Nutrients and contaminants in processed small indigenous fish species from Ghana

Implications for food and nutrition security

Astrid Elise Hasselberg

Thesis for the degree of Philosophiae Doctor (PhD)
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Scientific environment

This PhD thesis was completed between 2018 and 2022 at the Institute of Marine Research (IMR) in Bergen, Norway, in the research group Seafood and Nutrition. The PhD was administered through the Department of Medicine at the University of Bergen, Norway. The PhD was supervised by Dr. Ole Jacob Nøstbakken (IMR) and Dr. Marian Kjellevold (IMR), and co-supervisors were Dr. Inger Aakre and associate professor Robin Ørnsrud (IMR/UiB).

The fieldwork was carried out during 2018 in Ghana in close collaboration with Amy Atter at the CSIR Food Research Institute in Accra and Prof. Matilda Steiner-Asiedu at The University of Ghana.

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List of abbreviations

ASF	Animal source food
CPUE	Catch per unit effort
DHA	Docosahexaenoic acid
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid
FA	Fatty acid
FCD	Food composition data
FCT	Food composition table
FNS	Food and nutrition security
IMR	Institute of Marine Research
LMIC	Low-and middle-income country
MeHg	Methylmercury
MOE	Margin of exposure
NGO	Non-governmental organization
PAH	Polycyclic aromatic hydrocarbon
PoU	Prevalence of undernourishment
PMTDI	Provisional maximum tolerable daily intake
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PTWI	Provisional tolerable weekly intake
PUFA	Polyunsaturated fatty acid
RBA	Risk benefit analysis
RNI	Recommended nutrient intake
SDG	Sustainable development goal
SIS	Small indigenous fish species
TWI	Tolerable weekly intake
UL	Upper tolerable intake level
WA	West African

Abstract

The prevalence of malnutrition is increasing globally, along with the need to access nutritious, safe and sustainable foods. Fish are known as a rich source of protein, essential fatty acids and micronutrients, and including small indigenous fish species (SIS) in the diet has been proposed as a strategy towards alleviating malnutrition. SIS are a central part of the diet in Ghana and are commonly preserved by smoking or drying. Still, stunting and micronutrient deficiencies among young children persist. Furthermore, the livelihoods of millions of Ghanaians depend on the small-scale fisheries, from fishers and traders, to processors and market women. Having reliable data on SIS availability and food composition data on nutrients and contaminants in SIS is therefore essential to assess its potential role towards improving food and nutrition security (FNS) in Ghana.

The broad aim of this thesis was to explore how SIS contribute to FNS in Ghana, while specific aims of the study were addressed in three articles: Firstly, we aimed to assess the current challenges and opportunities in the Ghanaian fish value-chain through a literature review, and assess how future strategies can strengthen the role of SIS towards enhancing FNS. Secondly, we aimed to determine the nutritional quality and food safety of six commonly consumed processed SIS from Ghana by analyzing key nutrients, heavy metals, PAHs and microbial contamination. Lastly, we aimed to determine nutrient and contaminant distribution in tissues of smoked European anchovy and assess how exposure potentially affects vulnerable population groups.

Through a narrative literature review, we found that uncertainties in fish availability and access are challenging the sustenance of the millions of Ghanaians involved in the small-scale fisheries value-chain. The synthesized data further indicated that fish utilization is constrained by inadequate young child feeding practices and food safety issues, while seasonality and climate changes are hindering stability.

To assess the nutritional quality and food safety of commonly consumed SIS, samples of smoked European anchovy (*Engraulis encrasicolus*) bigeye grunt (*Brachydeuterus auritus*), round sardinella (*Sardinella aurita*), African moonfish (*Selene dorsalis*), dried/smoked West African (WA) pygmy herring (*Sierrathrissa leonensis*) and salt dried tilapia (*Tilapia spp.)* were collected from fish markets in five different regions in Ghana. Samples of European anchovy were divided into subsamples of whole fish, heads and skin and samples without heads and skin. Using accredited methods, composite samples of SIS were analyzed for nutrients (crude protein and fat, fatty acids, key vitamins, minerals, and trace elements), microbiological quality and contaminants (PAH4 and heavy metals). The marine SIS, tissues of European anchovy and WA pygmy herring had the potential to significantly contribute to the nutrient intakes of essential micronutrients, including Fe, Zn, I, Se, Ca, vitamin D, vitamin B_{12} and ω-3 polyunsaturated fatty acids (PUFAs), while tilapia was the least nutrient dense. High levels of Fe, Hg, Pb and Cd were detected in certain SIS and tissues and PAH4 in all smoked fish samples reached high concentrations, up to 1,300 µg/kg. In a scenario referencing Ghanaian infants and toddlers (6-23 months), it was determined that consuming smoked SIS will entail potential risk in terms of Cd and PAH-exposure.

In this thesis, we determined that processed SIS contain high levels of nutrients and represent a promising food-based approach to alleviate micronutrient deficiencies in Ghana. However, the current levels of certain heavy metals and PAHs above recommended limits may entail potential consumer risk. Generating data on dietary intakes of SIS, identification of contamination sources and improvement of processing methods are therefore warranted in order to secure safe utilization of SIS in Ghana. Furthermore, the current lack of focus on fish and FNS in scientific literature and legislation highlights the need for fish to be given greater priority, which is essential to secure the sustenance of Ghana's small-scale fisheries.

List of Publications

I. Hasselberg AE, Aakre I, Scholtens J, Overå R, Kolding J, Bank MS, Atter A, Kjellevold M.

Fish for food and nutrition security in Ghana: Challenges and opportunities

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III. Hasselberg AE, Nøstbakken OJ, Aakre I, Madsen L, Amy A, Steiner-Asiedu, Kjellevold M.

Nutrient and contaminant exposure from smoked European anchovy (*Engraulis encrasicolus*): Implications for children's health in Ghana

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* Shared first authorship

Disclaimer

Microbiological laboratory analyses for paper II were carried out by Laura Wessels, a colleague and fellow PhD-candidate in the SmallFishFood project. Laura Wessels and I contributed equally to Paper II in this thesis, and therefore hold shared first authorship. The sections on microbiological quality and PAHs were written by Laura Wessels, and the sections on nutrients and heavy metals were written by me. The paper reports major findings important for the understanding of the overall project, hence it will also be included in the PhD-thesis of Laura Wessels, planned to be submitted during spring 2022.

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1. Introduction

1.1 Food and nutrition security

1.1.1 Food security

Food security is a flexible concept, which is reflected by the multiple definitions used in research and policies. The term «Food security» was conceived during the World Food Conference in the mid-1970s and has evolved in tune with changing policy strategies over the last 40 years. In its inception, the definition of food security mainly referred to food supply, with minimal emphasis on food access, stating that: *"Availability at all times of adequate world food supplies of basic foodstuffs to sustain a steady expansion of food consumption and to offset fluctuations in production and prices (FAO 1974)".* The aspect of food access was incorporated into the terminology by the early 1980s, underscoring the important balance between food supply and demand for vulnerable populations: *"Ensuring that all people at all times have both physical and economic access to the basic food that they need (FAO 1983)".* In 1986 The World Bank's report on poverty and hunger highlighted the seasonal dynamics of food security (The World Bank 1986), adding to the definition that: *"…access of all people at all times to enough food for an active, healthy life".* This was the basis for the widely accepted distinction between *transitory food insecurity* caused by natural disasters, economic collapse or conflict stressors and *chronic food insecurity*, which is associated with extended periods of structural poverty and low income (FAO 2006). By the 1990s, the food security definition was broadened by expanding the focus from protein-energy malnutrition to the composition of food, including food safety and micronutrient requirements for an active and healthy life (FAO 2003). In 1996, the World Food Summit adopted a more comprehensive definition of food security, which has only been altered by addition of the sole word *"social"* during FAO's Declaration of the World Summit on Food Security in 2009 (CFS 2012), stating that: *"Food security exists when all people, at all times, have physical, social and economic access to sufficient safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life (FAO 1996)".* From this definition, a framework including four dimensions of food security can be identified, namely *availability, access, utilization* and *stability*:

Availability refers to the physical existence of food, including domestic food production, commercial food imports and exports, domestic food stocks and food aid (FAO 2006). On a household level, food availability includes food from own production and locally bought foods.

