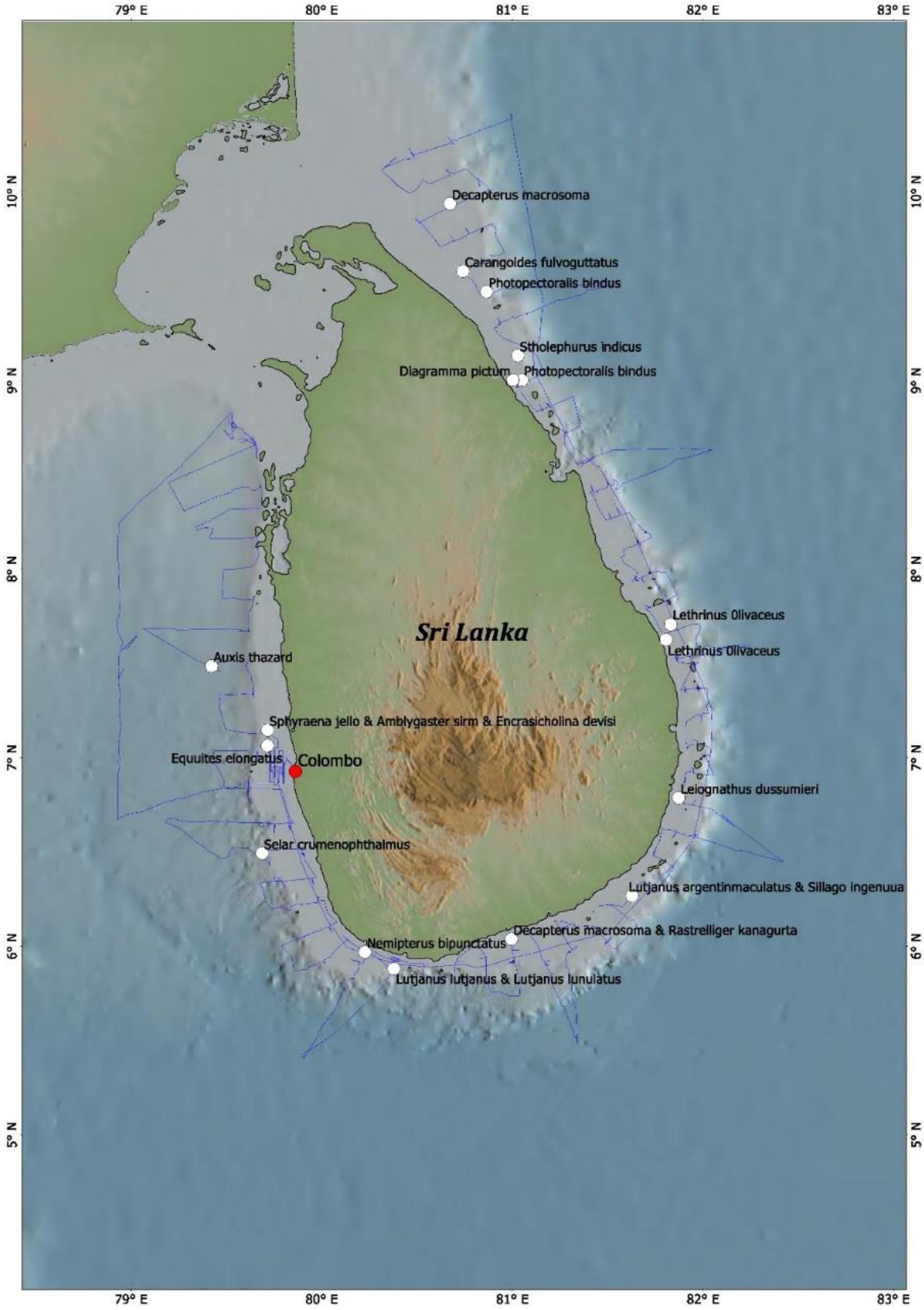

Figure 4: Map of the Nansen cruise track around Sri Lanka, including sample locations and the scientific names of the samples collected in each location (station). For more detailed coordinates of each sample location, see appendix VIII.

Characteristics of length, weight, and sex of the included species are presented in Table 5. For small species, the average weight per fish was 21 ± 15 g, while the mean length was 11.3 ± 3.1 cm. The sex of the large species are presented as either male or female.

Table 5: Weight, length, and sex characteristics of the 19 species included in the thesis[a].

Species name	Average weight[b]	Average length per individual fish[b]	Fish in each composite sample	Sex	
	g	cm	n	Male	Female
Large fish					
Carangoides fulvoguttatus[c]	168 ± 31	20.5 ± 1.5	15	15	-
Diagramma pictum	1694 ± 906	47.9 ± 7.5	15	9	6
Lethrinus olivaceus	1886 ± 2275	46.4 ± 17.4	15	7	8
Lutjanus lutjanus	317 ± 58	27.5 ± 1.8	15	13	2
Nemipterus bipunctatus[c]	78 ± 45	16.3 ± 3.2	15	10	5
Selar crumenophthalmus[c]	174 ± 45	21.3 ± 1.7	15	6	9
Sphyraena jello	2885 ± 557	88.5 ± 5.6	15	11	4
Small fish					
Amblygaster sirm	278 ± 20	10.5	75		
Auxis thazard	1180 ± 27	16.2	75		
Decapterus macrosoma (1)	763 ± 23	13.5	75		
Decapterus macrosoma (2)	273 ± 22	9.2	75		
Encrasicholina devisi[d]	219 ± 1	10.5	150		
Equulites elongatus	183 ± 8	7.7	75		
Leiognathus dussumieri	637 ± 56	10.6	75		
Photopectoralis bindus (1)	245 ± 20	7.4	75		
Photopectoralis bindus (2)	228 ± 10	7.5	75		
Rastrelliger kanagurta	610 ± 6	12.5	75		
Sillago ingenuua	1099 ± 24	16.3	75		
Stolephorus indicus	676 ± 10	13.2	75		

[a] Values are means ± SD, and are based on length and weight values (prior to any handling) from the included species sampled during the Nansen survey around Sri Lanka.

[b] Weight and length measurements are expressed as the mean of the composite sample consisting of n number of fish for small species, and per individual fish for large species. The length of small species was calculated as a mean value of the first composite sample during the survey, thus, no SD is presented.

[c] Species categorized as large fish (although their length was < 25 cm) based on input on the eating practice of the current species given by the local scientists on board.

[d] For this species, each composite sample consisted of 50 individual fish in order to obtain enough sample material.

Abbreviations: g: grams; cm: centimeters, SD: standard deviation.

4.2 Proximate composition

The protein, fat, and percentage of dry matter of the 19 samples analyzed, expressed as g/100g edible portion are presented in Table 6. The total protein content in the species ranged from 16.53 ± 0.81 g/100g in *Leiognathus dussumieri* to 22.33 ± 0.58 g/100 g in *Selar crumenophthalmus*, with a mean protein content for all large species combined of 20.52 ± 1.47 g/100 g, and an average value of 18.96 ± 1.14 g/100g for small species. Furthermore, total fat content varied from 0.50 ± 0.3 g/100g in *Diagramma pictum* to 3.0 ± 0.2 g/100g in *Rastrelliger kanagurta*, thus categorizing all large samples as lean, and 9 of the 12 small species as intermediate according to the Norwegian and Danish categorization of lipid content in percentage of total body weight (78). The mean fat content in all large species combined was 0.94 ± 0.5 g/100 g, and 2.24 ± 0.5 g/100 g for small species. The dry matter ranged from 21.2 ± 0.2 % in *Lutjanus lutjanus* to 26.3 ± 0.2 % and 26.3 ± 0.5 % in both *Selar crumenophthalmus* and *Leiognathus dussumieri*, respectively. The mean dry matter for large species was 22.7 ± 1.7 %, whereas it was 24.6 ± 0.9 % for small species.

Table 6: Analytical values of the proximate composition of the 19 fish species sampled from Sri Lanka[a].

		Protein	Fat (total)	Dry matter
	n[b]	g/100g	g/100g	%
Large fish				
Carangoides fulvoguttatus	3	22 ± 0.0	1.3 ± 0.3	23.4 ± 0.5
Diagramma pictum	3	20 ± 0.0	0.50 ± 0.3	21.5 ± 0.5
Lethrinus olivaceus	3	21 ± 1.5	1.1 ± 0.9	22.5 ± 0.3
Lutjanus lutjanus	3	19 ± 0.0	0.90 ± 0.1	21.2 ± 0.2
Nemipterus bipunctatus	3	19 ± 1.7	1.2 ± 0.3	22.4 ± 0.2
Selar crumenophthalmus	3	22 ± 0.6	1.0 ± 0.2	26.3 ± 0.2
Sphyraena jello	3	21 ± 0.6	0.51 ± 0.1	21.7 ± 0.6
Mean for large species		21 ± 1.5	0.94 ± 0.5	22.7 ± 1.7
Small Fish				
Amblygaster sirm	3	21 ± 0.7	2.6 ± 0.2	25.8 ± 0.4
Auxis thazard	3	20 ± 0.0	2.2 ± 0.0	25.0 ± 0.4
Decapterus macrosoma (1)	3	18 ± 0.0	2.0 ± 0.3	24.3 ± 0.2
Decapterus macrosoma (2)	3	19 ± 1.0	2.7 ± 0.2	24.1 ± 0.8
Encrasicholina devisi [c]	3[c]	19 ± 0.6	2.4 ± 0.2	23.8 ± 0.3
Equulites elongatus	3	18 ± 0.6	2.5 ± 0.1	23.0 ± 0.2
Leiognathus dussumieri	3	17 ± 0.8	2.2 ± 0.2	26.3 ± 0.5
Photopectoralis bindus (1)	3	19 ± 0.0	1.6 ± 0.1	24.3 ± 0.2
Photopectoralis bindus (2)	3	19 ± 0.0	2.4 ± 0.3	24.7 ± 0.5
Rastrelliger kanagurta	3	19 ± 0.6	3.0 ± 0.2	24.6 ± 0.2
Sillago ingenuua	3	20 ± 0.6	1.7 ± 0.1	25.3 ± 0.4
Stolephorus indicus	3	20 ± 0.0	1.7 ± 0.1	24.1 ± 0.3
Mean for small species		19 ± 1.1	2.2 ± 0.5	24.6 ± 0.9

[a] Values are reported as means ± SDs of the 19 fish species analyzed in triplicates, expressed as the nutrient content per 100 g raw, edible sample.

[b] Number of composite samples analysed. For large species (>25 cm), 5 fish are included in each composite sample, while for small species (<25 cm), 25 fish are included in each composite sample.

[c] For this species, each composite sample consisted of 50 individual fish in order to obtain enough sample material.

Abbrevations: g: grams, SD: standard deviation.

4.2.1 Fatty acid composition

The fatty acid composition in terms of SFA, MUFA, PUFA, ∑n-3 fatty acids, ∑n-6 fatty acids, EPA, and DHA content of the species are presented in Table 7. In gerenal, the 19 species differed widely in terms of their absolute values and percentages of fatty acids. The total fatty acid composition of the species ranged from 0.0738 ± 0.01 g/100g to 0.893 ± 0.1 g/100g saturated (SFAs; 28.6 to 43.5%), 0.0265 ± 0.004 g/100g to 0.357 ± 0.05 monounsaturated (MUFAs; 9.1 to 23.1%), and 0.121 ± 0.01 g/100g to 1.96 ± 0.1 g/100g polyunsaturated (PUFAs; 25.7 to 53%). ∑n-3 ranged from 0.085 ± 0.01 g/100g to 0.784 ± 0.07 g/100g (21.53 to 45.5%). In terms of the two n-3 fatty acids evaluated in this thesis, both EPA and DHA content varied substantially between the species. The EPA content ranged from 11 ± 2 mg/100g to 250 ± 24 mg/100g (1.9 to 13%), whereas the DHA content ranged from 41.0 ± 6 mg/100g to 467 ± 15 mg/100g (7.1 to 29.9%). *Rastrelliger kanagurta* was identified as the most significant source of EPA, whlile *Amblygaster sirm* was the most significant source of DHA. The mean of all small species presented a substantially higher content of both EPA and DHA compared to that of the large species (135 ± 64 mg/100g and 34.5 ± 26 mg/100g, respectively for EPA, and 322 ± 86 mg/100g and 143 ± 106 mg/100g, respectively for DHA).

Table 7: Analytical values of the fatty acid composition of the 19 species sampled from Sri Lanka[a].

Species	n[b]	Sum SFA g/100g (%[c])	Sum MUFA g/100g (%[c])	Sum PUFA g/100g (%[c])	Sum n-3 g/100g (%[c])	Sum n-6 g/100g (%[c])	EPA mg/100g (%[c])	DHA mg/100g (%[c])
Large fish								
Carangoides fulvoguttatus	3	0.279 ± 0.12 (37.7)	0.151 ± 0.07 (20.1)	0.258 ± 0.05 (36.5)	0.153 ± 0.04 (21.5)	0.100 ± 0.01 (14.4)	41.0 ± 14 (5.6)	74.3 ± 9.0 (10.8)
Diagramma pictum	3	0.282 ± 0.12 (43.5)	0.152 ± 0.07 (23.1)	0.151 ± 0.02 (25.7)	0.085 ± 0.01 (14.4)	0.0663 ± 0.01 (11.3)	23.3 ± 2.9 (3.9)	41.0 ± 6.0 (7.1)
Lethrinus olivaceus	3	0.198 ± 0.25 (28.6)	0.126 ± 0.18 (14.9)	0.222 ± 0.17 (48.2)	0.174 ± 0.15 (35.3)	0.0450 ± 0.02 (12.6)	12.7 ± 15 (1.9)	137 ± 110 (29.9)
Lutjanus lutjanus	3	0.145 ± 0.02 (33.4)	0.071 ± 0.02 (16.3)	0.198 ± 0.02 (45.8)	0.156 ± 0.02 (36)	0.0420 ± 0.01 (9.8)	19.0 ± 2.0 (4.4)	124 ± 10.0 (28.6)
Nemipterus bipunctatus	3	0.295 ± 0.07 (35.2)	0.114 ± 0.03 (13.5)	0.389 ± 0.03 (47.3)	0.294 ± 0.02 (35.8)	0.0910 ± 0.01 (11)	51.3 ± 9.8 (6.2)	210 ± 6.1 (25.7)
Selar crumenophthalmus	3	0.465 ± 0.04 (35.2)	0.194 ± 0.02 (14.6)	0.612 ± 0.04 (46.5)	0.489 ± 0.03 (37)	0.123 ± 0.01 (9.3)	83.0 ± 7.0 (6.3)	347 ± 20 (26.4)
Sphyraena jello	3	0.0738 ± 0.01 (29.1)	0.0265 ± 0.00 (10.4)	0.121 ± 0.01 (47.9)	0.089 ± 0.01 (35.1)	0.0321 ± 0.01 (12.7)	11.0 ± 2.0 (4.6)	70.0 ± 9.0 (27.8)
Mean for large species		0.248 ± 0.16 (34.7)	0.119 ± 0.085 (16.1)	0.279 ± 0.17 (42.6)	0.206 ± 0.15 (30.7)	0.0712 ± 0.03 (11.6)	34.5 ± 26 (4.7)	143 ± 110 (22.3)