Access is ensured when all individuals in the household have enough resources (e.g. entitlements) for acquiring food in sufficient quantity, quality and diversity for a nutritious diet (FAO 2006). This is mainly dependent on household resources and price levels, but is also influenced by the political, economic and social arrangements of communities.

Utilization refers to the biological utilization of accessible foods on individual and household levels (FAO 2006). Furthermore, the importance of adequate health care, sanitation, feeding practices and food safety is encompassed in this pillar, which aims at what is more precisely named «nutrition security».

Stability is reflected by the availability and access dimensions, and is given when the supply of food and economic resources remains stable throughout cyclical events (FAO 2006). This includes long-term resilience to climate changes, natural disasters, price volatility, conflicts and pandemics.

1.1.2 Nutrition security

As a result of the evolving definition of food security, the term "nutrition security" emerged in the mid-1990s (Shetty 2015). Nutrition security encompass both individual and household food consumption, and focuses on how foods are utilized by the body (CFS 2012). The terminology was built on UNICEF's conceptual framework on malnutrition, where a twofold pathway, including both health status and food intake, are considered key determinants of nutritional status (UNICEF 1991). This is in contrast with the food security framework, in which food as a commodity is the central focus. In 1995, the International Food Policy Research Institute proposed the following definition of nutrition security, stating that: *"Nutrition security can be defined as adequate nutritional status in terms of protein, energy, vitamins, and minerals for all household members at all times (Quisumbing et al. 1995)".* While this definition highlights the importance of nutritious foods to meet physiological needs, non-food factors are not addressed. M.S. Swaminathan (cited in Shetty (2015)) later redefined nutrition security as *"physical, economic and social access to a balanced diet, safe drinking water, environmental hygiene, primary health care and primary education"*, thus incorporating both food and non-food factors.

1.1.3 Food and nutrition security

The continuous evolution of both food security and nutrition security terminology has resulted in a common consensus that food cannot be separated from its nutritional role to meet physiological requirements in terms of quantity, quality and safety, in addition to being socially and culturally acceptable (Gross et al. 2000). Thus, a merge of the two concepts has resulted in a unified term, namely "food and nutrition security". The ultimate goal of improving the nutritional status of vulnerable populations is therefore emphasized linguistically and conceptually, acknowledging both the importance of food production, food systems, and socioeconomic aspects of food security and the biological approach centered around the human being, as emphasized by the nutrition security concept (CFS 2012). FNS has been used by leading non-governmental organizations (NGOs) since the mid-2000s (Shetty 2015) and both UNICEF and FAO have constructed definitions for the term, stating that "*Food and nutrition security is achieved when adequate food (quantity, quality, safety, socio-cultural acceptability) is available and accessible for and satisfactorily used and utilized by all individuals at all times to live a healthy and active life (UNICEF 2008)"* and "*Food and nutrition security exists when all people at all times have physical, social and economic access to food of sufficient quantity and quality in terms of variety, diversity, nutrient content and safety to meet their dietary needs and food preferences for an active and healthy life, coupled with a sanitary environment, adequate health, education and care (FAO 2011a)".*

1.1.4 Global food and nutrition security goals and trends

In 2015, all United Nations Member States adopted the 2030 agenda for sustainable development and announced 17 Sustainable Development Goals (SDGs) (UN 2015). SDG 2 "Zero hunger" is dedicated to *"End hunger, achieve food security and improved nutrition and promote sustainable agriculture"* by the year 2030 (UN 2021a). One of the key targets is to reduce the many forms of malnutrition, which not only encompass undernutrition, but the increasing prevalence of overweight and obesity (UN 2021b). The FAO uses two main indicators to track the progression towards SDG 2; the prevalence of undernourishment (PoU), which reflects the prevalence of hunger based on food availability and access, and The Food Insecurity Experience Scale, which reports the prevalence of moderate (transitory) and severe (chronic) food insecurity (FAO 2021). From remaining stable the last five years, the global PoU increased approximately 10% from 2019-2020, affecting an estimated 720-811 million people. Similarly, the prevalence of moderate and severe food insecurity had a dramatic increase since the onset of COVID-19, and it is estimated that every third person in the world did not have access to adequate food in 2020 (FAO 2021). Additionally, Muthayya et al. (2013) estimated that 2 billion suffered from multiple micronutrient deficiencies or "hidden hunger" in 2013, which has likely increased since. Worldwide, it is estimated that 22% of children under five years of age are affected by stunting, 6.7% are suffering from wasting and 5.7% are overweight, affecting over 230 million individuals (FAO 2021). Collectively, the different forms of malnutrition are attributable to nearly half of all deaths in children under 5 years (UNICEF et al. 2021). The burdens of young-child malnutrition are disproportionally prevalent in low-and middle-income countries (LMICs) in Asia and Sub-Saharan Africa, which is home to more than nine out of ten of all children who are stunted and suffer from wasting (FAO 2021).

1.1.5 Food and nutrition security during the first 1000 days

Nutrition during the first 1000 days of life, from conception through 2 years of age, has a profound impact on a child's ability to grow, learn and thrive (Victora et al. 2008, Black et al. 2017). This period marks the most active period of neurologic development and inadequate intake of key micronutrients including Zn, Fe, I, vitamins A, D and B_{12} and ω-3 PUFAs during this period may result in lifelong impairments in brain function, independent of subsequent nutrient repletion (Schwarzenberg et al. 2018). Young children may also have a limited ability of detoxification compared with adults, making them more sensitive to toxicant exposure (Scheuplein et al. 2002, Ginsberg et al. 2004). Furthermore, deficient healthcare and frequent infectious diseases throughout early childhood, which is frequent in many LMICs, may exacerbate the detrimental effects of poor nutrition (Grantham-McGregor et al. 2007, Victora et al. 2021). This also translates to inadequate complementary feeding practices, which remains one of the main causes of malnutrition in children under 5 years living in LMICs (UNICEF 2019).

1.1.6 Food based approaches

To combat malnutrition and rising levels of food insecurity, the FAO recommends a food-based approach (FAO 2011b). A food-based approach encompasses the interrelationships of food, health, and environment, which includes dietary diversification, food fortification and sustainable food production (Burchi et al. 2011, FAO 2011b). Thus, access, availability and utilization of foods not only enhances nutrition, but aids in sustaining rural livelihoods in LMICs which are commonly mediated through agriculture, small-scale fisheries and its related activities (Thompson and Amoroso 2014).

1.2 Nutrients in fish

Fish are known as a good source of essential nutrients, which may exhibit significant variation both within and among species, between different types of tissue, habitats, regions, seasons and through post-harvest processing (Larsen et al. 2011, Abraha et al. 2018, Nerhus et al. 2018, Tilami and Sampels 2018).

1.2.1 Proximate and fatty acid composition

Water is the main constituent of fish flesh, and accounts for 70-80% of total body weight in small marine fish species (Aakre et al. 2020). The water content largely depends on variations in lipid content, and a linear relationship exists between the two (Gökoğlu and Yerlikaya 2015). Fish are commonly categorized as lean (<2% fat), intermediate (2-8% fat) or fatty fish (>8% fat) (European Food Safety 2005), however, external factors, such as temperature, salinity and nutrient composition of the feed modulates fat content throughout the life cycle (Ababouch 2005). This is exemplified by the seasonal variations in fat content of marine species such as European anchovy, ranging from 0.9-5.7 g/100 g (Zlatanos and Laskaridis 2007). The fatty acid (FA) composition of fish is characterized by low amounts of saturated FAs and varying amounts of ω-3 PUFAs, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which is accumulated through the food chain (Larsen et al. 2011). Hence, there is a distinct division between habitats, reflected by higher concentrations of EPA and DHA in wild fatty marine fish species (Wall et al. 2010), while ω-6 PUFAs are more prominent in farmed freshwater species, including tilapia (Weaver et al. 2008). During traditional open-flame fish smoking the fat content of fish is physically reduced (Adeyeye and Oyewole 2016b), while lipid oxidation and lowered ω-3 PUFA retention is more pronounced in sun-dried fish products (Chaula et al. 2019). Fish are also a source of highly digestible animal protein and is recognized as a source of several essential amino acids, including methionine and lysine, which are limited in terrestrial animal source foods (ASFs) and plant-based foods (Tacon and Metian 2013, Tilami and Sampels 2018). By smoking or drying fish, the reduced water content contributes to a relative increase in protein content (Kiczorowska et al. 2019). Furthermore, Usydus et al. (2009a) have reported that processed fish products represent good sources of digestible protein and that smoked fish is characterized by higher retention of essential amino acids than salted fish.