Table 7 continued

Small Fish	n[b]	**Sum SFA** g/100g (%[b])	**Sum MUFA** g/100g (%[b])	**Sum PUFA** g/100g (%[b])	**Sum n-3** g/100g (%[b])	**Sum n-6** g/100g (%[b])	**EPA** mg/100g (%[b])	**DHA** mg/100g (%[b])
Amblygaster sirm	3	0.597 ± 0.03 (36.8)	0.188 ± 0.01 (11.6)	0.761 ± 0.04 (47)	0.652 ± 0.03 (40.3)	0.104 ± 0.04 (6.4)	147 ± 41 (8)	467 ± 15 (28.9)
Auxis thazard	3	0.432 ± 0.04 (32.4)	0.122 ± 0.01 (9.1)	0.707 ± 0.07 (53)	0.606 ± 0.06 (45.5)	0.0990 ± 0.01 (7.4)	130 ± 16 (9.8)	399 ± 37 (29.9)
Decapterus macrosoma (1)	3	0.313 ± 0.06 (32.8)	0.159 ± 0.03 (16.7)	0.432 ± 0.06 (45.6)	0.329 ± 0.05 (34.8)	0.0987 ± 0.01 (10.4)	56.3 ± 11 (5.9)	242 ± 33 (25.6)
Decapterus macrosoma (2)	3	0.665 ± 0.11 (35.3)	0.274 ± 0.05 (14.4)	0.856 ± 0.08 (45.7)	0.700 ± 0.07 (37.4)	0.146 ± 0.01 (7.8)	246 ± 39 (13)	339 ± 8.1 (18.2)
Encrasicholina devisi	3[d]	0.537 ± 0.02 (37.3)	0.178 ± 0.01 (12.4)	0.633 ± 0.02 (43.9)	0.527 ± 0.01 (36.6)	0.108 ± 0.01 (7)	120 ± 4.1 (8.3)	342 ± 80 (25.3)
Equulites elongatus	3	0.550 ± 0.08 (36.6)	0.212 ± 0.03 (13.7)	0.677 ± 0.06 (44)	0.538 ± 0.04 (35)	0.136 ± 0.01 (8.9)	112 ± 10 (7.2)	361 ± 25 (23.5)
Leiognathus dussumieri	3	0.764 ± 0.09 (38.8)	0.357 ± 0.05 (17.9)	0.714 ± 0.4 (36.4)	0.482 ± 0.03 (24.6)	0.223 ± 0.01 (11.4)	167 ± 16 (8.5)	219 ± 6.1 (11.2)
Photopectoralis bindus (1)	3	0.640 ± 0.07 (37.8)	0.252 ± 0.03 (14.8)	0.698 ± 0.06 (41.1)	0.509 ± 0.05 (30)	0.180 ± 0.02 (10.6)	128 ± 10 (7.6)	297 ± 29 (17.5)

Table 7 continued

	n[b]	**Sum SFA** g/100g (%[b])	**Sum MUFA** g/100g (%[b])	**Sum PUFA** g/100g (%[b])	**Sum n-3** g/100g (%[b])	**Sum n-6** g/100g (%[b])	**EPA** mg/100g (%[b])	**DHA** mg/100g (%[b])
Photopectoralis bindus (2)	3	0.673 ± 0.05 (37.4)	0.254 ± 0.02 (14.1)	0.754 ± 0.04 (42)	0.548 ± 0.04 (30.5)	0.197 ± 0.01 (11)	141 ± 10 (7.8)	323 ± 19 (18)
Rastrelliger kanagurta	3	0.893 ± 0.10 (35.8)	0.329 ± 0.09 (13.1)	1.96 ± 0.10 (43.9)	0.784 ± 0.07 (31.5)	0.303 ± 0.03 (12.1)	250 ± 24 (10)	416 ± 38 (16.7)
Sillago ingenuua	3	0.329 ± 0.05 (35.3)	0.155 ± 0.05 (16.6)	0.381 ± 0.06 (40.8)	0.270 ± 0.04 (28.9)	0.110 ± 0.02 (1.7)	50.0 ± 6.0 (5.4)	169 ± 27 (18.1)
Stolephorus indicus	3	0.385 ± 0.05 (37.6)	0.109 ± 0.01 (10.6)	0.478 ± 0.06 (46.6)	0.398 ± 0.05 (38.8)	0.0767 ± 0.01 (7.5)	73.3 ± 10 (7.1)	292 ± 5.8 (28.6)
Mean for small species		0.565 ± 0.18 (36.1)	0.216 ± 0.08 (13.8)	0.682 ± 0.20 (44.2)	0.529 ± 0.15 (34.5)	0.148 ± 0.07 (9.4)	135 ± 64 (8.2)	322 ± 86 (21.8)

[a] Values are reported as means ± SD of the 19 fish species analyzed in triplicates, expressed as the nutrient content per 100 g raw, edible sample.
[b] Values given in percent of total fatty acids.
[c] Number of composite samples analysed. For large species (>25 cm), 5 fish are included in each composite sample, while for small species (<25 cm), 25 fish are included in each composite sample.
[d] For this species, each composite sample consisted of 50 individual fish in order to obtain enough sample material.
Abbrevations: g: grams; DHA: docosahexaenoicacid; EPA: eicosapentaenoicadic, mg: milligrams; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SD: standard deviation, SFA: saturated fatty acids.

4.3 Vitamin content

The vitamin A, vitamin B_{12}, and vitamin D content for all species with the exception of *Diagramma pictum* are presented in Table 8. Vitamin A_1 ranged from 2.7 ± 0.4 µg/100g to 2000 ± 150 µg/100g, whereas vitamin A_2 was undetected (values <LOQ) in 5 species and ranged up to 46 ± 4.1 µg/100g. The total vitamin A content (A_1 + A_2) was generally low in all large species compared to small species, where the mean for vitamin A_1 was 6.9 ± 7.1 µg/100g and 280 ± 520 µg/100g, respectively. This was also seen for vitamin A_2, where the mean of large species was 0.74 ± 1.1 µg/100g, whereas the mean of small species was 15 ± 11 µg/100g. The large variation in the total mean of both vitamin A_1 and A_2 for small species is caused by the species *Leiognathus dussumieri*, which presented exraordinary large values compared to the rest of the species (2000 ± 150 µg/100g and 46 ± 4.1 µg/100g for A_1 and A_2, respectively). The content of vitamin B_{12} varied from 0.64 ± 0.04 µg/100g in the large species *Caragoides fulvoguttatus* to 20 ± 1.5 µg/100g in the small species *Decapterus macrosoma (2)*. The total mean for large species was 3.0 ± 4.1 µg/100g, whereas the mean for small species was 12 ± 5.1 µg/100g, thus presenting a substantially higher concentration in small species compared to large species. The only deviation was the large species *Selar crumenophthalmus*, which presented a value more in line with the results of small species than those of large species. The vitamin D content was undetected (values <LOQ) in 6 species and ranged up to 7.3 ± 0.6 in *Auxis thazard*, and presented a slightly higher mean in small species compared to large species (3.6 ± 2.5 µg/100g and 2.4 ± 2.3 µg/100g, respectively) when adjusting for values <LOQ.

Table 8: Analytical values of the vitamin A, vitamin B_{12}, and vitamin D content in the 19 species sampled from Sri Lanka[a].

Species	n[b]	Vitamin A_1 µg/100g	Vitamin A_2 µg/100g	Vitamin B_{12} µg/100g	Vitamin D_3 µg/100g
Large fish					
Carangoides fulvoguttatus	3	3.8 ± 3.7	< 0.5[c]	0.64 ± 0.04	< 1[d]
Diagramma pictum[e]	3	-	-	-	-
Lethrinus olivaceus	3	11 ± 16	< 0.5[*]	1.4 ± 0.1	3 ± 2
Lutjanus lutjanus	3	2.7 ± 0.1	< 0.5[c]	1.5 ± 0.2	< 1[c]
Nemipterus bipunctatus	3	2.7 ± 0.4	< 0.5[c]	1.2 ± 0.2	4.3 ± 0.6
Selar crumenophthalmus	3	9.3 ± 0.6	< 0.5[c]	12 ± 1.2	< 1[c]
Sphyraena jello	3	12 ± 5.5	2.8 ± 1.1	1.3 ± 0.2	5.7 ± 0.6
Mean for large species		6.9 ± 7.1	0.74 ± 1.1[f]	3.0 ± 4.1	2.4 ± 2.3[f]
Small fish					
Amblygaster sirm	3	70 ± 0.0	24 ± 2.0	14 ± 1.2	3 ± 1
Auxis thazard	3	110 ± 5.8	6.3 ± 1.5	12 ± 1.5	7.3 ± 0.6
Decapterus macrosoma (1)	3	170 ± 21	8.7 ± 1.5	16 ± 1.0	< 1[d]
Decapterus macrosoma (2)	3	130 ± 5.8	12 ± 4.7	20 ± 1.5	4.3 ± 1.5
Encrasicholina devisi	3[g]	93 ± 31	9.7 ± 3.5	9.7 ± 0.5	2.3 ± 0.6
Equulites elongatus	3	160 ± 35	10 ± 2.0	8.6 ± 0.4	6.7 ± 2.1
Leiognathus dussumieri	3	2000 ± 150	46 ± 4.1	8.1 ± 0.2	< 1[d]
Photopectoralis bindus (1)	3	150 ± 25	7.3 ± 0.6	5.7 ± 0.3	3 ± 1
Photopectoralis bindus (2)	3	140 ± 29	6.0 ± 1.0	4.7 ± 0.4	2.7 ± 0.6
Rastrelliger kanagurta	3	100 ± 5.8	16 ± 0.6	18 ± 0.6	4.7 ± 0.6
Stolephorus indicus	3	100 ± 31	11 ± 1.5	5.4 ± 0.6	7 ± 2
Sillago ingenuua	3	230 ± 46	23 ± 3.5	17 ± 1.2	< 1[d]
Mean for small species		280 ± 520	15 ± 11	12 ± 5.1	3.6 ± 2.5[f]

[a] Values are reported as means ± SDs of the 19 fish species analyzed in triplicates, expressed as the nutrient content per 100 g raw, edible sample.

[b] Number of composite samples analysed. For large species (>25 cm), 5 fish are included in each composite sample, while for small species (<25 cm), 25 fish are included in each composite sample.

[c] Value below LOQ of 0.5.

[d] Value below LOQ of 1.0.

[e] No data available as vitamin analyzes for the species *Diagramma pictum* were not completed in time.

[*] 2 composite samples were below the LOQ of 0.5, while 1 composite sample was 1.6 µg/100g ww.

[f] Values < LOQ were divided by 2 to be able to calculate the mean.

[g] For this species, each composite sample consisted of 50 individual fish in order to obtain enough sample material.

Abbrevations: g: grams, SD: standard deviation, µg: micrograms.

4.4 Mineral composition

The calcium, iron, iodine, magnesium, phosphorus, potassium, selenium, sodium, and zinc composition for all species are presented in Table 9. The calcium content varied considerably with a range from 7.9 ± 0.5 mg/100g in *Sphyraena jello* to almost 300 times higher in the small species *Leiognathus dussumieri* with a value of 2300 ± 460 mg/100g. The mean values for small and large species showed great variations with a mean content of 100 ± 200 mg/100g for large species, and 960 ± 590 mg/100g for small species. These data suggest that small species consumed with bones, head, and viscera intact may be a considerably better source of calcium than other larger species where only the filet is consumed. For the mineral phosphorus, a comparable correlation was observed, where small species traditionally consumed whole contained considerably higher concentrations than large species (700 ± 240 mg/100g and 310 ± 96 mg/100, respectively). A similar variation between large and small species was also observed for iron, where the mean iron content was 3.3 ± 2.5 mg/100g and 0.51 ± 0.3 mg/100g for small and large species, respectively. For iron, a considerable variation amongst the small species was also observed, in which *Leignathus dussmuieri* ranged far above all other species with a peak value of 10 ± 1.2 mg/100g. Furthermore, the total zinc content ranged from 0.27 ± 0.01 mg/100g in the large species *Lethrinus olivaceus* to 3.0 ± 0.1 mg/100g in the small species *Leiognathus dussumieri*, also presenting a substantially higher mean content in small species compared to large species (2.1 ± 0.6 mg/100g and 0.4 ± 0.2 mg/100g, respectively). Morover, the only nutrient in which the mean value for large species exceeded those of small species, was for potassium, where the mean value was 470 ± 46 mg/100g for large species, and 370 ± 40 mg/100g for small species. Overall, the small species *Leiognathus dussumieri* ranged over all other species with the highest calcium, iron, iodine, phosphorus, selenium, and zinc content, and the second highest magnesium content.

Table 9: Analytical values of the mineral content in the 19 species sampled from Sri Lanka[a].

Species		Calcium	Iron	Iodine	Magnesium	Phosphorus	Potassium	Selenium	Sodium	Zinc
		Ca	Fe	I	Mg	P	K	Se	Na	Zn
	n[b]	mg/100g	mg/100g	µg/100g	mg/100g	mg/100g	mg/100g	µg/100g	mg/100g	mg/100g
Large fish										
Carangoides fulvoguttatus	3	17 ± 5.7	0.81 ± 0.02	160 ± 20	36 ± 1.7	310 ± 12	530 ± 15	3.7 ± 0.1	61 ± 1.7	0.5 ± 0.01
Diagramma pictum	3	23 ± 17	0.45 ± 0.04	470 ± 35	29 ± 0.6	250 ± 12	470 ± 12	46 ± 4	50 ± 1.5	0.4 ± 0.01
Lethrinus olivaceus	3	42 ± 39	0.22 ± 0.1	320 ± 100	31 ± 2.9	280 ± 40	510 ± 47	44 ± 1	44 ± 2.1	0.3 ± 0.01
Lutjanus lutjanus	3	65 ± 45	0.38 ± 0.04	97 ± 3	32 ± 1.2	260 ± 32	450 ± 15	43 ± 1	37 ± 2.1	0.3 ± 0.01
Nemipterus bipunctatus	3	490 ± 350	0.40 ± 0.2	130 ± 12	38 ± 3.5	500 ± 150	440 ± 70	39 ± 2	68 ± 20	0.6 ± 0.2
Selar crumenophthalmus	3	53 ± 58	1.1 ± 0.2	110 ± 10	36 ± 0.6	280 ± 23	430 ± 5.8	77 ± 5	60 ± 7	0.7 ± 0.3
Sphyraena jello	3	7.9 ± 0.5	0.21 ± 0.01	130 ± 10	34 ± 1.7	270 ± 10	490 ± 25	44 ± 2	42 ± 6.1	0.4 ± 0.01
Mean for large species		100 ± 200	0.51 ± 0.3	200 ± 140	34 ± 3.5	310 ± 96	470 ± 46	47 ± 10	52 ± 13	0.4 ± 0.2
Small fish										
Amblygaster sirm	3	500 ± 100	3.0 ± 0.2	1100 ± 0.0	63 ± 1.6	540 ± 58	390 ± 12	110 ± 10	290 ± 5.8	1.9 ± 0.1
Auxis thazard	3	550 ± 87	3.4 ± 0.2	160 ± 10	43 ± 1.7	540 ± 49	350 ± 17	83 ± 4	160 ± 12	1.6 ± 0.2
Decapterus macrosoma (1)	3	1100 ± 240	5.8 ± 1	220 ± 10	60 ± 3.1	740 ± 93	370 ± 15	230 ± 23	140 ± 5.7	1.8 ± 0.1

Table 9 continued

Decapterus macrosoma (2)	3	650 ± 170	3.6 ± 0.3	510 ± 50	48 ± 1.5	590 ± 84	380 ± 12	46 ± 1	170 ± 12	1.7 ± 0.2
Encrasicholina devisi	3[c]	550 ± 98	1.7 ± 0.1	740 ± 40	83 ± 7	510 ± 59	300 ± 5.8	56 ± 2	460 ± 17	2.4 ± 0.1
Equulites elongatus	3	640 ± 150	2.1 ± 0.1	360 ± 29	55 ± 1.2	560 ± 67	390 ± 5.8	46 ± 3	200 ± 5.8	2.7 ± 0.1
Leiognathus dussumieri	3	2300 ± 460	10 ± 1.2	1400 ± 58	75 ± 6.1	1200 ± 290	310 ± 15	88 ± 5	180 ± 5.8	3.0 ± 0.1
Photopectoralis bindus (1)	3	1300 ± 260	1.7 ± 0.4	300 ± 40	66 ± 3.8	910 ± 110	400 ± 5.8	38 ± 2	200 ± 5.8	2.6 ± 0.1
Photopectoralis bindus (2)	3	1000 ± 140	1.4 ± 0.1	700 ± 29	55 ± 1.2	760 ± 61	390 ± 12	38 ± 2	190 ± 5.8	2.5 ± 0.1
Rastrelliger kanagurta	3	490 ± 25	3.2 ± 0.2	390 ± 6	49 ± 0.6	520 ± 12	420 ± 10	53 ± 1	180 ± 5.8	1.3 ± 0
Stolephorus indicus	3	620 ± 55	2.0 ± 0.3	81 ± 3	51 ± 1.7	590 ± 36	440 ± 10	44 ± 0	160 ± 0	2.2 ± 0.1
Sillago ingenuua	3	1800 ± 440	1.6 ± 0.4	330 ± 50	52 ± 12	910 ± 270	360 ± 27	56 ± 6	150 ± 12	1.3 ± 0.2
Mean for small species		960 ± 590	3.3 ± 2.5	510 ± 380	58 ± 12	700 ± 240	370 ± 40	74 ± 52	210 ± 85	2.1 ± 0.6

[a] Values are reported as means ± SD of the 19 fish species analyzed in triplicates, expressed as the nutrient content per 100 g raw, edible sample.

[b] Number of composite samples analysed. For large species (>25 cm), 5 fish are included in each composite sample, while for small species (<25 cm), 25 fish are included in each composite sample.

[c] For this species, each composite sample consisted of 50 individual fish in order to obtain enough sample material.

Abbrevations: g: grams, SD: standard deviation, µg: micrograms.

4.5 Contribution to RNI

The various species' potential contribution to the RNI of calcium, iron, zinc, and vitamin A was evaluated in reference to 19-50-year-old women of reproductive age, and the results are presented in respective graphs in regard to both a Sri Lankan serving size of 30g of fish and a portion of 100g of fish. As illustrated by the RNI for calcium presented in Figure 5, several small species may potentially contribute with ≥100% of the daily RNI of 1000 mg/day when a portion of 100g of fish is consumed, but none of the specie reach the daily RNI when a singular Sri Lankan serving size of 30g is consumed. However, 5 of 12 small species were identified to potentially contribute with ≥ 30% of the RNI when a standard Sri Lankan serving size is consumed. Furthermore, all of the small species (with the exception of *Rastrelliger kanagurta*) may contribute with at least 500 mg of calcium when a portion of 100g is consumed, thus accounting for ≥ 50% of the RNI. Two of the small species, *Leiognathus dussumieri* and *Sillago ingenuua*, were also identified to potentially contribute with over 50% of the RNI when a 30g portion is consumed.

The results for zinc are presented in Figure 6. The RNI of zinc for the example group with an assumed low dietary bioavailability is estimated to be 9.8 mg/day, a value that far exceeds the calculated contribution from any of the species. However, 3 species (all small fish): *Leiognathus dussumieri*, *Equulites elongatus*, and *Photopectoralis bindus (1 and 2)* were identified that would potentially contribute ≥ 25% of the daily RNI for zinc in a 100g portion, while a single Sri Lankan serving size was estimated to contribute ≤10% of the RNI for all species.

The assumed RNI of iron of 29.4 mg/day is also a value that far exceeds the contribution of any single species of fish (Figure 7). For iron, the species with the highest iron content (*Leiognathus dussumieri*) would potentially contribute with approximately 10% of the daily RNI in a standard portion of 30g, while a 100g portion may account for ≥ 30 % of the RNI.

The results for vitamin A_1 are presented in Figure 8, and as illustrated in the graph, the small species *Leiognathus dussumieri* contains by far the highest content of vitamin A_1 of the species. Even a single serving of 30g/day of this particular species may ensure the recommended safe intakes of 500 µg RE/day for the reference group. Several other small species may also contribute substantially with a little under 50% of the recommended safe intakes, while all large species were identified to contribute minimally.

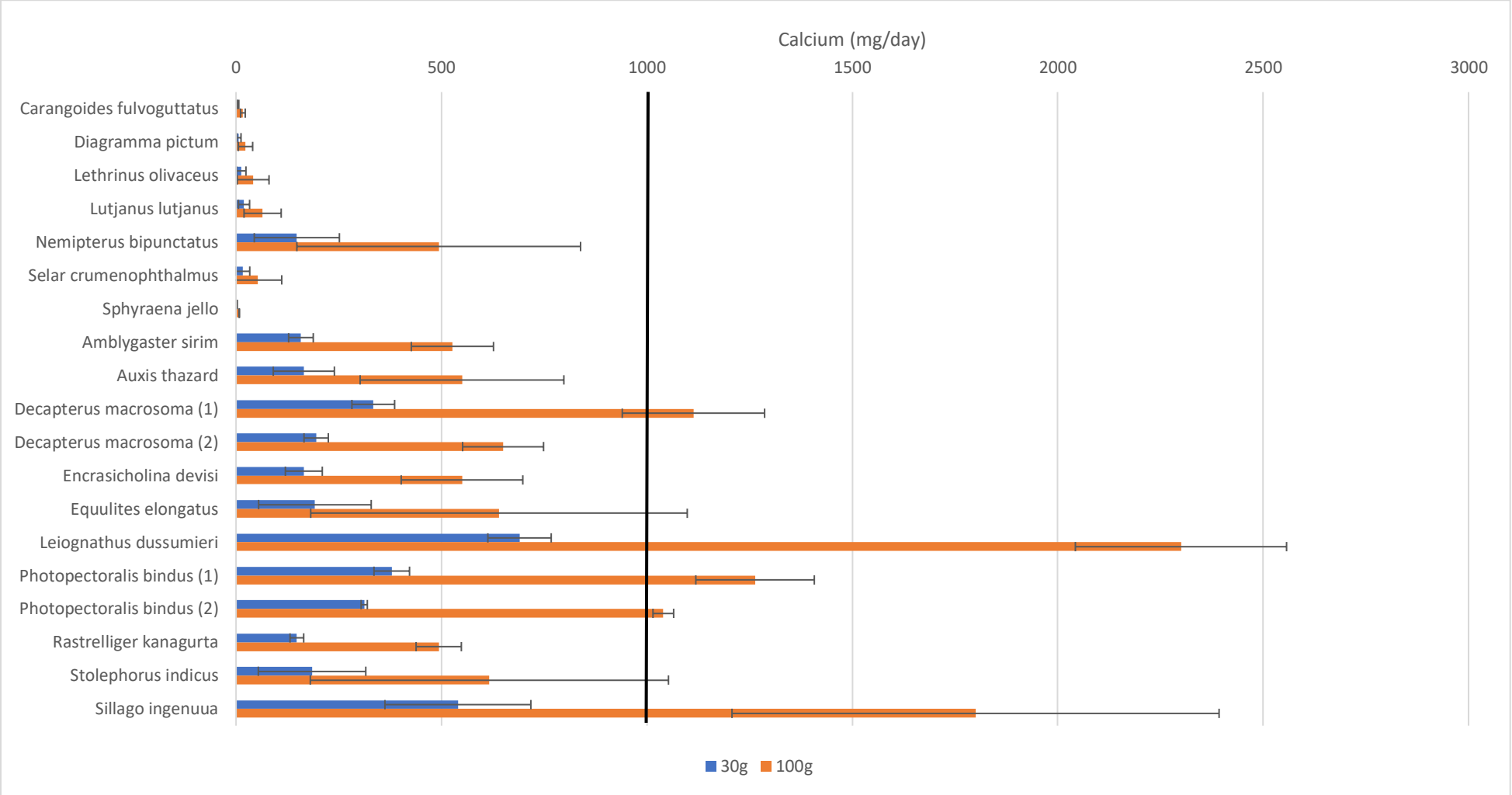

Figure 5: The various species' calcium content in one serving size of 30g and a portion of 100g in reference to the average recommended nutrient intake (RNI) of 19-50-year-old women of reproductive age. The recommended nutrient intake of calcium for this group is estimated to be 1000 mg/day, as indicated by the bold line, and the whiskers represent the standard deviations of the means.

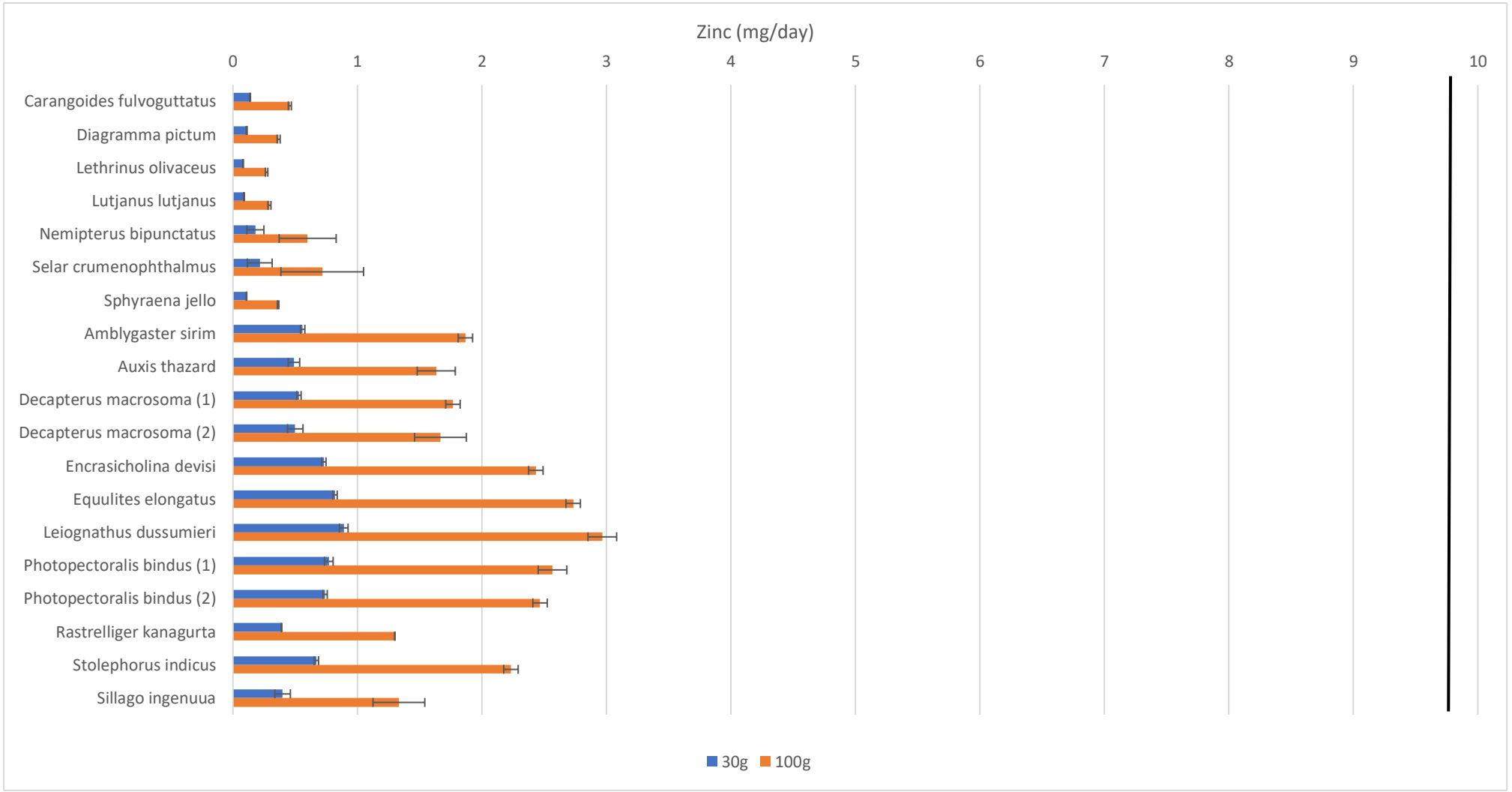

Figure 6: The various species' zinc content in one serving size of 30g and a portion of 100g in reference to the recommended nutrient intake (RNI) of 19-50-year-old women of reproductive age. The recommended nutrient intake of zinc for this group with an assumed low (15%) dietary bioavailability is estimated to be 9.8 mg/day, a value that exceeds the contribution from the serving sizes of any single species. The whiskers represent the standard deviations of the means.