1.2.2 Minerals and trace elements

Fish are a rich source of multiple minerals and trace elements, particularly Se and I, which are generally more abundant in fish than terrestrial ASFs (Larsen et al. 2011, Nerhus et al. 2018). Mineral and trace element density is largely dependent on fish size, and may be attributed to the inclusion of bones, skin, head and viscera (Béné et al. 2015). Recent studies have reported that whole small marine fish species including *Engraulis* and *Sardinella spp* have a higher concentration of I, Zn, Fe, Mg and Ca compared with fillets of larger species (Aakre et al. 2020, Reksten et al. 2020a) and Bogard et al. (2015) have confirmed this pattern in freshwater habitats. The available literature on mineral and trace element content in processed fish is limited and scattered. Polak-Juszczak (2016) reported that more essential minerals and trace elements are retained in hot-smoked sprat compared with raw sprat, while Kiczorowska et al. (2019) determined reduced mineral concentrations in various smoked fish species compared with raw fish. Moreover, by comparing processing methods, one study determined that mineral-retention is higher in solar dried fish than traditionally smoked fish from Malawi (Katola and Kapute 2017).

1.2.3 Vitamins

Fish contain a range of vitamins, which display large variations dependent on seasonal fluctuations, species and tissues (Zlatanos and Laskaridis 2007, Weichselbaum et al. 2013). Fatty fish and cod liver oil are among the few dietary sources that contain naturally high concentrations of vitamin D (Larsen et al. 2011, Tilami and Sampels 2018). The distribution of vitamin D is also known to differ between tissues, and higher concentrations have been determined in whole fish compared with fish fillet (Reksten et al. 2020a). Vitamin A is present in fish in the form of vitamin A_1 (retinol) and A_2 (3,4-didehydroretinol) (La Frano et al. 2018) and it has been determined that whole small fish from marine and freshwater habitats contain higher levels of vitamin A compared with fillets of larger fish (Roos et al. 2002, Aakre et al. 2020, Reksten et al. 2020a). Roos et al. (2002) furthermore documented that up to 90% of vitamin A is concentrated in the eyes and viscera of small fish. Among the water-soluble vitamins, fish are rich in vitamin B_{12} (Watanabe 2007). Similar to the fat-soluble vitamins, the highest concentrations of B_{12} have been documented in the viscera (Nishioka et al. 2011) and in whole small fish (Aakre et al. 2020, Reksten et al. 2020a). It has been documented that various cooking processes may induce vitamin degradation (Nishioka et al. 2011, Jakobsen and Knuthsen 2014) and that sun-drying reduces vitamin A-activity (Chittchang et al. 1999), but as of yet, specific studies on vitamin-retention in smoked fish could not be identified.

1.3 Contaminants in fish

Contaminants in fish originate from both natural and anthropogenic sources, and are known to vary between different habitats, trophic positions and throughout the life cycle (van der Oost et al. 2003). Furthermore, the concentration of contaminants in fish may be influenced by post-harvest activities, including processing (Sheng and Wang 2021).

1.3.1 Heavy metals and metalloids

Heavy metals (Hg, Cd and Pb) and metalloids (As), hereby referred to as "*metals*", are present in varying concentrations in fish (Castro-González and Méndez-Armenta 2008). Owing to their toxicity and ability to bioaccumulate and biomagnify in the food chain, even low exposure levels are associated with a range of adverse health effects in humans, including neurotoxicity and carcinogenicity (Hughes 2002, Zahir et al. 2005, Flora et al. 2012, Rani et al. 2014). Hg is mainly present in fish as methylmercury (MeHg), its most toxic form, and is known to primarily accumulate in muscle (Amlund et al. 2007, Ho et al. 2021). Conversely, elevated levels of As and Cd have been determined in whole SIS, which may be attributed to the inclusion of heads and viscera (Chowdhury et al. 2004, Boalt et al. 2014, Ho et al. 2021, Moxness Reksten et al. 2021). However, unlike plant foods, the majority of As in fish is organic arsenic (such as arsenobetaine) which is considered harmless (Mania et al. 2015). Whole small marine fish are known to contain somewhat higher concentrations of Pb compared with fillet since viscera and bones are the primary accumulation sites, but the overall low levels of Pb in marine fish indicate minor exposure (Julshamn et al. 2004, Moxness Reksten et al. 2021). Anthropogenic activities, including the release of Pb and other metals from e-waste recycling and use of Hg in artisanal gold mining, are increasing in larger parts of Africa and represent possible routes of contamination (Hilson 2002, Yabe et al. 2010, Itai et al. 2014, WHO 2015). A limited set of publications have reported both high and low concentrations of metals in traditionally smoked and dried fish, but the contamination source was not determined (Adeyeye et al. 2016a, Ibanga et al. 2020). Others attribute metal increment in heat-treated fish to water loss (Kalogeropoulos et al. 2012).

1.3.2 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a group of ubiquitous compounds mainly derived from incomplete combustion of organic matter from anthropogenic activities, including coal mining, emissions from power plants, e-recycling and petroleum spills (Phillips 1999, Tobiszewski and Namiesnik 2012). The European Food Safety Authority (EFSA) has categorized 15+1 PAH congeners as priority-contaminants based on their carcinogenic and genotoxic effects in humans and the frequency of occurrence at hazardous waste sites (EFSA 2008). Owing to their hydrophobic and lipophilic characteristics, notable concentrations of PAHs are not naturally found in water, and their presence in surface water or groundwater is an indication of anthropogenic pollution (Kronenberg et al. 2017, Mojiri et al. 2019). Marine fish are known to contain low or undetectable concentrations of PAHs (Stołyhwo and Sikorski 2005, Essumang et al. 2012, Bandowe et al. 2014), whereas higher concentrations of pyrolytic PAHs have been detected in freshwater fish (Zhao et al. 2014, Nwaichi and Ntorgbo 2016). The PAH-load of fish reportedly remains unaltered from sun-drying (Mahugija and Njale 2018), while formation of PAHs during fish smoking is dependent on multiple variables, including fuel source, temperature, duration and the construction of the smoking chamber (Stołyhwo and Sikorski 2005, Essumang et al. 2013, Asamoah et al. 2021). In traditional kilns, the use of fuelwood combined with generation of high temperatures (300-700 °C) and direct contact between product and heat-source all contribute to the high levels of PAHs determined in smoked fish from multiple LMICs (Essumang et al. 2012, Mahugija and Njale 2018, Ingenbleek et al. 2019). Outer parts of fish are the main targets for PAH-accumulation (Stołyhwo and Sikorski 2005), whereas the overall PAH-concentration is positively linked with increasing fat content, thus displaying large seasonal variations (Essumang et al. 2012, Essumang et al. 2013).

1.3.3 Microbiological contamination

Fish are prone to microbial spoilage due to a high water content, near neutral pH and highly digestible protein (Gram and Dalgaard 2002, Ghaly et al. 2010). Cold storage is limited in many LMICs, which enables microbial growth and subsequent high post-harvest losses due to spoilage (Abbas 2009). Improper fish handling and inadequate hygienic practices represent additional exposure routes in many LMICs, and may result in contamination with pathogenic bacteria including *Salmonella spp* and *Escherichia coli* (Kombat et al. 2013, Antwi-Agyei and Maalekuu 2014, Sheng and Wang 2021). Thus, fish processing is essential to reduce microbial proliferation. Sun-drying reduces the water activity and inhibits microbial growth; however, the process is time-consuming and expose fish to ambient contamination (Kwenin et al. 2013). Smoking of fish in traditional open-flame kilns inhibits microbial growth by drying the product, as well as adding antioxidative woodsmoke components such as phenols and aldehydes (Arvanitoyannis and Kotsanopoulos 2012).

1.4 The role of fish in food and nutrition security

The nutritional properties of fish may play an important role for the health of billions of consumers (Béné et al. 2015, Thilsted et al. 2016, Hicks et al. 2019). Apart from being a highly nutritious food, the diversity of fish species represents an advantage compared with terrestrial ASFs by enabling accessibility for both high-income and low-income consumers (Mohanty et al. 2019). Considering the high prevalence of micronutrient deficiencies in the Global South, affordable SIS are regarded as key towards enhancing FNS (Thilsted et al. 1997, Roos et al. 2007a, Kawarazuka and Béné 2011). Recent projections by Golden et al. (2021) estimate that by 2030, a high-production scenario of seafood may improve FNS substantially through increased availability and accessibility, thus preventing 166 million cases of micronutrient deficiency. Furthermore, Maire et al. (2021) found that the micronutrient content of marine fish catches is generally higher in coastal countries affected by micronutrient deficiencies, highlighting an unmet potential. The pathways in which fisheries and FNS are interconnected are schematically presented in figure 1, illustrating direct (e.g. fish as food) and indirect (e.g. fish as a source of income used to purchase food) linkages. This encompasses small-scale fisheries, which contribute indirectly to FNS through providing livelihoods for over 100 million and sustenance for ~1 billion people worldwide (Short et al. 2021).

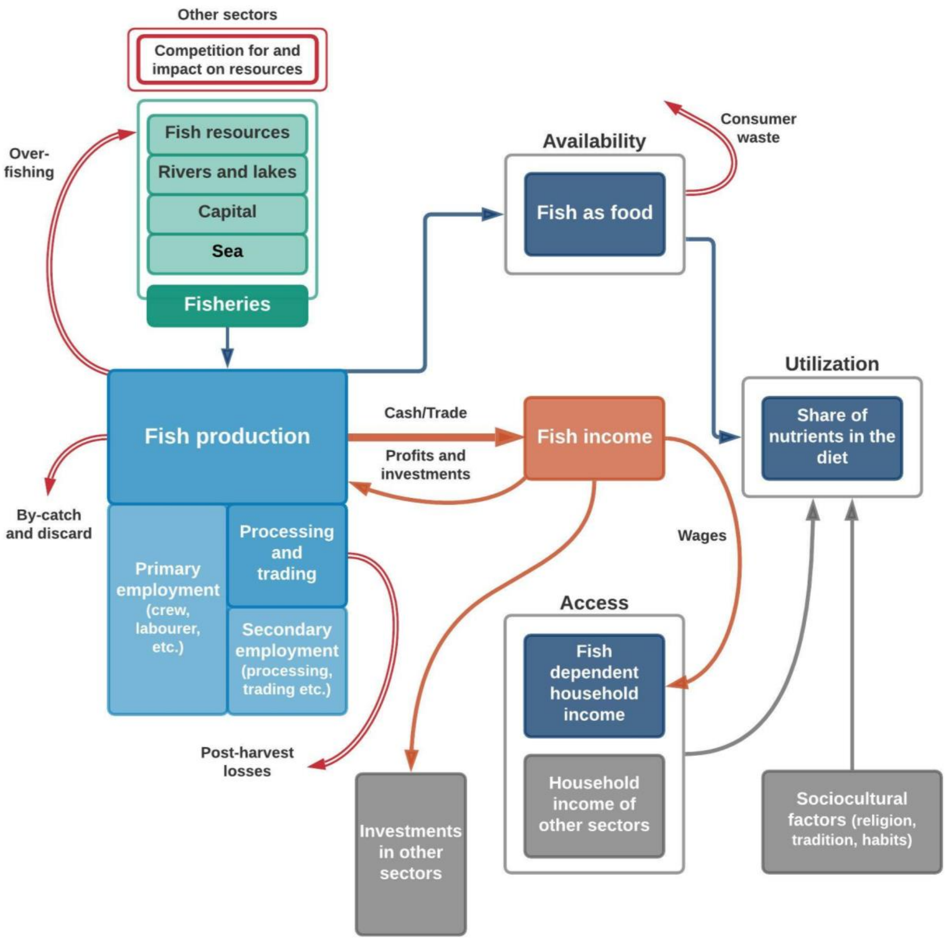

Figure 1: Conceptual representation of the pathways through which fisheries influence food and nutrition security. Adapted from HLPE (2014).

1.5 Ghana

The Republic of Ghana is located in West Africa with a total area of 238 540 km^2 and is home to an estimated 30 million people (The World Bank 2021). Ghana shares borders with Burkina Faso to the North, Côte d'Ivoire to the West, Togo to the East, and the Gulf of Guinea and Atlantic Ocean to the South. Grass-covered savannahs are found in the northern parts of the country and in part of the Southern coastal region, while one third of the territory is covered with evergreen and tropical rain forests

(Nunoo et al. 2014). Ghana's freshwater system is dominated by the Black- and White Volta headstreams which outflows into one of the world's largest manmade lakes, Lake Volta (8.482 km^2) (Davies et al. 2021). The Ghanaian economy has a rich natural resource base, including gold and industrial minerals, cocoa, and recently discovered offshore oil reserves (Davies et al. 2021). In 1957, Ghana became the first sub-Saharan country in colonial Africa to gain its independence, and consistently ranks among the top three countries in Africa for freedom of speech and press freedom (The World Bank 2021).

1.5.1 Ghana's fisheries

Ghana is endowed with rich and diverse fisheries, which mainly comprise marine capture fisheries along the 550 km continental coastline and the inland sector centered around Lake Volta (FAO 2016a). The fisheries sector account for approximately 4.5% of Ghana's Gross Domestic Product and provides livelihood for an estimated 10% of the population (3 million), including boat builders, fishers, fish processors, market traders and their dependents (MoFAD 2015). The fish value chain represents a cornerstone to FNS in Ghana; however, declining catches and illegal, unreported and unregulated fishing is challenging its sustenance (Atta-Mills et al. 2004, Nunoo et al. 2014, Asiedu et al. 2019, EJF and HenMpoano 2019).

1.5.2 Marine small fish resources

The marine sector is the main contributor to local fish supply in Ghana (80%), and consists of three subsectors: small-scale, semi-industrial and industrial fisheries (FAO 2016a). Annual landings in the marine sector are currently estimated at 300.000 metric tons and has been in consistent decline since peaking in 1996 (420.000 mt) (FishStat 2019). The productivity of Ghana's marine fisheries relies on a seasonal upwelling system, which brings cold nutrient-rich water to the surface and supports the growth of seaweed and plankton (Bakun 1978). Consecutively, this promotes the productivity of various marine fish species. Two annual upwellings occur on Ghana's continental shelf, the major season from July through September and the minor from December to early February, marking the periods with the highest catch rates (MoFAD 2015).

Marine small-scale or artisanal fisheries constitutes the largest subsector in terms of both employment and fish landings in Ghana, with 107.000 fishers supplying up to 70% of the marine fish catches (Dovlo et al. 2016, FAO 2016a). The sector is characterized by the use of motorized or non-motorized dug-out canoes (Fig 2) and use of diverse fishing gears including purse seines, hook and line, drift gill nets and beach seines (Asiedu et al. 2021). According to the latest canoe frame survey, artisanal fisheries are operated by 12.000 canoes from 292 landing beaches along the Ghanaian coast (Dovlo et al. 2016). Pelagic SIS are the main targets, including sardine (*Sardinella aurita*), Madeiran sardinella (*Sardinella maderensis*) European anchovy (*Engraulis encrasicolus*) Cunene horse mackerel (*Trachurus trecae*) and Atlantic chub mackerel (*Scomber colias*) (FAO 2016a). Stocks of pelagic SIS have followed a similar decline to total marine catches in the last decades, with the maximum level recorded in 2000 (237.000 mt) and lowest recorded in in 2018 (135.000 mt) (Asiedu et al. 2021). Still, pelagic SIS represents the bulk of marine fish landings in Ghana.

Figure 2: Artisanal canoes (left) and artisanal fishermen (right) in the port of Tema, Ghana. Photos by Astrid E. Hasselberg.

The marine semi-industrial and industrial sectors comprise a variety of vessels, including motorized plank boats and large foreign built trawlers operating bottom trawls and purse seines (FAO 2016a). Catches from the two sectors are estimated at <28.500 mt, representing 8% of total marine output (MoFAD 2015). Semi-industrial vessels mainly operate inshore during the seasonal upwellings using purse seines, where they compete with the artisanal fishing fleet for SIS, including sardinellas, mackerel and other *Carangidae* species (FAO 2007, FAO 2016a).