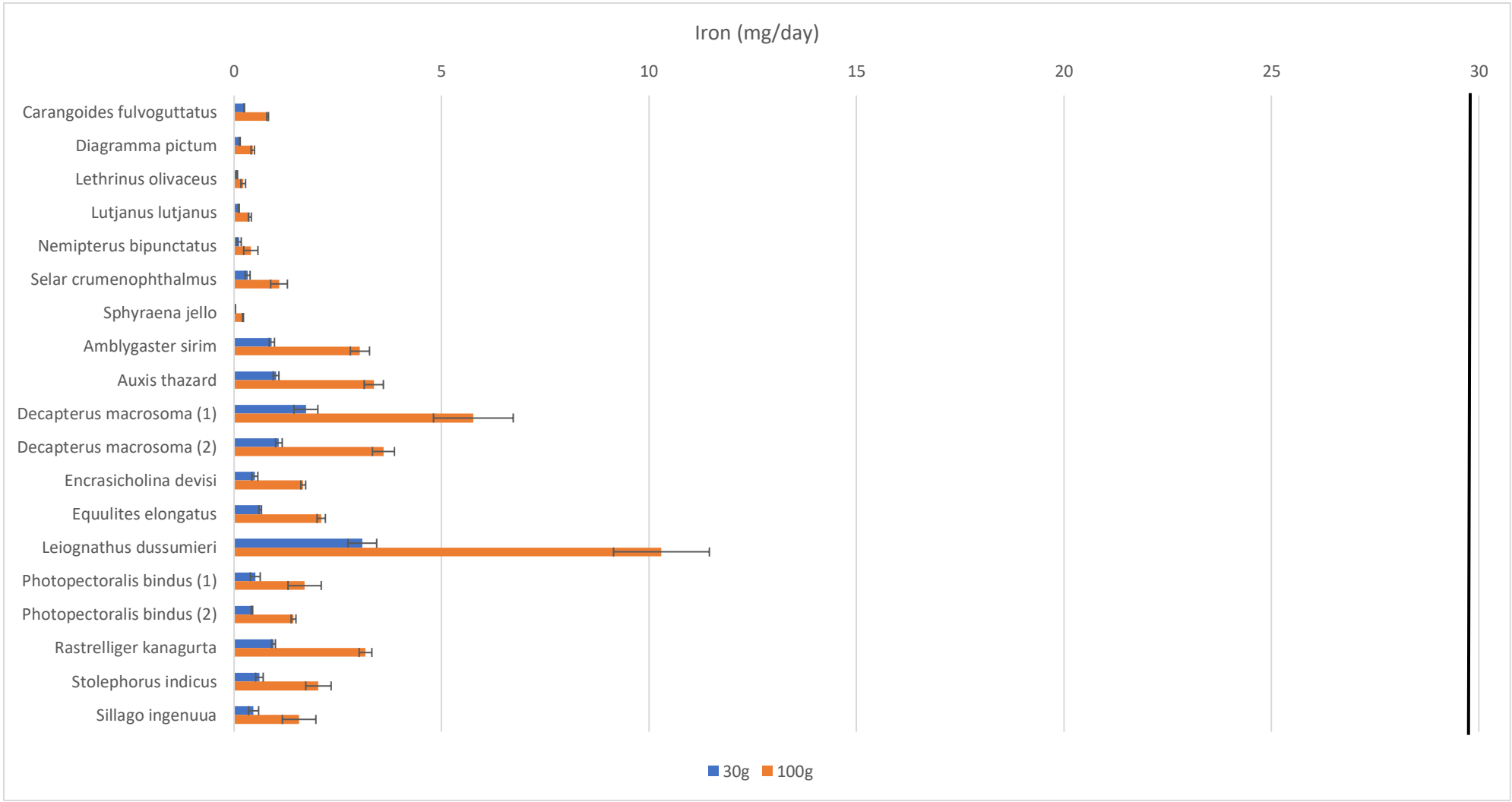

Figure 7: The various species' iron content in one serving size of 30g, and a portion of 100g in reference to the recommended nutrient intake (RNI) of 19-50-year-old women of reproductive age. The recommended nutrient intake of iron for this group with an assumed low (10%) dietary bioavailability is estimated to be 29.4 mg/day, a value that far exceeds the contribution from any serving size of any of the species. The whiskers represent the standard deviations of the means.

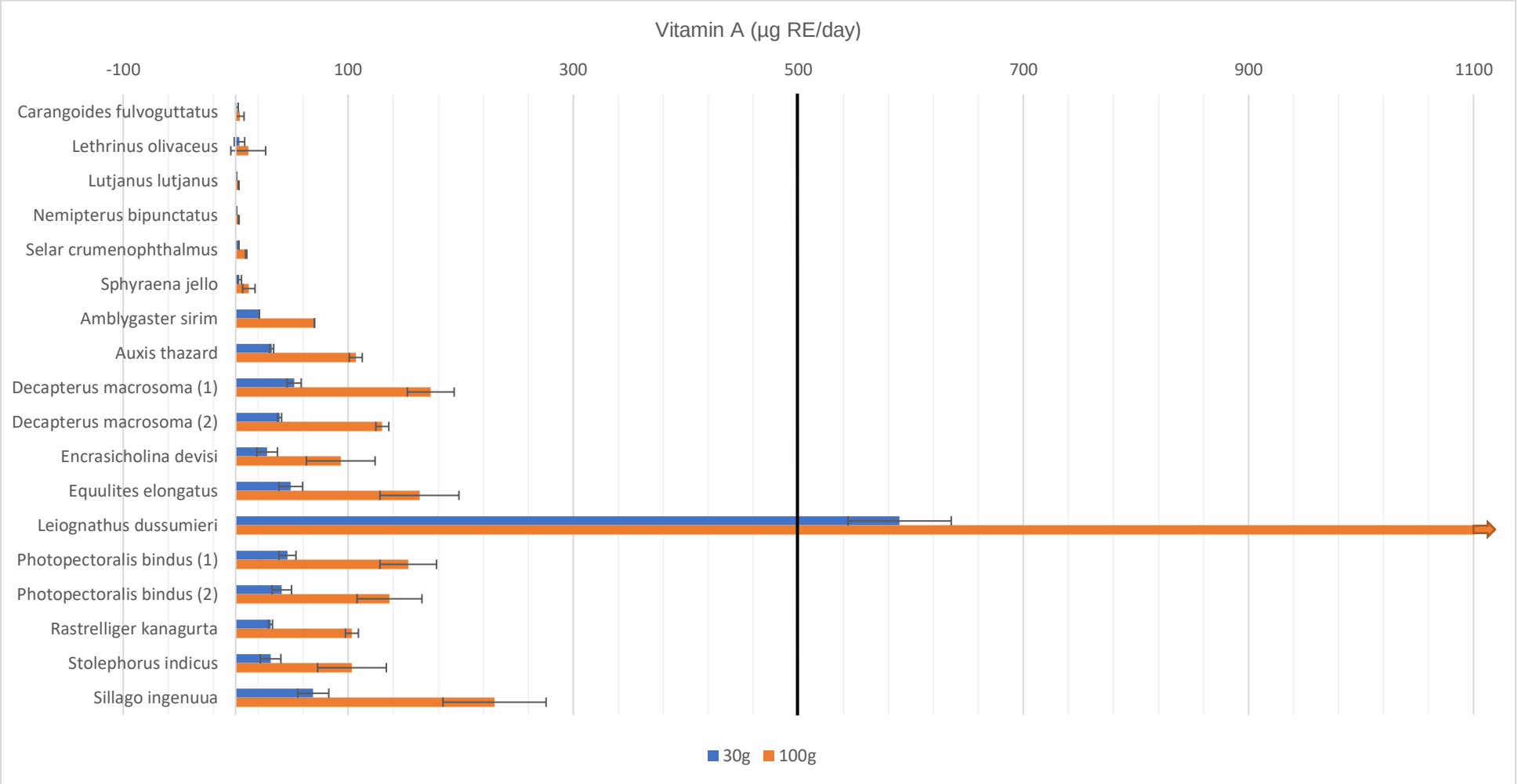

Figure 8: The various species' vitamin A_1 content in one serving size of 30g and a portion of 100g in reference to the recommended safe intakes for 19-50-year-old women of reproductive age. The recommended safe intake of vitamin A_1 for this group is estimated to be 500 µg retinol equivalent (RE)/day (where 1 µg retinol (A_1) = 1 RE), as indicated by the bold line. The small arrow implies the continuation of the line beyond the axis, and the whiskers represent the standard deviations of the means.

5 Discussion

The overall aims of this thesis were to generate and document comprehensive information on the nutritional composition of several commonly consumed fish species caught off the coast of Sri Lanka, and to fill current knowledge gaps by presenting analytical data on the nutrient profiles of these species. This was facilitated through the EAF-Nansen Programme, which emphases an ecosystem approach to assist developing regions in managing their aquatic resources in order to, inter alia, reduce poverty and improve FNS (87, 88, 90). The following chapter will firstly discuss the results and compare them to previous findings. Subsequently, methodological strengths and limitations will be discussed.

5.1 Discussion of results

To the best of my knowledge, very few studies have been conducted on the quantification of the micronutrient content in fish commonly consumed in LMICs. This is currently the first study where the vitamin A, -B_{12}, and -D content in marine fish in Sri Lanka have been analyzed; nutrients that are of particular public health significance given the prevalence estimates of vitamin A deficiency in the country (15), and the clear negative health consequences of deficiency, including growth disturbances, xerophthalmia, susceptibility to infections, and increased mortality (107, 109, 110). Furthermore, only one study from Sri Lanka evaluating a more comprehensive nutrient composition than the proximal composition of marine fish, and to some extent the fatty acid profile, was discovered in the scientific literature. A very limited amount of studies evaluating the nutrient composition of the specific species included in this thesis exists, thus comparisons of the results have to be made across borders. This may include species with different habitats, environment, and feed/diets. Additionally, the assurance of analytical quality is varying in different countries (111, 112), thus making it challenging to compare exact results.

5.1.1 Vitamin content

Due to the fat-soluble nature of both vitamin A and vitamin D, fatty fish filet typically contains greater concentrations than lean fish, in which the majority of these vitamins are concentrated in the liver (27, p. 84, 72, 80, 113). However, the results presented in this study somewhat deviate from this general assumption, where there seemed to be a random variation in the vitamin D content between species with no clear pattern attributed to neither medium-fat or lean categorizations nor size categorizations, and where even lean species contained

remarkable amounts of vitamin D. As previously stated, inter-species variation in vitamin D content exists, and intra-species variation may be of a similar magnitude (106). It should also be mentioned that neither of the sampled species in this thesis had a particularly high fat content. Plausible explanations may be variations in diet, age, sex, season, and climate, as debated in other studies where the vitamin D content in fish were not in line with the general assumption (113-115). Variation in dietary composition is arguably the most important factor, as zooplankton and phytoplankton are the only known sources of vitamin D in the diets of fish, and the amount and quality of the cholecalciferol in plankton varies based on solar radiation, thus making the effect of climate important as well (114, 116).

For vitamin A, a clear pattern was observed, where small species contained substantially higher concentrations compared to large species. This pattern was also seen in a study from Roos et al. (117), where reported values for vitamin A (both vitamin A_1 and A_2) ranged from 2680 RE/100g in the small indigenous species (SIS) Mola (*Amblypharyngodon mola*, analyzed and commonly consumed whole) to <30 RE/100g in the edible parts (only the filet) of larger cultured species in Bangladesh. These exceptionally high concentrations of vitamin A also correspond to the high levels discovered in the small species *Leiognathus dussumieri* in this thesis, and may imply caution of the consumption of the species over longer periods to avoid toxicity, especially for vulnerable groups like pregnant women (107). Roos et al. (117) further reported that >50% of the vitamin A in Mola was concentrated in the retina of the fish, while another 40% was concentrated in the viscera, which may explain the findings in this thesis were small fish that had their head and viscera included in the analysis had a substantially higher content of vitamin A. They also discovered varying concentrations of the two vitamin A isomers among species; some species contained >60% mean vitamin A_1, while others contained >60% mean vitamin A_2. In this thesis, almost all of the large species presented values <LOQ for vitamin A_2, while small species where the liver and retina were included in the analyses presented considerably higher values of vitamin A_2. The results presented in this thesis also suggest that the general assumption of fatty fish being the most significant source of vitamin A is not necessarily accurate, and that which parts of the fish are consumed may be an even more important factor than the fat categorization of the fish. Little data on vitamin B_{12} in fish is available for comparison in the literature, thus no further comments or comparisons were available for this vitamin.

5.1.2 Mineral content

A significant variation in mineral content between small and large species was clearly documented in this thesis. Although the majority of existing studies evaluating small species have assessed the nutrient content in SIS, and not marine fish, the results of this thesis correlate with those of other studies from neighboring countries where small species have been analyzed as a whole. Bogard et al. (118) analyzed the nutrient composition of 30 SIS and some larger inland carps from Bangladesh, and reported a remarkable richer micronutrient composition in the smaller species compared to the larger ones. The results of this thesis suggest that small marine species prepared whole may be equally as nutrient-dense as these SIS. In comparison, several of the small marine species included in this thesis presented greater values for calcium, iron, phosphorus, magnesium, potassium, and sodium than the SIS reported in the study.

The high content of several important minerals in small species may be naturally attributed to the inclusion and exclusion of various fish parts (bones, skin, head, viscera, etc.,) in the analyses, and which parts of the fish are consumed is therefore of great importance for FNS, as argued by Bogard et al. (118). For example, approximately 99% of the accumulated calcium and 80% of the phosphorus is stored in the bones, teeth, and scales of the fish, while the remaining 1% is distributed throughout the organs and tissues of the fish (83, 119). Small soft-boned species are generally eaten with bones, where the calcium in previous studies have been confirmed to be of high bioavailability (76, 120), and may therefore be considered a rich source of calcium in comparison to larger species where the bones are discarded as plate waste and commonly not eaten (121). The importance of minimizing plate waste was accentuated in a Sri Lankan study from 2012, where five species of tuna (amongst them *Auxis thazard,* which was also included in this thesis) were analyzed for a variety of nutrients. According to the results, the skin of all species of tuna contained the highest levels of potassium, calcium, zinc, and magnesium, and they therefore concluded with the importance of consuming various parts of the fish to reap the most nutritional benefits (122). Furthermore, Roos et al. (123) reported a 60% loss of total iron in the Cambodian SIS, *E. longimanus,* when the head and viscera were removed, concluding that it is of absolute importance that the poor, and particularly vulnerable groups like women of reproductive age, optimize the use of locally available nutrient-dense foods.

5.1.3 Direct comparison of the sampled species

Of studies evaluating micronutrients in any of the species included in this thesis, three Indian studies were discovered *for Rastrelliger kanagurta*, and one for *Leiognathus Dussumieri*. Palani et al. (124) reported very low calcium, iron, and phosphorus values for *Leiognathus dussumieri* (90.41 ± 1.8, 2.66 ± 0.11, and 190.27 ± 0.27 mg/100g, respectively; all analyses conducted in accordance to AOAC standards from 1990), compared to the extremely high values observed in this thesis. One plausible reason for this, in addition to natural intra-species variation, is the method of preparation where the fish were beheaded, gutted, and filleted, thus excluding the nutrient-dense bones, scales, and teeth of the fish. The other species evaluated in the study, *Rastrelliger kanagurta*, presented substantially higher calcium values, similar iron values, and substantially lower values for phosphorus (1170.9 ± 0.89, 2.33 ± 0.08, and 86.91 ± 2.47 mg/100g, respectively) than those reported in this thesis. Furthermore, of the two additional studies analyzing the mineral content in *Rastrelliger kanagurta*, one reported higher calcium and potassium levels (680 mg/100g and 750 mg/100g, respectively; analyses conducted in accordance to AOAC standards from 2000) in the filets (125), while another reported higher iron and potassium levels (5.0 ± 0.1 and 2397 ± 0.2 mg/100g, respectively), lower sodium levels (107 ± 0.1 mg/100g), and substantially higher zinc levels (13.0 ± 0.1 mg/100g) than those reported in this thesis (126). It is challenging to further directly compare the results of the species included in this thesis with existing values due to the limited amount of studies that exists, and, in general, very few studies using whole fish (as consumed) for analyses.