1.5.3 Inland fisheries

Ghana's inland fisheries are exclusively artisanal and mainly undertaken in Lake Volta, providing approximately 90% of inland fish catches (FAO 2016a). The most commercially important fish species in Lake Volta include tilapia (*Tilapia spp*) and catfish (*Chrysichtys spp, Synodontis spp*) (van Zwieten et al. 2011). SIS are abundant in Ghana's inland waters, including WA pygmy herring *(Sierrathrissa leonensis*), *Alestes* and *Brycinuys* species (Nunoo and Asiedu 2013). However, the catch profile and catch rates in the inland fisheries are not well documented, and the annual yield has consistently been reported as 90.000 mt the last ten years by the FAO (FishStat 2019). Recent canoe frame surveys from the inland fisheries are also not available, thus adding to the uncertainties regarding the contribution of inland fisheries to FNS in Ghana.

1.5.4 Fish processing and trading

Women dominate the onshore activities in Ghana's fish value chain, including *fish wives* responsible for processing, retailing and selling fish at the local markets (Ameyaw et al. 2020) and *fish mammies* who owns fishing canoes and engage in large-scale trading (Walker 2001, Overå 2003). The majority of landed SIS in Ghana are processed by smoking (80%) or preserved by sun-drying, salting, fermentation or frying (Adeyeye and Oyewole 2016b). Fish smoking is commonly performed at high temperatures using traditional metal drum kilns or "Chorkor smokers" operating on fuelwood (Adeyeye and Oyewole 2016b, Asamoah et al. 2021). Drying is carried out by spreading fresh SIS on various surfaces, ranging from purpose-built drying-racks, to rocks or directly on the ground and sun-drying for 3-5 days (Atter 2021). Processing may extend the shelf life of SIS up to 9 months, even when stored at room temperature (Sakyi et al. 2019). This also enables long-distance transportation of SIS throughout the country without depending on cooling facilities, and access is secured by keeping a low price level (Nunoo et al. 2015).

1.5.5 The Ghanaian diet

The Ghanaian diet largely relies on indigenous starchy staple foods, including roots (cassava, yams), fruit (plantain) and cereals (maize, rice), which supply three quarters of dietary energy (Nti 2008, FAO 2010). Traditional dishes such as *fufu* and *banku* consist of pounded and fermented staples served together with soups or stews, accompanied by small amounts of ASFs (Amuquandoh and Asafo-Adjei 2013). Fish has a central part in the Ghanaian diet and is consumed across the demographic spectrum by all age groups throughout the country (FAO 2016a, Christian et al. 2019). The annual per capita fish consumption is estimated at 28 kg and constitutes over 50% of total animal protein intake, which is considerably higher than Africa’s 12 kg/cap/year average (FAO 2016a, FAO 2020). In tune with the majority of LMICs, rapid urbanization and migration has spurred the westernization of urban diets in Ghana, while traditional dietary patters persist in rural areas (Amuna and Zotor 2008, Galbete et al. 2017). This pattern is also apparent for protein consumption, with higher intakes of meat and large fish species in urban populations, whereas rural dwellers rely more heavily on SIS resources (Asiedu et al. 2016, Sumberg et al. 2016). SIS are also utilized in traditional Ghanaian complementary food blends for young children, which predominantly comprise fermented maize porridge (*koko*), millet or ready-to-use cereal and legume blends (Lartey et al. 1999, Amangloh et al. 2011).

1.5.6 The state of food and nutrition security in Ghana

Ghana has taken major strides since the 1990s by attaining status as a lower middle-income country, halving poverty rates and by reducing the prevalence of malnutrition (GSS et al. 2014, Vasco and Paci 2015). Recent improvements notwithstanding, regional disparities are increasing with poverty rates exceeding 40% in most northern districts, compared with 20% in the urban south (GSS et al. 2014, Cooke et al. 2016).

Malnutrition remains persistent among children under the age of five in Ghana, where one-fifth (19%) are stunted, 5% suffer from wasting, and two-thirds (66%) of children between 6-59 months of age are anemic (GSS et al. 2014). However, large regional variations are present, and the Northern region of Ghana have the highest rates of child stunting (33.1%) and anemia (82%) (GSS et al. 2014). While breastfeeding is common practice, only half of children under 6 months are exclusively breastfed and complementary feeding practices are inadequate (FAO 2010). This, in combination with household food insecurity and inadequate health services, are among the main causes of malnutrition among young children. Although economic constraints are the main limitations to access nutritious foods in Ghana, cultural beliefs and taboos also inhibit use of certain ASFs for women and young children, which may aggravate the prevalence of malnutrition (Colecraft et al. 2006, Dalaba et al. 2021).

1.6 Food composition data

Food composition data (FCD) represents the nutritionally important components of foods, and provides values for energy, nutrients and non-food components including fiber and water (INFOODS 2017). Access to reliable and up to date FCD, including local and regional foods, constitutes the ground pillar of nutrition-related research (Pennington et al. 2007, Stadlmayr et al. 2011, Charrondiere et al. 2016, Rittenschober et al. 2016). The use of food composition data is widespread, ranging from policymaking, farmers' selection of cultivars, food labelling, implementation of health programs and calculating nutrient intake from dietary assessments (Fig 3).

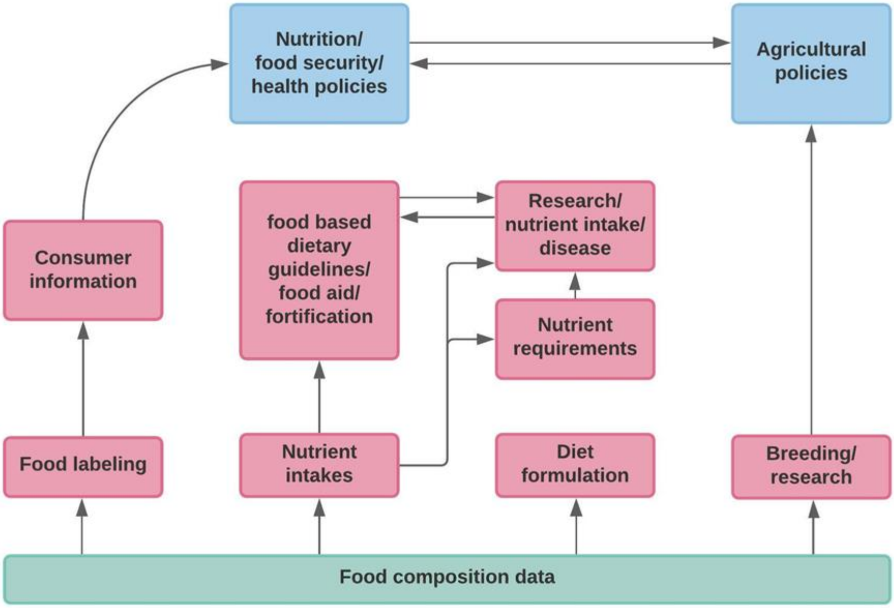

Figure 3*: “Food composition data - The Base for a Multitude of Nutrition Activities”* adapted from INFOODS (2017).

1.6.1 Compilation of food composition data

FCD is compiled from either direct nutritional analyses of foods, by imputing values, or calculations from complementary literary sources. Values from original laboratory analyses are considered the gold standard but is not always feasible to obtain due to limitations of financial resources or infrastructure capacity. According to international standards set by INFOODS (2017), a reliable food composition table (FCT) should contain comprehensive information and descriptions of important nutrients and food items and provide documentation for the data source of each nutrient value. A FCT should also include cooked foods, mixed dishes, processed foods, fortified foods, and biodiverse foods that reflect the habitual food consumption and represent the majority of the consumed foods in the given country (Greenfield and Southgate 2003).

1.6.2 Food composition data in Ghana

In conformity with most LMICs, available FCD from Ghana remains limited (de Bruyn et al. 2016). The only FCT from Ghana available online consists of proximate and fatty acid composition of 81 foods and mixed dishes, including 17 varieties of fresh or processed fish (Westenbrink et al. 1983). Currently, the FAO/INFOODS FCT for Western Africa represents the most up to date FCD available, including 106 entries on raw fish, cooked fish, and dishes containing fish (Vincent et al. 2020). However, the FCD is not country-specific and up to date FCD on micronutrients and contaminants in processed indigenous fish from Ghana is missing.

2. Objectives

The broad aim of this thesis was to explore how SIS contribute to FNS in Ghana. This was assessed both though a literature review (paper I) and by providing novel analytical data on nutrients and contaminants in different processed SIS and tissues (papers II and III). The specific aims of the study were addressed in three articles, as listed below:

Paper I: Assess the current challenges in the Ghanaian fish value chain and how future strategies can strengthen the role of SIS towards enhancing FNS in Ghana.

Paper II: Determine the nutritional quality and food safety of six commonly consumed processed SIS from Ghana by analyzing key nutrients, heavy metals, PAHs and microbial contamination.

Paper III: Determine nutrient and contaminant distribution in tissues of smoked European anchovy and how this affects potential nutrient and contaminant exposure in vulnerable population groups.

3. Methods

Methods used in the three papers in this thesis are described below. For a detailed description of methods used for laboratory analyses of nutrients and contaminants, the reader is referred to the respective papers.

3.1 Literature review

In paper I a topical approach was chosen to assess the linkages between fish, fisheries and FNS in Ghana in a mixed-methods narrative literature review. For this purpose, a conceptual framework based on the four pillars of food security was designed in collaboration with key persons prior to the literature search (Fig 4).

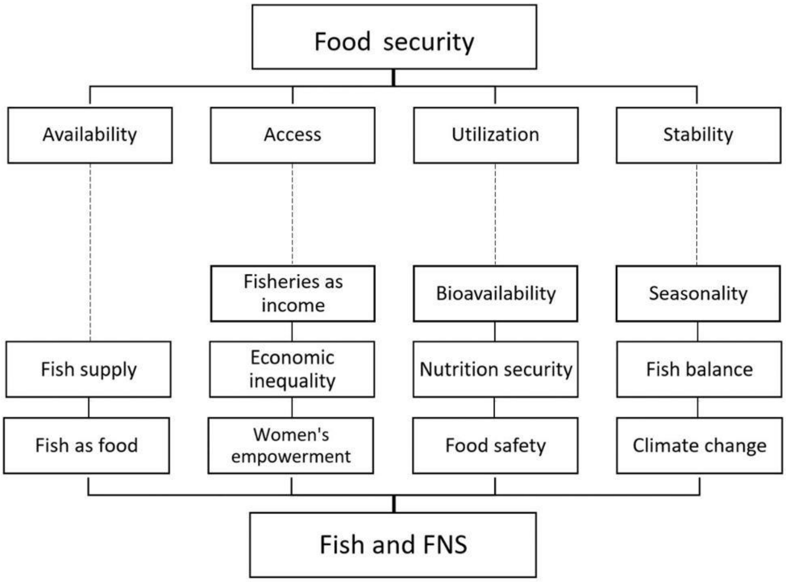

Figure 4: Conceptual framework for article I. The four pillars of food security (FAO 2006) and selected focus areas related to fish and FNS in Ghana.

Relevant studies were identified by a single literature search in Embase (OVID), Ovid MEDLINE® and Web of Science (Clarivate) from inception up to April 8th, 2019. Primary screening and subsequent inclusion criteria applied to full text assessment of the literature adhered to the recommendations outlined for the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al. 2009). Additionally, relevant grey literature from manual searching and research articles recommended by key collaborators were included in the final synthesis.

3.2 Selection of fish species and sampling locations

The foundation for selecting relevant SIS for sampling originated from the EAF Nansen project, which is a joint initiative of the Norwegian Agency for Development Cooperation, FAO and the IMR (FAO 2016b). The objective of the project is to aid LMICs in improving food security by identifying seafood resources through ocean surveys by the research vessel Dr. Fridtjof Nansen. Pelagic trawl data on SIS from a 2017 cruise within Ghana's exclusive economic zone was used as a starting point for our selection of species, however, this data remains unpublished. The marine SIS in question were Cunene horse mackerel (*Trachurus trecae*), European anchovy (*Engraulis encrasicolus*), Brazilian lizardfish (*Saurida brasiliensis*), bigeye grunt (*Brachydeuterus auritus*), red pandora (*Pagellus bellottii*), round sardinella (*Sardinella aurita*), Atlantic chub mackerel (*Scomber colias*) and African moonfish (*Selene dorsalis*).

To assess the commercial importance of the SIS and the common preservation methods used for the given species in Ghana, four open fish markets in three regional capitals, namely Accra, Techiman and Kumasi, were visited during February 2018 (Fig 5). At the markets, the different seafood products available for sale in each market stall and their respective processing methods were registered. Accordingly, the following marine SIS, all of which were most commonly smoked (92%), were selected based on availability at the markets: European anchovy, bigeye grunt, round sardinella and African moonfish. In one of the fish markets in Accra, Adabraka, the fish wives and mammies were only selling freshwater species (Fig 5). The two most common freshwater SIS identified at Adabraka were therefore selected for sampling, namely salted tilapia (*Tilapia spp*) and dried WA pygmy herring (*Sierrathrissa leonensis*).

For paper III, European anchovy was selected as the most relevant SIS due to its documented use in complementary foods (Lartey et al. 1999, Amangloh et al. 2011) and through recommendation by key persons in Ghana[1].

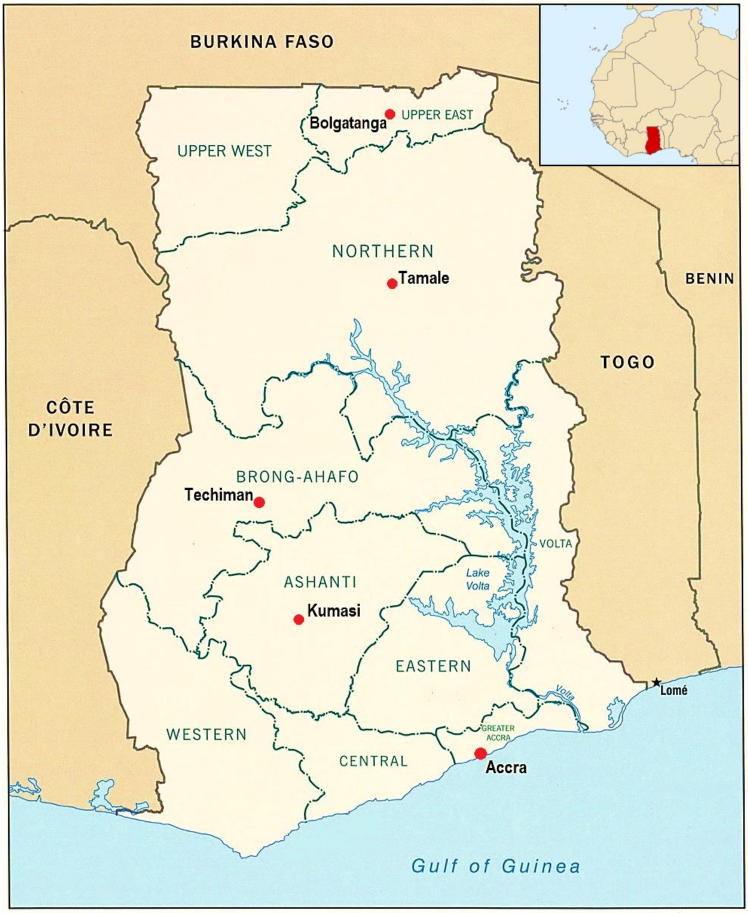

Figure 5: Sampling locations in Ghana. Adapted from Rei-artur (2006) and U.S. Central Intelligence Agency (2007).

Sampling locations were selected on the basis of obtaining geographically stratified data, and included open fish markets in the capital city of five regions in Ghana, namely: Accra in the Greater Accra region, Kumasi in the Ashanti region, Techiman in the

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[1] Matilda Steiner-Asiedu, University of Ghana and Amy Atter, CSIR Food Research Institute, Accra, Ghana

Brong-Ahafo region (now Bono East), Tamale in the Northern region and Bolgatanga in the Upper East region (Fig 5).

3.3 Size of samples

Ensuring a sufficient number of samples is essential to secure representable FCD (Holden et al. 2002, Greenfield and Southgate 2003, Codex Alimentarius Comission 2004). Composite samples are frequently used in the aggregation of FCD and consist of mixtures formed by combining primary samples of the same or different foods. The number of samples needed to achieve reasonable levels of confidence per composite- and subsequent analytical sample depends on the nutrient variability of foods. Fish are known for their variation in nutrient content, particularly vitamins and seasonal fluctuations in fat content (Ababouch 2005, Zlatanos and Laskaridis 2007). To emulate nutrient variations and account for the effect of processing in calculations of sample number, certain preliminary information of the food in question is needed. Thus, analytical values on vitamin D, DHA and I in smoked herring and mackerel were obtained from the literature (Usydus et al. 2009b) and calculation of sample numbers was subsequently performed using the following equation from Greenfield and Southgate (2003):

Sample size $\geq (t\ \alpha\ n\ -1)^2$ standard deviation^2/(precision × mean value)^2

The alpha was set at 0.05 and precision at 0.1, while standard deviation, mean values and n-1 were borrowed from Usydus et al. (2009b) and t-values were collected from student's t table.