5.1.4 Contribution to recommended nutrient intakes

Bogard et al. (118) evaluated the contribution of various SIS and some larger marine and freshwater species to the RNI of pregnant and lactating women and infants, and their results showed that several of the smaller species could potentially contribute with ≥25% of RNI of three or more micronutrients for both groups when a standard portion (50 g/day and 25 g/day, respectively) is consumed, but only one of the larger species was identified to do the same. These results are in line with the results of this thesis, were all small species were identified to potentially contribute substantially more than larger species to RNI of all micronutrients for women of reproductive age. However, all species enhances the bioavailability of minerals like iron and zinc from cereal- and tubers based diets, thus including even small amounts of fish in the diet may enhance overall micronutrient bioavailability (26, 107, 127-129). Karunaratne et al. (130) reported the highest value for zinc in a standard Sri Lankan rice meal accompanied

by dried anchovies, while also presenting one of the lowest phytate : zinc molar ratios (an estimate of zinc bioavailability (105; p. 95)) in comparison to the other composite meals where various vegetable curries and soy products were included to supplement the rice. With >50% of the meals presenting a high level of phytate : zinc molar ratio (>15), this study also supports the assumption of a low dietary bioavailability of zinc in the Sri Lankan diet, as characterized by, inter alia, a phytate : zinc molar ratio of >15 and approximately 50% of the energy content accounted for by the staple food rice (105; p. 95), but more comprehensive dietary studies are needed to confirm this.

5.1.5 Fish for food and nutrition security in Sri Lanka

Small species often have a significantly lower market value than larger species, and are therefore assumed to be more easily accessible and commonly consumed in poor households, thus playing a very important role for FNS by diversifying the diets of the poor (26, 127, 131). This was demonstrated by a dietary diversification intervention study by Gibson et al. (132) aiming to investigate the effects of increased fish consumption on a variety of indicators for FNS in young children in rural Malawi. They reported that individuals fed the intervention diet containing significantly more whole, dried fish with soft bones had lower incidences of both anemia and common infectious illnesses compared to the control groups consuming high-phytate diets where > 50% of the energy was derived from the staple food maize. Furthermore, despite no mention of the preparation/processing of the species, Tacon et al. (133) reviewed the nutritional composition of both small and large pelagic fish species from the USDAs National Nutrient Database, and assessed small pelagic species nutritional superior with reference to a very high level of micronutrients (calcium, iron, magnesium, potassium, zinc, copper, selenium, vitamin A, -B_{12}, -D, and EPA and DHA) compared to the larger species. They further concluded that the use of small pelagic species for animal feed should be minimized, thus enabling the promotion of small pelagic species for human consumption to be sustainable, especially for the benefit of people that are food insecure.

Amongst food-based strategies to improve FNS in LMICs, dietary diversification is recognized as a long-term sustainable and cost-effective solution, and have the natural advantage of being suitable for several micronutrients (31, 134, 135). As evidenced by this thesis, fish is a great source of several micronutrients; some species more than others. The availability of accurate FCD is therefore absolutely essential to successfully apply such strategies, and as the results presented in this thesis show, increasing the consumption of fish

may be a great way to nutritionally diversify diets otherwise dominated by staple foods. Small fish species as a source of micronutrients have been given little attention so far, but due to their high nutrient content, affordability, and increased storage potential (due to common local processing methods), could be used as a key component in strategies aimed at reducing micronutrient deficiencies and improving FNS in developing countries like Sri Lanka (26).

5.2 Methodological considerations

5.2.1 Sampling methods

A strength of this thesis is the strong record of sampling and processing, in which comprehensive information on the identification details (scientific taxonomic names, alternative names, parts of the fish included, date and time, weight, length, and sex) and handling details (methods of preparation, seasonal and geographical information of sampling, transport conditions, and storage conditions) are provided in accordance to the guidelines laid out in "*Food Composition Data"* by the FAO (60). A limitation is the lack of information on the state of maturity of the species sampled (60; p. 69-78, 105; p. 87), which to the best of my knowledge is the only descriptive factor not included in this thesis due to insufficient skills and experience in the particular field. The nutrient composition of foods, including fish, is as mentioned previously known to vary with season, environment and maturity, but it was outside the scope of the thesis to attempt to sample to account for such variations (60; p. 19-20, 61). Another limitation related to the sampling is the number of sub-samples included per analytical sample, which according to Greenfield and Southgate should be calculated from the variance of the nutrient composition of the food product in order to achieve means with reasonable levels of confidence (60; p. 74, 214-215). This was not performed in this thesis; however, all analytical values were based on samples consisting of at least 15 individual fish. This is in accordance with most standards where at least ten units are used to reflect the variability in composition, but optimally, a higher number of samples are required for certain nutrients because of the intra-species variability present in various foods (60; p. 74, 105; p. 76).

The species sampled during the survey were selected if the local scientists on shift all agreed that the particular species was commonly consumed, and/or if the species were in either of the lists of common commercial species. The lists were printed on board the ship a week into the survey (with very limited internet access to do extended research) to accelerate the process of

sampling and alleviate the author's reliance on the local scientists in the midst of a hectic working environment. Optimally, lists of commonly consumed fish species based on for example information from national consumption surveys or market surveys, should have been pre-printed prior to the survey. However, when returning to Norway and researching the matter, I was not able to discover any such lists, nor any additional information on commonly consumed species beyond the two lists utilized during the survey. This may partially be due to the vast diversity of species commonly consumed in Sri Lanka compared to many other countries (136), and due to the lack of national dietary surveys.

A further consideration is the trawling locations of where the species were sampled from, of which some locations may be further out at sea than where local fishermen operate, but at the same time within range of where larger commercial fishing vessels maneuver. The ratio of supply from various fishing vessels (and more primitive methods) to markets and/or households may therefore be of great importance when evaluating the sample selection, which was not further assessed in this thesis. However, a number of other studies confirm several of the species included in this thesis, specifically *Amblygaster sirm, Auxis thazard, Rastrelliger kanagurta*, *Stolephorus indicus*, and *Selar crumenophthalmus*, as the most dominant and commercially important small pelagic species in Sri Lanka, accounting for approximately 40% of the total 60% share of the entire coastal fish catch (47, 137, 138), which may be viewed as an indicator of their dietary importance in the country.

A limitation is however the lack of scientific data to confirm the consumption patterns and preparation methods of small fish species in the Sri Lankan population, as all FCD ought to be presented on the basis of per 100g edible portion of food (60; p. 166-167), or the forms most commonly consumed (60; p. 14). Although it is known on a world-wide-basis, and particularly in LMICs, that small pelagic species generally are processed, sold, and consumed whole without the removal of heads, bones, and viscera (25; p. 114-116, 26, 121, 139, 140), I was not able to discover any studies or scientific articles confirming this statement for Sri Lanka. However, several studies from the neighboring countries Bangladesh and Cambodia confirm the consumption of small species (< 25 cm), although SIS, as eaten whole (76, 117, 123, 127, 141). This, the verification from the local scientists on board during the survey, and the Sri Lankan FBDG explicitly encouraging the population to consume small species whole, indicates the consumption of whole, small fish as a common practice in Sri Lanka as well (59). The decision to prepare and analyze the species as commonly consumed may therefore

be viewed as a strength for this thesis, as, to the best of my knowledge, direct analyses of the complete edible portion of small marine species have not yet been performed to a great extent.

For this thesis, it was decided to use composite samples for all analyses. Using either composite or individual samples present various strengths and limitations. Pooling is similar to averaging, thus reducing the effect of the biological variation present in each sample. Data on the sample variance is important to identify the sample distribution, and to obtain information about any extreme values, which can be useful for nutrients and/or contaminants where a set maximum value for human consumption exists (142). More studies examining the quantitative amount of the sampling size needed to obtain a representative size that accounts for the natural variation present in foods are also needed (60; p. 204). Another important argument is that individual samples enable direct adjustments for possible confounding factors such as length, weight, and maturity. However, the major argument for using composite samples is that they significantly reduce the financial costs (60; p. 79, 143). Additionally, pooling samples was part of the original protocol for small species as a means to obtain enough sample material to conduct the chemical analyses, something that could not have been done through the use of a single individual (143). It is however considered an advantage that if retrospective studies on the within-specimen variance and distribution of the species in this thesis are a priority to be made at a later time, individual samples for all large species are stored at the IMR and available for analyses.

5.2.2 Analyses and calculations

In agreement with the "*Food Composition Data"* guidelines, all data presented express the same modes- and bases of expression (per 100g wet weight), have undergone the same rounding procedures, and are listed to an appropriate number of significant figures for each nutrient value as specified by the respective analytical method and as recommended in the guidelines for each nutrient (60; p. 165-166). Furthermore, the data presented in this thesis may be considered high-quality data due to the use of accredited and reliable methods of analyses performed at a national reference laboratory for the particular food matrix (fish), and that the laboratory satisfy criteria of good laboratory practice, which are all in compliance with the international guidelines (60; p. 164-169, p. 14., 104, 105; p. 76, 144). The analytical data reported in this study may therefore represent an important contribution to the future compilation and further development of the Sri Lankan FCT/FCDB.

There are several different methods for handling values < LOQ (145), but for this thesis a decision was made to simply present the unadjusted LOQ value for the given nutrient. When the value of a nutrient is below the LOQ, it simply implies that very low concentrations could be detected, but not reliably quantified (146). Although the real concentration is an unknown value, we do know that the concentration is very low, something that should be reflected within the analysis by expressing the LOQ value. Nevertheless, I would argue to say that for this study, quantifying very low values are not of interest as they have very little impact on the total dietary consumption of the nutrient.

When evaluating nutrient-intake data for population groups, it is recommended to use the estimated average requirement (EAR). This is because the RNI by definition is set at an intake level that exceeds the requirements of 97-98% of all individuals. However, the goal of the calculations in this thesis were to demonstrate the various species' potential contributions to the nutrient requirements of a healthy individual in an age- and sex-specific group, and not to assess the adequacy of actual intake and risk of nutrient inadequacy for the group as a whole. Thus, the RNI was used for reference values, implicating that species presenting values at or above the RNI indicates a low risk of inadequacy granted usual intakes over time (105). Due to no recommended dietary allowances for Sri Lankans being available, the FAO/WHO "*Vitamin and mineral requirements in human nutrition"* were used for reference values (107). Furthermore, the particular group of non-pregnant, nor lactating women of reproductive age was selected as the example group due to their increased vulnerability to micronutrient deficiencies such as iron (41, 42), in addition to the importance of the maternal nutritional status for both the periconceptional period and for any (potential) future pregnancy outcomes (147, 148). However, it is also recognized that several other vulnerable population groups could have been selected for the demonstration of the species' contribution to the RNI.

Due to the lack of consumption data on the average daily fish consumption in Sri Lanka, an assumption of the daily serving size of fish had to me made. A daily portion of 30g of fish was derived from the Sri Lankan FBDG, which recommends 3-4 daily servings of foods from the food group "fish, meat, eggs, and pulses", where 30g of cooked fish or 15g of dried fish is categorized as one serving (59). From this, one can assume an average of 1-2 servings of fish daily (supposing the advice is followed), a number that is also supported in the HIES report from 2016, where the mean daily consumption of fresh fish per person (both marine and freshwater) was approximately 33g, while mean daily consumption of dried fish was 10g

(55). It was therefore decided to include both a lower limit of a 30g serving size, and an upper serving size of 100g, as endorsed by Kawarazuka et al. (26), to illustrate any differences. Furthermore, it is also known that the method of preparation/processing (sun-drying, fermenting, boiling, smoking, etc.) influences the supply of nutrients (60; p. 40-41, 105; p. 87) and the weight and volume of the food (105; p. 68). Additionally, when using food composition values to estimate the potential RNI, it is important to be aware that such values indicate the total amount of the nutrient in the food, rather than the amount actually absorbed (105; p. 65). The bioavailability of nutrients in most foods have not yet been quantified, and these factors were therefore not considered in the calculations for this thesis, where only values for raw, unprocessed fish were estimated, and an assumption of 100% bioavailability was presumed.

6 Conclusions

The results of this thesis suggest that small species may be an important source of several micronutrients such as calcium, iron, zinc, vitamin A, vitamin B_{12}, vitamin D, and the essential fatty acids EPA and DHA, and that the content of these micronutrients are substantially higher in small species consumed whole, in comparison to filet from large species. A plausible explanation for these differences is the way small species are consumed, namely, whole with, heads, bones, and viscera intact. Several species have been identified to have the potential to contribute substantially to the RNI of women of reproductive age for multiple nutrients important for FNS in Sri Lanka, of which the small species *Leiognathus dussumieri* presented noticeably higher levels of most micronutrients compared to other species. Improved knowledge through enhanced and up-to-date FCD of commonly consumed fish species will allow for the promotion of particularly nutrient-dense fish to be used in food-based strategies to combat common deficiencies in the country. However, as evidenced by this thesis; a wide range of nutrients are present in various concentrations in different species, thus a diverse diet promoting an all-inclusive nutrient intake is preferred to improve overall FNS in Sri Lanka, as even small amounts of fish in the diet can diversify diets otherwise dominated by staple foods. The analytical data presented here may represent an important contribution to the future development of the Sri Lankan FCT/FCDB.

7 Future perspectives

There is a need for future studies assessing the nutrient composition of the marine resources available in Sri Lanka. Because of the vast diversity of species, national consumption surveys identifying the most commonly consumed species and generally more data on fish consumption per capita, are necessary to narrow the initial compilation of FCD, but more studies are evidently also needed on the nutrient composition of all commonly consumed species. This will also enable more accurate calculations of the present and potential contribution of fish to the RNI of various population groups and facilitate the further development of specific dietary interventions and guidelines.

This thesis provides data on fish in their raw state, but data on the species in their processed state as prepared for consumption is also fundamental. Further development of the Sri Lankan FCT/FCDB should therefore also include analytical values for the species as prepared for consumption for improved representativeness and compatibility (60; p. 40). Furthermore, as seafood contains a range of both beneficial nutrients and several contaminants such as methylmercury and dioxins, which may lead to adverse health outcomes, a risk-benefit analysis is a necessity. In general, there is an agreement that the positive health effects of a higher consumption of fish largely outweigh the potential negative effects associated with contaminants (23, 149), but data on various contaminants should also be incorporated in the further development of a comprehensive Sri Lankan FCDB (60; p. 54-61).

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Appendices

Appendix I: Sampling protocol of fish for determination of nutrients and contaminants on the Nansen cruise.

Appendix II: Sampling and handling protocol for small fish species.

Appendix III: Sampling and handling protocol for large fish species.

Appendix IV: List of common commercial marine fish species found in Sri Lanka (92).

Appendix V: Additional list of common commercial marine fish species found in Sri Lanka (91).

Appendix VI: Trawl form for small fish species used during the Nansen survey around Sri Lanka.

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Appendix VIII: Scientific and English names, coordinates, and station numbers of the locations of where the samples were collected during the 2018 Nansen survey around Sri Lanka.

Appendix I:

Sampling fish for determination of nutrients and contaminants on the Nansen cruise 2017

Innhold

Introduction

Samples of fish taken on the Nansen cruise will be analysed for a range of nutrients in order to document the importance of these fish species in food security. In addition, they will be analysed for contaminants in order to ensure food safety. It is important to assess whether marine pollution affects food safety and the marine environment in general.

The samples will therefore be analysed for many different nutrients and contaminants, with advanced analytical methods at NIFES' laboratories in Norway. Sampling the fish and homogenising and freeze-drying the sample material will be done on board the ship. These are essential steps of the analysis which can significantly affect the results, and it is therefore very important that this is done properly. It is also important that as much relevant information as possible about the fish is registered.

Ideally and preferably the fish is processed immediately following sampling. However, too much freezing and thawing can affect the levels of some nutrients. But if is not possible, the fish can be frozen after sampling, then be partially thawed and processed later. The third alternative is to freeze the fish until there are NIFES people on board who can process the samples.

This document is meant primarily as background reading material. For a more hands-on description of the work to be done on board the ship, see separate protocols for small fish and large fish, respectively.

Definitions

Sampling position: The geographical area where the fish to be sampled were caught, given by geographical coordinates. The coordinates may represent the place where a trawl haul was pulled in. Sometimes in the open sea a position may represent a relatively large area, e.g. an area with a radius of 2 km.

Sample: The object to be described through the analysis – for example this may consist of muscle tissue from one fish or a pool of 25 whole fish.

Fillet: Soft tissue between skin and bone, consisting of skeleton musculature and fatty tissue. This is the part of the fish that is most often consumed. It contains both brown and white muscle. A fillet can be with or without skin. It is important that as much as possible of the fillet is sampled from each fish.

Fillet with skin and bone: Whole fish from which the internal organs have been removed and the head and tail have been cut off.

Composite or pooled sample: A sample composed of tissue from several fish. The objective here is to ensure enough material for all the analyses and that each sample is representative of the sampled population.

Homogenising: Grinding and mixing a sample until the composition is identical throughout.

Homogenous sample: If the sample is split into several parts, the composition of each part will be identical.

Aliquot: A representative part of a sample, separated out for further analysis.

Secondary sampling: When there are large composite samples, an aliquot of the composite sample is taken for further analysis. Secondary sampling should take place according to regular routines to ensure representativeness.

Freeze-drying: Removing water from the sample using vacuum and low temperature in order to make a dry sample which is durable, and to determine water contents of the sample. Many analyses apply dried samples. Freeze-drying is done with a specialised apparatus, a freeze-dryer.

Sampling

The aim is to sample and analyse fish that are important in terms of volume and as a food source for people in the region. It is important that we sample edible tissues of the fish, i.e. the fillet of large fish and fillet with skin and bone or whole fish of small fish. However, in order to assess pollution levels we also want a tissue that is particularly sensitive to pollution. We therefore also take samples of liver of the large fish, although this is not directly a food safety issue.

For each species we want samples from two-three different sampling positions on each cruise leg. The positions should be well separated geographically.

The fish samples are separated into two main types: "Small" fish and "large" fish. Examples of fish species in the small and large categories as defined here are given in Table *1*.

Table 1. Overview of possible species in the small fish and large fish categories, tissue to be analysed, type of sample and the number of samples to be taken.

Species of interest	Tissue	Sample	Number of samples at each position
Small fish			
Pilchard, *Sardina pilchardus*	Whole fish	Composite of 25 fish	3 samples
	Fillet with skin and bone	Composite of 25 fish	3 samples
Anchovy, *Engraulis encrasicolus*	Whole fish	Composite of 25 fish	3 samples
	Fillet with skin and bone	Composite of 25 fish	3 samples
Round sardinella, *Sardinella aurita*	Whole fish	Composite of 25 fish	3 samples
	Fillet with skin and bone	Composite of 25 fish	3 samples
Flat sardinella, *Sardinella maderensis*	Whole fish	Composite of 25 fish	3 samples
	Fillet with skin and bone	Composite of 25 fish	3 samples
Large fish			
Horse mackerel, *Trachurus trachurus*	Fillet	Individual	25 fish
	Liver	Individual	15 fish
Atlantic chub mackerel, *Scomber colias*	Fillet	Individual	25 fish
	Liver	Individual	15 fish
Axillary seabream, *Pagellus acarne*	Fillet	Individual	25 fish
	Liver	Individual	15 fish

Small fish, e.g. anchovy and sardinella, ca. three species:

Small fish are so small that they are eaten either whole or whole without the head, tail and innards (fillet with skin and bone). One of these fish will provide too little sample material for all the planned analyses.

From one position and one small fish species, 6 samples containing 25 fish each will be taken. Each sample to be analysed contains a pool of 25 fish (pooled sample, or composite sample).

Two different composite samples are made:

1. Whole fish: 3 composite samples
2. Fish fillet with skin and bone: 3 composite samples

For each small fish, length should be measured, but for the weight it is sufficient to weigh the 25 fish together and write down both the total weight of the sample and the number of fish in it. Alternatively, average weight and length of fish in the catch can be obtained from the fish survey. If there are large fish scales they should be removed as much as possible, as they are not eaten.

Large fish, e.g. sardinella, horse mackerel, chub mackerel, etc. - 2-4 species

Large fish are fish that are large enough to be filleted and skinned individually, and where each fillet will provide enough material for all the analyses. For this, the fish should be at least 30 cm long.

From one position and one species of large fish, a sample of 25 individuals is taken and tissue from **each fish** is to be prepared and analysed separately

1. Skinless fillet from each individual fish
2. Liver from 15 of the 25 fish

In addition to the individual fillet samples, three pooled samples are to be prepared from subsamples of fillet tissue from the first 15 fish.

As much information as possible about each fish is registered. Length and weight is a minimum, sex if possible.

Samples of whole fish and of fillets are to be freeze dried (see below), but liver samples cannot be freeze dried due to the high fat contents. Liver samples must be frozen as they are. A sample of 30 g of wet fillet homogenate is also taken, since some of the nutrients are best analysed wet.

Preferably, sample preparation should take place immediately after sampling in order to preserve the nutrients as much as possible. However, in case there is little time immediately following the haul, the fish may be frozen until the samples can be prepared.

Fish handling

Small fish

Weight of each fish is not required, but total weight (g) of the sample and the number of fish must be noted. Length (cm) of each fish should be measured and written down. Alternatively, average weight and length from the catch can be obtained from the fish biologists.

Before preparing the samples, large scales should be scraped off, as these generally are not eaten.

1. Composite sample of 25 whole fish: Put all 25 fish into the food processor and homogenise.
2. Composite sample of fillets with skin and bone from 25 fish: Remove head, tail and internal organs. Clean the fillet with skin and bone with fresh water and put it in the food processor. Do this for all the 25 fish and start the food processor to homogenise.

When homogenising whole fish or fillets with skin and bone, it is particularly important to run the food processor for a long time to get a properly homogenised paste.

Large fish

For each individual fish, total length (cm), weight (g) and sex (m/f) must be written down.

For the first 15 fish, cut open the fish and take out the liver. This must be done very carefully to avoid leakage of the liver which may contaminate the fillet. If the fish has been frozen, the liver should be removed before the fish has completely thawed, because thawing increases cell damage and risk of leakage from the liver. Weigh the liver and write down the liver weight (g).

From these 15 fish, also take out the contents of the intestines. These must be weighed and freeze-dried.

Cut out the fillet from both sides of all 25 fish, as described below. Then homogenise and freeze-dry the fillet sample from each fish. After homogenisation, ca. 30 g sample is added to a tube which is

become homogenous than if it is just one tissue. Skinless fillet is very easy to homogenise at it is relatively homogenous already. If the fillet has skin it may be very difficult because the skin can be tough and hard to grind into small enough pieces.

Freeze-drying

After homogenising the sample, weigh in the necessary amount to be freeze-dried in a tared and labeled container. Write down the weight of the container and the weight of the wet sample. The sample should not be thicker than 2 cm. Put on a lid and freeze the sample. The sample must be frozen before it is inserted into the freeze-drier. When it is ready for freeze-drying, remove the lid, and put the sample inside the freeze drier and the process is started, see the user **instruction for freeze drier**, below. After 24 hours the plate temperature is changed to +25°C, see **instruction for freeze drier**.

The freeze-dryer should run for 72 hours or more. When the freeze-drying has finished the sample must be weighed, and remember to write down the weight (g). After weighing, the dried sample is homogenised once more. Freeze dried samples may draw humidity from the air. It is therefore important that the sample is placed in exicator cabinet if it is not weighed immediately after freeze-drying.

Calculation of dry matter and water content

Dry matter is determined according to the formula:

$$\% \text{ dry matter} = \frac{(c-b)\ x\ 100\%}{a-b},$$

where: a = weight of sample + container before drying (g), b = weight of container (g), c = weight of the sample + container after drying (g).

% water content = 100% – % dry matter

The result is reported with one decimal, and the reported unit is g/100 g.

Practical remarks

The freeze drier is maintained by wiping off shelves, door and capacitor and keeping them clean. The oil is changed after 6-12 months, depending on the colour of the oil. The oil should have a clear colour. If changing the oil, see user guidance for the vacuum pump.

After freeze-drying

After the samples have been freeze-dried, check that they are completely dry by breaking them in half. They should have a bisquit-like texture. Then homogenise to a fine powder and transfer to one or two pre-labelled 50 ml tubes. Vacuum-pack the tubes from one station and species, label the bag and store at -20°C. See separate instruction for the vacuum sealer.

User guide for the freeze drier

Start freeze drier

1. Turn on the two main switches on the left side of the freeze drier
2. Place baffle in the correct direction (se illustration)
3. Put the glass lid over the collector and plug the tube.
4. Check the oil level. It should be between min. and max.
5. Push "Man". Wait until the temperature of the collector is ca. -50°C. When all the lamps on the temperature curve are alight, the freeze drier is ready.
6. Check that the black handle on the vacuum pump is pointing straight upwards
7. Put frozen samples in the shelves, making sure that the wires in the inner part of the shelves and the temperature sensors are not in a squeeze
8. Close the door. Turn the handle "Vac Release" to position close.
9. Push "VACUUM"
10. Push "SET TEMP" and lower the shelf temperature using the arrow button to run -20°C
11. After 24 hours, change "Set temp" (shelf temperature) to +25°C using the arrow buttons.

Stop freeze drier

1. Turn off "VACUUM"
2. Turn the handle "Vac Release" to position "open"
3. Wait until the hissing sound stops before opening the door. Do not use power
4. Take out the samples
5. Unplug the tube, and check that the tube is hanging into the bucket. The water will run into the bucket
6. Turn off "Man"
7. Push "Defrost". It turns off automatically
8. Lift off the glass lid
9. Pull up baffle, and take out the ice lumps
10. Wipe off the rest of the moisture in the collector with paper
11. Wipe off the door and around the collector with glass wipe or methyl ethanol. Wipe off the shelves when needed
12. Leave the door open, the glass lid is set aside so the freeze drier can air off. Turn off the main switches.

Appendix II: Sampling and handling protocol for small fish species.

Small fish (e.g. sardine, anchovy)

Sample 3 x composite samples of 25 whole fish, and 3 x composite samples of 25 fillets with skin and bone. Sample 3 x individual whole fish for microplastics analysis. A composite sample should contain at least 25 individuals or 120 g wet sample material. Because of the small size of mesopelagic fish, you may need more than 25 fish to get 120 g.
Try to avoid direct sunlight on the samples for a long time. If necessary, cover with aluminum foil.

1. Collect 150 fish in a basket. Print the"TrawlformNifesSmallFish" for the correct station. . Also save the file at the portable PC. Alternatively, use a preprinted working sheet (make sure to get the position data correctly). Note the journalnumber on the form (look at tube/tray "2018-xxx").

2. If this species is weighed and measured on this station, you can have average numbers from this measurement. However, always weigh and write down the total weight of each 25 fish sample. If you don't get average length from the catch, you will need to measure 25 fish. Write down the length of each of 25 fish (on the reverse side of the sheet). Write the mean weight and length on the form.

3. You will need:
   - Cutting board
   - Filleting knife
   - Six tubs

4. Count up 25 fish in each tub
   - Weigh the 25 fish in each tub
   - If the fish have large scales, these should be scraped off as well as you can
   - For three of the tubs: Make fillet sample: Open the fish from the gut opening, take out all the viscera (inner organs). Cut off the head and the tail. Wash the fish to remove blood and remains of the viscera.
   - For the next three tubs you keep the fish as is = whole fish sample.
   - Wash all the equipment with soap and a brush, and clean the workspace when you are done.

5. When you have finished filleting all the fish, take out the following:
   - Food processor (the big white one).
   - 6 x 50 ml tubes for wet sample. Label with preprinted labels, and sort by increasing number. Be aware that there are 3 tubes for whole fish (sample 1-3), and 3 tubes for fillet (sample 4-6).
   - 6 x salad tray and lid.
   - Spatula (baking type)
   - Spatula (lab type) or spoon
   - Permanent marker

6. Homogenization:
   The fish from each tub are now to be homogenized in the food processor. Put the 25 fish or fillets in, and run the food processor until you have a homogenous paste. Fill one 50 ml tube with paste (check that you have the right number on the tube, it should correspond to the number on the fish, and marked with whole fish ="Hel fisk", or fillet = "filet").
   Label salad trays with preprinted labels. Add about 120 g paste to a salad tray, filling to no more than 2 cm height.
   Repeat this for all the fish samples (all 6 tubs).
   Gather all 50 ml tubes with wet sample in one bag, label well with species, station no., date and journal no. (see tubes). Count the tubes and weigh the bag. Enter this information in the form:

"MÅ FYLLES UT Oversikt Jnr og prøver for sending fra alle leg"
Put the bag in a marked box for wet samples in the big freezer..

7. Weigh each salad tray with contents on the two decimal scale. Note wet weight in the form per sample. Freeze the samples for at least 12 hours at -20^{o}C or lower.