The number of samples varied considerably depending on the nutrient used for calculation, from 8 when DHA in smoked mackerel was used to 958 when I in smoked mackerel was used. A more general sampling protocol was therefore adopted. At each market, 5 batches containing a minimum of 12 primary samples each were collected at different market stalls and combined into a composite sample consisting of 60 primary samples. Furthermore, we estimated that approximately 500 g of sample material was needed for the selected laboratory analyses, which resulted in a higher number of primary samples in the composite samples of certain species. This was mainly influenced by fish size but also fish availability, with the mean number primary samples in each composite sample ranging from 43 for tilapia spp to approximately 250 for European anchovy.

3.4 Risk-benefit scenario

Research to inform public health policies is often focused on either food safety and risk assessment, or nutritional assessments where the beneficial properties of foods are weighed (Pires et al. 2019). On the contrary, a risk-benefit assessment (RBA) is an integrated and multidisciplinary approach to assess both risks and benefits of foods. According to EFSA guidelines (EFSA 2010a), problem formulation should precede the RBA. Hence, assessing the balance of risks and benefits caused in a population by a dietary component, in this case tissues of smoked European anchovy, was the starting point for paper III. The study was limited to an initial RBA (Step 1), where the question of whether the health risks clearly outweigh the health benefits or vice versa was assessed (EFSA 2010) (Fig 6).

Collectively known as the *Terms of Reference*, an RBA should also specify the population group and dietary exposure of the given food, presented in one or multiple scenarios (EFSA 2010a, Boobis et al. 2013). Recognizing the importance of adequate nutrition during the first 1000 days of a child’s life (Victora et al. 2021), infants and toddlers aged 6-23 months were chosen as the target population group in paper III. This approach was further supported by data reporting that inadequate complementary feeding and subsequent nutritional deficiencies are prevalent in this population group in Ghana (GSS et al. 2014). Moreover, traditionally smoked fish may be a source of PAH and heavy metals, which are detrimental to the developing brain (Bose-O'Reilly et al. 2010, Xia et al. 2010, Flora et al. 2012, Rodríguez-Barranco et al. 2014).

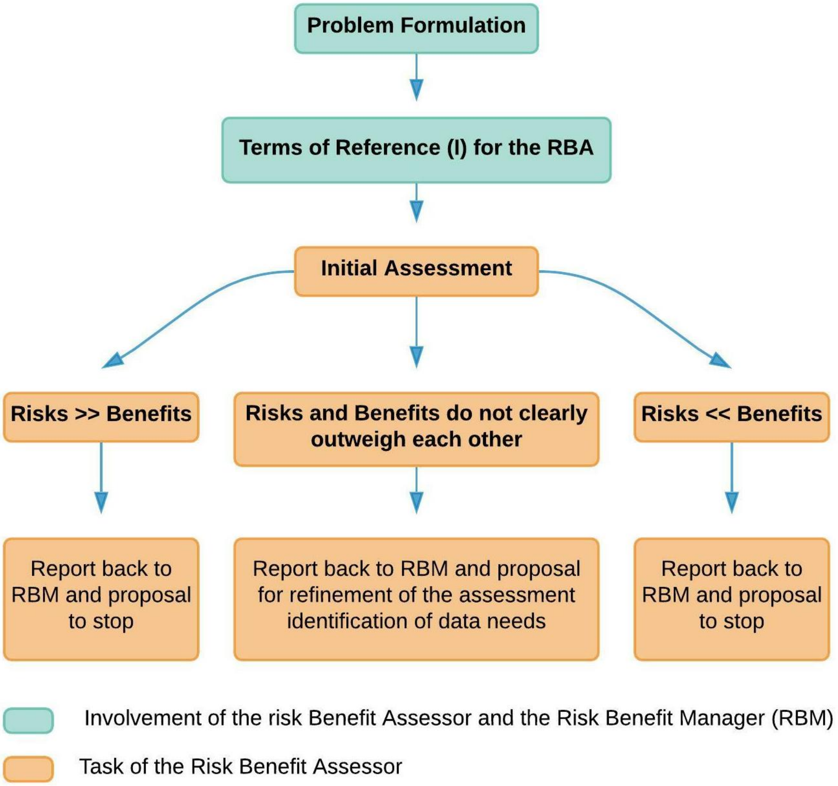

Figure 6: First step of the EFSA approach for risk-benefit assessment – Initial assessment. Adapted from EFSA (2010a).

The high fish consumption in the Ghanaian population also applies to young children living in coastal areas (Bandoh et al. 2018), but the species and amount of fish used in complementary foods is sparsely reported. We therefore used data from formulations used in three studies on complementary foods to estimate portion size (e.g. dietary exposure) in the scenario (Lartey et al. 1999, Amangloh et al. 2011, Bogard et al. 2015). Values used to calculate recommended nutrient intakes (RNIs) and upper tolerable intake levels (ULs) were selected according to compliance to the age group, country of origin and conservativeness (e.g. strictness). In lack of national recommendations for nutrient intakes in Ghana we utilized international (FAO/WHO), European and United States reference values. Specific recommendations for RNIs in the selected age range

(6-23 months) were in many cases not available and had to be calculated using the mean of two values. Models and reference levels used to assess exposure to heavy metals and PAHs were primarily obtained from European sources (EFSA), given the dearth of international alternatives.

4. Summary of results

4.1 Paper I

Paper I is a mixed methods narrative literature review, aiming to identify and review available literature on fish, fisheries and FNS in Ghana. A conceptual framework was developed to highlight relevant topics for review, and to capture the links between the four pillars of food security and fish, fisheries and FNS. An independent primary literary search was performed in Embase, MEDLINE and Web of Science. Articles were included based on relevance to the selected topics and additional inclusion criteria, adhering to the PRISMA guidelines.

The literature search yielded 848 potentially relevant articles, of which 38 research papers met the inclusion criteria. An additional 41 reports and 38 research articles were retrieved from other sources and were included in the synthesis. Overall, the results indicate that there are multiple constraints throughout the small-scale fisheries, affecting FNS in Ghana. Fish availability and access is challenged by the uncertainties in reported fish landings, competition from the industrial sector, and increasing inequality, affecting the livelihoods of up to 10% of the population. Utilization is constrained by inadequate young child feeding practices and healthcare, and through food safety issues arising from poor fish handling, fish processing and anthropogenic pollution. The stability of fish, fisheries and FNS in Ghana is restricted by seasonal variations in fish landings, climate changes and disposition of fisheries resources. SIS and small-scale fisheries have the potential to significantly improve FNS in Ghana, but its importance remains underrecognized in health policies, fisheries legislation and global fora.

4.2 Paper II

Paper II reports the concentration of selected nutrients, heavy metals, PAHs and microbiological contamination in six processed SIS from Ghana. Composite samples of smoked European anchovy (*Engraulis encrasicolus*) bigeye grunt (*Brachydeuterus* *auritus*), round sardinella (*Sardinella aurita*), African moonfish (*Selene dorsalis*) and dried/smoked SIS WA pygmy herring (*Sierrathrissa leonensis*), and salt-dried tilapia (*Tilapia spp.)* were collected from fish markets in five different regions throughout Ghana.

Analyses of proximate composition, fatty acids, minerals, trace elements and vitamins A, D and B_{12} uncovered large intra-and interspecies variations. All marine SIS contained substantial concentrations of the selected micronutrients and could be considered valuable additions to the staple-food dominant Ghanaian diet. Tilapia stood out as markedly less nutrient dense, while the freshwater SIS WA pygmy herring displayed several nutritional characteristics similar to the marine SIS. It was discovered that the concentration of several micronutrients in the processed fish were elevated compared with levels reported in fresh fish, indicating that smoking and drying is not detrimental to micronutrient retention. High counts of coliform bacteria were found in 70% of the fish samples, indicating fecal contamination from handling and underscoring the need of improving hygienic conditions throughout the fish-value chain. Additionally, elevated levels of Fe and heavy metals Cd and Pb were determined in certain samples. While this may be attributed to the reduced water content from processing, further research and identification of anthropogenic sources along the value chain is warranted. Levels of PAH4 in smoked fish samples reached high concentrations, up to 1.300 µg/kg, whereas salt-dried tilapia samples had a range of 1-24 µg/kg PAH4. Given the high concentrations of Fe, Cd, Pb and PAH4 determined in this study, identifying critical points and developing applicable mitigation strategies to improve the quality and safety of processed SIS in Ghana is essential.