8. Freeze-dry the samples for 72 hours. Remember to turn up the temperature after 24 hours. See a separate instruction on the freeze drier. Remember to take off the lid before freeze drying.

9. Check that the samples are completely dry by breaking a sample in two, and check that it is dry inside (bisquit consistency). As soon as the samples are done freeze-drying, you weigh each sample with tray on the two decimal scale. Note the weight per sample in the form.
If you don't have time to weigh the samples immediately you must put them in the exicator cabinet. Check that the silicagel in the bottom are orange. If it is blue you have to change it, and dry the old ones in a drying cabinet. You will find new silicagel in the chemical closet (1st deck, red box labeled "NIFES").

10. Homogenisation after freeze drying.
You will need:
    - Use the new blender for this. (The black hand blender is not good eneugh)
    - Funnel made of wet paper sheets (prepare using wet paper/photo paper and tape – should fit into the 50 ml tube) or plastic funnel.
    - Up to 6 x 50 ml tubes. Label 1 tube for each each pooled sample. Sort in a rack in increasing order.
    - A brush to clean out the dust between samples.
    - A sieve to sift out remaining whole scales

    Break the freeze-dried sample into the blender bowl. Mix in the blender until you have a homogeneous powder. If there are a lot of visible fish scales that have not been homogenized, they can be removed by sifting the powder through a sieve. Add the powder to a 50 ml tube. NB! Check that the number and letter on the tube corresponds with the number and letter on the tray. Repeat for all samples. Clean the workspace and the equipment when you are finished.

11. Vacuum packing the samples
When all the samples have been put in tubes they must be vacuum packed and put in the freezer. How to use the vacuum machine: Put the tubes into a vacuum bag, lid against lid. Avoid filling the bag completely. There should be app. 7 cm left. Put the end of the bag inside the machine (the end of the vacuum bag should touch the black pegs inside the machine, but not cover the vacuum hole). Make sure the lid is closed properly on both sides. Push the "Vacuum and seal" button. Wait until finished. Count the tubes and weigh the bag. Enter this information in the form:
"MÅ FYLLES UT Oversikt Jnr og prøver for sending fra alle leg"
Put the bag in a marked box for dry samples in the freezer.

12. .Make sure to enter all information into the trawlform and bring all the working sheets back home.

Appendix III: Sampling and handling protocol for large fish species.

Large fish (E.g. mackerel, horse mackerel, sardinella)

Each fish must be at least 25 cm long to have enough sample material. Sample 25 individual fish, or at least 15 if it is difficult to get as many as 25 in one area.
Try to avoid direct sunlight on the samples over a long time. Cover with aluminum foil if necessary. Keep the fish or fillet as cold as possible, use the refrigerator if necessary.

1. Collect the fish in a basket. Print the"TrawlformNifesBigFish" for the correct station. Also save the file at the portable PC. Alternatively, use a preprinted working sheet (make sure to get the position data correctly). Note the journal number on the form (look at tube/tray "2018-xxx").

2. Weigh and measure the fish, label each fish with number. Write weight and length in the form.

3. You will need:
   - 15 x 12.5 or 50 ml Nunc tray for liver samples. Label them with preprinted labels, and sort by number.
   - 15 x Lids for the Nunc trays.
   - Scalpel with scalpel blade
   - Tweezer
   - Cutting board
   - Filleting knife
   - Cover a shelf in the refrigerator with aluminum foil

4. For each fish, do the following:
   - Open the fish from the gut opening, take out the internal organs. Write the gender in the form. Cut loose the liver from the first 15 fish, and put it in the correct Nunc tray (the number of the fish should correspond to the number on the tray).
   - Fillet the fish and remove the skin from the fillet. Remove visible bones. NOTE: It is important to include as much of the muscle tissue as possible. Make sure to include the red/brown tissue along the skin. Put the fillet with the number-note on, in the fridge.
   - Wipe off the cutting board, scalpel and tweezer between each fish, using a paper towel.
   - Wash all the equipment with a soap and a brush, and clean the work space when you are finished

   Put all the liver samples in a bag labeled well with species, station no., date, journal no. and "liver samples". Count the tubes and weigh the bag. Enter this information in the form:
   "MÅ FYLLES UT Oversikt Jnr og prøver for sending fra alle leg"
   Put the bag in a marked box for wet samples in the big freezer.

5. Once you have finished filleting all the fish, take out the following:
   - Hand blender or food processor (depending on size)
   - 30 x 50 ml tubes for wet samples, label them with preprinted labels. Sort in a rack in increasing order by number.
   - 30 x Salad trays and lids
   - Baking spatula
   - Laboratory spatula or spoon.

6. Homogenization:
   All fillet from each fish is now to be homogenized in the food processor/hand blender. Put the fish inside and run the blender until you have a homogenous paste.
   - Fill a 50 ml tube with at least 30 ml paste (check that you have the right number on the tube – should correspond to the fish number).
   - Label salad trays with preprinted labels.
   - Add ca. 150 g (ca 2,5 cm) paste to a salad tray. (20g for pooled samples later)

Side 1

- Repeat this for all the fillets.

7. Make 5 pooled samples of the 25 fillets:
   - Take 20 g homogenized paste from each of the 5 first salad trays into a hand blender, homogenize.
   - Fill a 50 ml tube with at least 30 ml paste labelled "Jnr. /fish 1-5"
   - Add ca 120 g to a new tray, labelled "fish 1-5"
   - Repeat for the next 5 fish labelled "fish 6-10"
   - Repeat for the next 5 fish labelled "fish 11-15"
   - Repeat for the next 5 fish labelled "fish 16-20"
   - Repeat for the next 5 fish labelled "fish 21-25"

   Gather all 50 ml tubes with wet sample in one bag, label well with species, station no., date and journal no. (see tubes). Count the tubes and weigh the bag. Enter this information in the form:
   "MÅ FYLLES UT Oversikt Jnr og prøver for sending fra alle leg"
   Put the bag in a marked box for wet samples in the big freezer.

8. Weigh all salad trays with lid and contents on the 2 decimal scale. Note the wet weight in the form per sample. Freeze the samples for at least 12 hours at -20^{o}C or lower.

9. Freeze-dry the samples. Remember to turn up the temperature after 24 hours. See a separate instruction for the freeze-dryer. Remember to take off the lid of the tray before freeze-drying.

10. Check that the samples are entirely dry by breaking a sample in two, and check that it is dry inside (biscuit consistency). As soon as the samples are freezedryed, you put on **new lids** and weigh the samples on the two decimal scale. Note the weight per sample in the form. If you don't have time to weigh the samples immediately you must put them in the exicator cabinet. Check that the silicagel in the bottom is orange. If it is blue you have to change it, and dry the old ones in a drying cabinet. You will find new silicagel in the chemical closet (1st deck, red box labeled "NIFES").

11. Homogenization after freeze drying. You will need:
    - Use the new blender for this. (The black hand blender is not good enough)
    - Funnel made of wet paper sheets (prepare using wet paper/photo paper and tape – should fit into the 50 ml tube) or plastic funnel.
    - Up to 30 x 50 ml tubes. Label 1 tubes for each fish and for each pooled sample. Sort in a rack in increasing order.
    - A brush to clean out the dust between samples.

12. Break the freeze-dried sample into the blender bowl. Mix until you have a homogeneous powder. Put the powder in a 50 ml tube, fill it up. NB! Check that the number on the tube corresponds with the number on the tray. Repeat for all samples. Clean the workspace and the equipment when you are finished.
    The lids for the salad trays can be re-used.

13. Vacuum packing
    When all the samples have been put in tubes they must be vacuum packed and put in the freezer.
    How to use the vacuum machine: Put the tubes into a vacuum bag, lid against lid. Avoid filling the bag completely. There should be app. 7 cm left. Put the end of the bag inside the machine (the end of the vacuum bag should touch the black pegs inside the machine, but not cover the vacuum hole). Make sure the lid is closed properly on both sides. Push the "Vacuum and seal" button. Wait until finished.
    Count the tubes and weigh the bag. Enter this information in the form:
    "MÅ FYLLES UT Oversikt Jnr og prøver for sending fra alle leg"
    Put the bag in a marked box for dry samples in the freezer.

Appendix IV: List of common commercial marine fish species found in Sri Lanka (91).

Common Commercial Fish Types of Sri Lanka

Commercial group	English Name (Common Name)	Scientific Name	Sinhala Name
Seer	Spanish mackerel	***Scomberomorus commersoni***	Thora
	Wahoo	***Acanthocybium commersoni***	Sawara
Paraw	Jack, Trevallies	***Carangoides gymnostethus***	Vattiya
		Carangoides fulvoguttatus	Thumba parawa
		Caranx ignobilis Atanagul	Parawa
		Caranx hebiri	Guru parawa
Balaya	Skipjack tuna	***Katsuwonus pelamis***	Balaya
Kelawalla	yellowfin tuna	***Thunnus albacares***	Kelawalla
Other Blood fish	Sail fish	***Istiophorus platypterus***	Thalapath
	Marlins	***Makariya indika***	Kalu koppara
		Makariya mazara	Nil koppara
		Tetrapturus audax	Iri koppara
	Sword fish	***Xiphias gladius***	Sappara
	Big eye tuna	***Thunnus abesus***	Esgedi kelawalla/Kenda
	Bullet tuna	***Auxis rochei***	Ragodu/kombaya
	Frigate tuna	***Auxis thazard***	Alagoduwa
	Kawakawa	***Euthynnus affinis***	Attawalla
Sharks	Mackerel shark	***Isurus sp.***	Mee mora
	Thresher shark	***Alopias sp.***	Kasa mora **(Banned)**
	Requiem sharks-silky shark	***Carcharhinus Falciformis***	Honda mora/Bala maora
	Ocean white strip shark	***Carcharhinus Longimanus***	Polkola mora
	Blue shark	***prionace gluaca***	Seeni mora/Hudja Mora
	Hammerhead shark	***Sphyrna sp.***	Udalu mora
Skate	Batoid Fisher shovelnose rays	***Rhinobatos sp.***	Baloliya
	String rays	***Dasyatis sp.***	Welli maduwa
	Spotted eagle rays	***Aetobatus narinari***	Vavoi maduwa
	Javanees cownose rays	***Rhinoptera javanica***	valuvadi cownose ray
	Numbfishers	***Narcine sp.***	Electric ray
	Manta and devil rays	***Mobula sp.***	Ali maduwa and Anga maduwa
Rock Fish/Galmalu	Spangled emperor	***Lethrinus nebulosus***	Meewetiya/Atissa
	Longface emperor	***Lethrinus olivaceus***	Uru hota
	Sharptooth jobfish	***Pristipomoides typus***	Kalamee
	Blubberlip snapper	***Lutjanus rivulatus***	Badawa
	Mangrove red snapper	***Lutjanus argentimaculatus***	Thabalaya
	Blackspot snapper	***Lutjanus fulviflamma***	Ranna
	Malabar grouper	***Epinephelus malabaricus***	Gas bola/Gal kossa
Rock Fish/Galmalu	Wavylined grouper	***Epinephelus undulosus***	Lawaya
	Coral hind	***Cephalopholis miniata***	Thabuwa
	Sri Lanka sweetlips	***Plectorhinchus ceylonensis***	Boraluwa
	Threadfin breams	***Nemipterus sp.***	Suddaha

Common Commercial Fish Types of Sri Lanka

	Parrotfishes	***Scarus sp.***	Girawa
	Rabbitfsh	***Siganus so.***	Orawa
	Barracudas	***Sphyraena sp.***	Jeelawa
	Mullets	***Liza sp.***	Godaya
	Trenched sardinella	***Amblygaster sirm***	Hurulla
	Bleeker's smooth belly	***Amblygaster clupeoides***	Gal Hurulla
	Smoothbelly Sardinells	***Amblygaster clupeoides***	Keeramin
	Rainbow sardine	***Dussumieria acuta***	Thondaya
	White sardine	***Escualosa thoracata***	Wella sudaya
	Shad	***Nematalosa nasus***	Koiya
	Goldstripe sardinella	***Sardinella gibbosa***	Kalawenna/Salaya
	White sardinella	***Sardinella albella***	Sudaya
Shore Seine	Bigeye scade	***Selar crumenophtthalamus***	Bolla
	Indian mackerel	***Rastrelliger kanagurta***	Kumbala
	Anchovy	***Stolephorus sp.***	Halmessa
	Ribbon fish	***Lepturacanthus savalaa***	Savalaya
	Gar fisher	***Belonidae***	Habarali
	Thryssa	***Thryssa sp.***	Lagga
	Silverbiddies	***Gerres sp.***	Thirali
	Pony fish	***Leiognathus sp.***	Karalla
	Ilishas	***Ilish sp.***	Puvali
	Half beaks	***Hemiramphus sp.***	Moralla
	Flying fish	***Cheilopogon sp.***	Piyamessa

Appendix V: Additional list of common commercial marine fish species found in Sri Lanka (92)

Mackerel Sharks

Order: Lamniformes. Family: Lamnidae

Name	Binomial	Sinhala Name
Longfin mako shark	*Isurus paucus*	Maha mee moraa (මහ මී මෝරා)
Shortfin mako shark	*Isurus oxyrinchus*	Heen mee mora (හීන් මී මෝරා)

Threshers

Family: Alopiidae

Name	Binomial	Sinhala Name
Common thresher	*Alopias vulpinus*	Kasa moraa (කස මෝරා) - banned

Requiem sharks

Family: Carcharhinidae

Name	Binomial	Sinhala Name
Silky shark	*Carcharhinus falciformis*	Bala moraa (බල මෝරා)
Oceanic whitetip shark	*Carcharhinus longimanus*	Polkola mora (පොල්කොළ මෝරා)
Blue shark	*Prionace glauca*	Seeni mora (සීනි මෝරා)

Hammerhead sharks

Family: Sphyrnidae

Name	Binomial	Sinhala Name
Scalloped hammerhead	*Sphyrna lewini*	Udalu mora (උදලු මෝරා)
Great hammerhead	*Sphyrna mokarran*	Udalu mora (උදලු මෝරා)
Smooth hammerhead	*Sphyrna zygaena*	Udalu mora (උදලු මෝරා)

Guitarfish

Order: Rajiformes. Family: Rhinobatidae

Name	Binomial	Sinhala Name
Common shovelnose ray	*Glaucostegus typus*	Baaloliyaa (බාලොලියා)

Stingrays

Order: Myliobatiformes. Family: Dasyatidae

Name	Binomial	Sinhala Name
Common stingray	*Dasyatis pastinaca*	Waeli maduwaa (වැලි මඩුවා)
Pale-edged stingray	*Dasyatis zugei*	Waeli maduwaa (වැලි මඩුවා)

Rays

Family: Myliobatidae

Name	Binomial	Sinhala Name
Spotted eagle ray	*Aetobatus narinari*	Vavoi maduwaa (වවෙයි මඩුවා)
Pygmy devil ray	*Mobula eregoodootenkee*	Ali maduwaa (අලි මඩුවා)
Flapnose ray	*Rhinoptera javanica*	Maduwaa (මඩුවා)

Numbfish

Order: Torpediniformes. Family: Narcinidae

Name	Binomial	Sinhala Name
Blackspotted numbfish	*Narcine timlei*	Viduli maduwaa (විදුලි මඩුවා)

Bony fish

Class: Actinopterygii

Scombrid fish

Order: Perciformes. Family: Scombridae

Name	Binomial	Sinhala Name
Wahoo	*Acanthocybium solandri*	Sawaraa (සවරා)
Bullet tuna	*Auxis rochei*	Ragodu, Kombayaa (රගෙඩු, කොම්බයා)
Frigate tuna	*Auxis thazard*	Alagoduwaa (අලගොඩුවා)
Mackerel tuna	*Euthynnus affinis*	Aetawallaa (ඇටවල්ලා)
Skipjack tuna	*Katsuwonus pelamis*	Balayaa (බලයා)
Indian mackerel	*Rastrelliger kanagurta*	Kumbalawaa (කුම්බලවා)
Narrow-barred Spanish mackerel	*Scomberomorus commerson*	Thoraa (තෝරා)
Yellowfin tuna	*Thunnus albacares*	Kelawallaa (කෙලවල්ලා)
Bigeye tuna	*Thunnus obesus*	As-gedi Kelawallaa (ඇස් ගෙඩි කෙලවල්ලා)

Jacks and allies

Family: Carangidae

Common name	Binomial	Sinhala Name
Bludger	*Carangoides gymnostethus*	Vattiyaa (වට්ටියා)
Yellowspotted trevally	*Carangoides fulvoguttatus*	Thumba paraawaa (තුම්බ පරාවා)
Blacktip trevally	*Caranx heberi*	Guru paraawaa (ගුරු පරාවා)
Giant trevally	*Caranx ignobilis*	Parsawaa (පරාවා)
Indian Scad	*Decapterus russelli*	Linna (ලින්නා)
Bigeye scad	*Selar crumenophthalmus*	Bollaa (බෝල්ලා)

Sailfish and allies

Family: Istiophoridae

Common name	Binomial	Sinhala Name
Indo-Pacific sailfish	*Istiophorus platypterus*	Thalapath (තලපත්)
Black marlin	*Istiompax indica*	Kalu kopparaa (කලු කොප්පරා)
Striped marlin	*Kajikia audax*	Iri kopparaa (ඉරි කොප්පරා)
Atlantic blue marlin	*Makaira nigricans*	Nil koppara (නිල් කොප්පරා)

Swordfish

Family: Xiphiidae

Common name	Binomial	Sinhala Name	Tamil Name
Swordfish	*Xiphias gladius*	Sapparaa (සප්පරා)	Thalapaththu/Myil Meen (மயில் மீன்)

Emperors

Family: Lethrinidae

Common name	Binomial	Sinhala Name
Spangled emperor	*Lethrinus nebulosus*	Meevetiya, Atissaa (මීවෙටියා, අටිස්සා)
Longface emperor	*Lethrinus olivaceus*	Uru hotaa (උරු හොටා)

Snappers

Family: Lutjanidae

Common name	Binomial	Sinhala Name
Sharptooth jobfish	*Pristipomoides typus*	Kalmaee (කලමී)
Mangrove red snapper	*Lutjanus argentimaculatus*	Thabalayaa (තබලයා)
Dory snapper	*Lutjanus fulviflamma*	Rannaa (රන්නා)
Blubberlip snapper	*Lutjanus rivulatus*	Badawaa (බඩවා)

Groupers

Family: Serranidae

Common name	Binomial	Sinhala Name
Coral hind	*Cephalopholis miniata*	Thabuwaa (තඹුවා)
Malabar grouper	*Epinephelus malabaricus*	Gas bola, Gal kossaa (ගස් බෝලා, ගල් කොස්සා)
Wavy-lined grouper	*Epinephelus undulosus*	Lawayaa (ලවයා)

Sweetlips

Family: Haemulidae

Common name	Binomial	Sinhala Name
Sri Lanka sweetlips	*Plectorhinchus ceylonensis*	Boraluwaa (බොරළුවා)

Threadfin bream

Family: Nemipteridae

Common name	Binomial	Sinhala Name
Delagoa threadfin bream	*Nemipterus bipunctatus*	Sudhdhaa (සුද්දා)
Fork-tailed threadfin bream	*Nemipterus furcosus*	Sudhdhaa (සුද්දා)

Parrotfish

Family: Scaridae

Common name	Binomial	Sinhala Name
Ember parrotfish	*Scarus rubroviolaceus*	Girawaa (ගිරවා)
Eclipse parrotfish	*Scarus russelii*	Girawaa (ගිරවා)

Rabbitfish

Family: Siganidae

Common name	Binomial	Sinhala Name
Bronze-lined rabbitfish	*Siganus insomnis*	Orawaa (ඔරවා)
Streaked spinefoot	*Siganus javus*	Orawaa (ඔරවා)
Golden-lined spinefoot	*Siganus lineatus*	Orawaa (ඔරවා)
Vermiculated spinefoot	*Siganus vermiculatus*	Orawaa (ඔරවා)

Barracudas

Family: Sphyraenidae

Common name	Binomial	Sinhala Name
Pickhandle barracuda	*Sphyraena jello*	Jeelawaa (ජීලවා)
Obtuse barracuda	*Sphyraena obtusata*	Jeelawaa (ජීලවා)

Mullets

Family: Mugilidae

Common name	Binomial	Sinhala Name
Largescale mullet	*Chelone macrolepis*	Godayaa (ඇඟඩියා)
Flathead grey mullet	*Mugil cephalus*	Godayaa (ඇඟඩියා)

Cutlassfish

Family: Trichiuridae

Common name	Binomial	Sinhala Name
Savalani hairtail	*Lepturacanthus savala*	Sevalayaa (සෙවලයා)

Mojarras

Family: Gerreidae

Common name	Binomial	Sinhala Name
Deep-bodied mojarra	*Gerres erythrourus*	Thirali (තිරලි))
Slender silver-biddy	*Gerres oblongus*	Thirali (තිරලි)
Common silver-biddy	*Gerres oyena*	Thirali (තිරලි)

Ponyfish

Family: Leiognathidae

Common name	Binomial	Sinhala Name
Berber ponyfish	*Leiognathus berbis*	Kaarallaa (කාරල්ලා))
Shortnose ponyfish	*Leiognathus brevirostris*	Kaarallaa (කාරල්ලා)
Common ponyfish	*Leiognathus equulus*	Kaarallaa (කාරල්ලා)

Herrings and allies

Order: Clupeiformes. Family: Clupeidae

Name	Binomial	Sinhala Name
Bleeker's smoothbelly sardinella	*Amblygaster clupeoides*	Gal hurulla (ගල් හුරුල්ලා)
Smoothbelly sardinella	*Amblygaster leiogaster*	Keeramin (කීරමීන්)
Spotted sardinella	*Amblygaster sirm*	Hurulla (හුරුල්ලා)
Rainbow sardine	*Dussumieria acuta*	Thondayaa (තොන්ඩයා)
White sardine	*Escualosa thoracata*	Wella sudaa (වැල්ල සුදා)
Bloch's gizzard shad	*Nematalosa nasus*	Koyyaa (කොය්යා)
White sardinella	*Sardinella albella*	Sudayaa (සුදයා)
Goldstripe sardinella	*Sardinella gibbosa*	Saalayaa (සාලයා)
Ilish	*Tenualosa ilisha*	Puvaali (පුවාලි)

Anchovy

Family: Engraulidae

Name	Binomial	Sinhala Name

Commerson's anchovy	*Stolephorus commersonnii*	Haalmassaa (හාල්මැස්සා)
Indian anchovy	*Stolephorus indicus*	Handalla (හැඳැල්ලා)
False baelama anchovy	*Thryssa encrasicholoides*	Laggaa (ලග්ගා)
Gautama thryssa	*Thryssa gautamiensis*	Laggaa (ලග්ගා)
Malabar thryssa	*Thryssa malabarica*	Laggaa (ලග්ගා)/ Balal parattaya (බළල් පරට්ටයා)
Moustached thryssa	*Thryssa mystax*	Ata Laggaa (ඇට ලග්ගා)

Garfish

Order: Beloniformes. Family: Belonidae

Name	Binomial	Sinhala Name
Freshwater garfish	*Xenentodon cancila*	Habarali (හබරලි)

Flying fish

Family: Exocoetidae

Name	Binomial	Sinhala Name
Black-sail flyingfish	*Cheilopogon nigricans*	Piyamassa (පියාමැස්සා)

Halfbeaks

Family: Hemiramphidae

Name	Binomial	Sinhala Name
Jumping halfbeak	*Hemiramphus archipelagicus*	Morallaa (මොරල්ලා)
Congaturi halfbeak	*Hyporhamphus limbatus*	Morallaa (මොරල්ලා)

River garfish

Family: Zenarchopteridae

Name	Binomial	Sinhala Name
Feathered river garfish	*Zenarchopterus dispar*	Habarali (හබරලි)

Appendix VI: Trawl form for small fish species used during the Nansen survey around Sri Lanka.

						Operator:		
R/V Dr. Fridtjof Nansen			Survey:			Region:	Station:	
Date da/mo/yr:	Start pos. (Lat./Lon.)		Stop pos. (Lat./Lon.):		Purpose:	Gear Cond:	Validity:	Gear Type:
	Start:	**Stop:**	**Duration:**	**Notes:**				
Time.								
Log.								
Gear depth. . .								
Bottom depth								
Wire out.								
Speed.								
Fisk:					**Journal no:**			

Fisk		Snitt Vekt(g)	Snitt Lengde (cm)	Merknader	Samleprøve av minst 25 fisk				
					Vekt skål m/lokk	Skål vekt + vått	Vått	Skål vekt + tørr	% Tørrst.
1	Heil fisk				5.2		-5.2		100.0
2	Heil fisk				5.2		-5.2		100.0
3	Heil fisk				5.2		-5.2		100.0
4	Filet				5.2		-5.2		100.0
5	Filet				5.2		-5.2		100.0
6	Filet				5.2		-5.2		100.0
Snitt:	**Heil fisk**								**100.0**
Snitt:	**Filet**								**100.0**

If this species is weighed and measured on this station, you can have average numbers from this measurement. However, always weigh and write down the total weight of each 25 fish sample. If you don't get average length from the catch, you will need to measure 25 fish. Write down the length of each of 25 fish (on the reverse side of the sheet). Write the mean weight and length on the form.

Appendix VII: Trawl form for large fish species used during the Nansen survey around Sri Lanka.

									Operator:		Notater						
R/V Dr. Fridtjof Nansen				Survey:			Region:	Station:									
Date da/mo/yr:	Start pos. (Lat./Lon.) Stop pos. (Lat./Lon.):					Purp	Gear Cond:	Validity:	Gear Type:								
	Start:	Stop:	Duration:	Notes:													
Time.																	
Log.																	
Gear depth. . .																	
Bottom depth																	
Wire out. . . .																	
Speed.																	
Fisk:						Journal no:											

						Enkeltprøver						Samleprøver					
Fisk	Fisk Vekt(g)	Fisk Lengde (cm)	Kjønn (m/f)	Ta ut lever	Ved ulike St.	Vekt skål m/lokk	Skål vekt + vått	Vått	Skål vekt + tørr	% Tørrst.	Fisk	Vekt skål m/lokk	Skål vekt + vått	Vått	Skål vekt + tørr	% Tørrst.
1				x		5.2				100.0						
2				x		5.2				100.0						
3				x		5.2				100.0	Fisk 1-5					
4				x		5.2				100.0						
5				x		5.2				100.0						
6				x		5.2				100.0						
7				x		5.2				100.0						
8				x		5.2				100.0	Fisk 6-10					
9				x		5.2				100.0						
10				x		5.2				100.0						
11				x		5.2				100.0						
12				x		5.2				100.0						
13				x		5.2				100.0	Fisk 11-15					
14				x		5.2				100.0						
15				x		5.2				100.0						
16						5.2				100.0						
17						5.2				100.0						
18						5.2				100.0	Fisk 16-20					
19						5.2				100.0						
20						5.2				100.0						
21						5.2				100.0						
22						5.2				100.0						
23						5.2				100.0	Fisk 21-25					
24						5.2				100.0						
25						5.2				100.0						
Snitt:	#DIV/0!	#DIV/0!								100.0						######

Appendix VIII: Scientific and English names, coordinates, and station numbers of the locations of where the samples were collected during the 2018 Nansen survey around Sri Lanka.

Scientific name	English name[a]	Start position[b]	End position[b]	Station number[c]
Large fish				
Carangoides fulvoguttatus	Yellowspotted trevally	9.58, 80.74	9.59, 80.00	14
Diagramma pictum	Painted sweetlips	9.00, 81.00	8.98, 81.00	19
Lethrinus olivaceus	Longface emperor	7.71, 81.83	7.68, 81.80	33
Lutjanus lutjanus	Bigeye snapper	5.88, 80.38	5.88, 80.40	63
Nemipterus bipunctatus	Deagoa threadfin bream	5.97, 80.23	5.96, 80.30	61
Selar crumenophthalmus	Bigeye scade	6.49, 79.69	6.44, 79.70	73
Sphyraena jello	Pickhandle barracuda	7.15, 79.71	7.12, 79.70	78
Small fish				
Amblygaster sirm	Trenched sardinella	7.15, 79.71	7.12, 79.71	78
Auxis thazard	Frigate tuna	7.49, 79.42	7.46, 79.50	80
Decapterus macrosoma (1)	Shortfin scad	9.94, 80.67	9.92, 80.70	8
Decapterus macrosoma (2)	Shortfin scad	6.04, 80.99	6.04, 81.0	53
Encrasicholina devisi	Devis' anchovy	7.15, 79.71	7.12, 79.71	78
Equulites elongatus	Slender ponyfish	7.06, 7.71	7.03, 79,70	77
Leiognathus dussumieri	Dussumier's ponyfish	6.79, 81.87	6.77, 81.9	41
Photopectoralis bindus (1)	Orangefin ponyfish	9.47, 80.86	9.45, 80.90	15
Photopectoralis bindus (2)	Orangefin ponyfish	9.00, 81.05	8.98, 81.10	18
Rastrelliger kanagurta	Indian mackerel	6.04, 80.99	6.04, 81	53
Stolephorus indicus	Indian anchovy	9.13, 81.03	9.15, 81.0	17
Sillago ingenuua	Bay whiting	6.22, 81.47	6.22, 81.5	47

[a] Names obtained through the global species database FishBase (93).
[b] GPS coordinates expressed as latitude and longitude, respectively, in reference to the starting and ending position of the trawl of which each species was sampled from.
[c] Station numbers as referred to in the Nansen Cruise Report.