4.3 Paper III

The aim of paper III was to determine the content of selected nutrients and contaminants in different tissues of smoked European Anchovy including whole fish, heads and skin and samples without heads and skin. A scenario referencing 6-23-month-old Ghanaian infants and toddlers was developed to assess the benefits and risks associated with consumption of the different tissues.

All analyzed tissues had the potential to substantially contribute to the recommended nutrient intakes of selected vitamins, minerals and essential fatty acids for young children. Samples containing heads and skin had the highest concentrations of all minerals, vitamins and fatty acids, with an estimated portion (17 g) contributing >50% to the recommended nutrient intake-targets for all selected nutrients except iodine, followed by >40% for whole fish and >25% for samples without heads and skin. Samples of heads and skin contained the highest concentrations of As (7.8 mg/kg), iAs (0.10 mg/kg) and significantly higher levels of Cd (0.34 mg/kg) and Pb (0.202 mg/kg) compared with samples without heads and skin. Furthermore, samples without heads and skin contained significantly higher concentrations of MeHg (0.049 mg/kg) than samples of heads and skin (0.018 mg/kg). Samples of heads and skin contained the highest mean concentration of PAH4 (608 µg/kg), followed by whole fish (478 µg/kg) and samples without heads and skin (400 µg/kg). PAH4 levels exceeded EU-limits between 33 and 51-fold and calculation of margin of exposure (MOE) illustrated that all tissues contained levels of BaP and PAH4 below the safety threshold, entailing potential consumer risk.

Collectively, samples of heads and skin contained the highest concentrations of nutrients and contaminants, followed by whole fish and samples without heads and skin. Despite the nutritional potential of incorporating different tissues of smoked European anchovy in the diet of young children, the elevated concentrations of Cd, Pb and severely elevated levels of BaP and PAH4 currently inhibits safe use.

5. Discussion of methods

5.1 Literature review

A literature review provides a methodological approach to answer a number of research questions, from a clear hypothesis, to a broader context where the collective evidence in a certain research area is examined (Snyder 2019). Given the broad conceptual scope of paper I, a mixed methods approach, including both qualitative and quantitative research literature was considered most suitable. The literature was obtained via multiple sources, including both systematic- and manual searches and by recommendation, and compiled in a narrative-style synthesis.

The pitfalls of literature reviews are many, and the exemption of a systematic framework introduces many routes of bias, including *reporting bias* (selective dissemination of research findings), *selection bias* (from missed studies) and *publication bias* (due to non-publication) (Drucker et al. 2016). There are no acknowledged frameworks available for non-systematic or narrative reviews, but Ferrari (2015) proposes that bias may be mitigated by borrowing methodological approaches aimed at systematic reviews. Based on this premise, predefined search words and inclusion/exclusion criteria adhering to the PRISMA framework was used in paper I (Moher et al. 2009). However, the topical approach and general fluidity of the FNS-concept is not compliant with systematic tools and deciding on exclusion and inclusion criteria for such a wide scope was challenging.

This imparity was further confirmed by the literature search, which did not yield sufficient data for a thorough review. On this basis, we included additional research articles recommended by key collaborators and grey literature. While this broadened the theoretical basis of the review, it also reintroduced the many biases associated with narrative reviews, and compromised reproducibility (Yuan and Hunt 2009). This can be exemplified by the inclusion of recommended research articles (e.g. selection bias), which may conclude with recommendations that are inconsistent with those of other experts or with the literature. Furthermore, including grey literature (e. g., non-peer reviewed reports or conference papers) in the synthesis added to selection bias due to its incompatibility with rigorous criteria. Limitations notwithstanding, the development of a conceptual framework and application of PRISMA guidelines provided an added degree of transparency and strengthened the narrative format of article I.

Although a standardized approach to evaluate study quality is not available for this format, the use of mixed methods allows inclusion of more data compared with a monomethod approach and may therefore provide a broader understanding of complex topics (Molina-Azorin and Cameron 2010, Paez 2017). In the context of fish, FNS and Ghana, this approach enabled a greater recognition of the contexts and perspectives of the potential users of research findings (Leeman et al. 2015). Hence, we were able to discuss the linkages between the small-scale fish value-chain, FNS and Ghana in a holistic perspective.

5.2 Size of samples

When calculating the number of samples for FCD, the analytical data used to determine the mean and standard deviation should ideally come from a pilot study. However, the geographical distance to the sampling location and the high cost associated with sampling and nutrient analyses inhibited this approach in the current study. As elaborated in Greenfield and Southgate (2003), the second-best option relies on analytical data obtained from the scientific literature, which we used for sample calculations in paper II and III.

Analytical data may be abundant for certain commercially important fish species in high income countries, but there is a dearth of available food composition data on fish from LMICs (de Bruyn et al. 2016, Rittenschober et al. 2016). Resultantly, no publications on vitamins and fatty acids in traditionally smoked small fish from Ghana or other African LMICs were obtainable for calculations of number of samples. The concentration of vitamin D, DHA and I in smoked herring and sprat from Poland (Usydus et al. 2009b) was subsequently considered as the best fit. However, smoking practices in Poland and Ghana are widely different, and other factors such as seasonality was not accounted for, which may have biased sample calculations. The general approach to calculate number of samples for FCD, led by the work of Greenfield and Southgate (2003), state that *"the required information is often incomplete and one has to proceed intuitively"*. Thus, using high quality analytical data for our calculations rather than resorting to intuitive guesses adds to the precision of size estimations.

Nutrients constitute the basis of FCD, but the inclusion of analytical values on heavy metals, PAHs and selected bacteria in study II raises the question of whether the variation of contaminants should be accounted for in calculations of sample numbers. The predicament of contaminants is that sources may be of anthropogenic origin, which adds another layer of variability (Elmadfa and Meyer 2010). In a Ghanaian setting, this pertains to PAHs from fish smoking, post-harvest bacterial contamination and heavy metal contamination from haphazard waste management, among others (Kombat et al. 2013, Itai et al. 2014, Asamoah et al. 2021). Given the multitude of anthropogenic variables, we decided to adhere to standard calculations based on nutrient variability in our study. However, whether the variability of certain contaminants in fish exceeds that of vitamins should be elucidated to secure a valid size of samples for both benefit and risk estimations.

Collecting and analyzing FCD is costly, and it should be borne in mind that budget constraints often limit the possibility to analyze several primary or composite samples of the same food. Papers II and III include analytical data on a wide array of vitamins, minerals, trace elements, heavy metals and PAHs, which was considered essential to assess the data in a risk-benefit perspective. As a result, the number of composite samples analyzed for each species was restricted to five, which is less than the ideal number of 12 analytical samples as proposed by Holden et al. (2002).

A small number of analytical samples limits the ability to estimate the mean and variability of foods, but through compositing, a reliable estimate of the actual nutrient concentrations in foods may be produced (Pehrsson et al. 2000, Danster and Wolmarans 2008). Hence, while we analyzed five composite samples of European anchovy, each consisted of more than 250 fish and the mean of each sample therefore represents an extensive sampling of the species. It must, however, be recognized that analytical determination of nutrient variability is masked or confounded following compositing (Holden et al. 2002). This is also relevant for contaminants, since compositing may mask high values and inhibit identification of potential pollution sources.

5.3 Sampling protocol

Sampling protocols for FCD are associated with random and systematic errors, from incorrect identification of foods during sampling to inaccurate preparation of analytical samples (Greenfield and Southgate 2003, Codex Alimentarius Comission 2004). The current sampling plan was constrained by the nature of Ghana's fish markets, which is dependent on the off- and onshore activities in the small-scale fish value-chain.

Prior reconnoitering and registering of SIS in Accra, Techiman and Kumasi fish markets facilitated the sampling process in the Southern regions, while sampling at the Northern markets was more unpredictable. All selected marine SIS were available for sampling in the three southernmost regions, whereas only European anchovy and round sardinella were available at Bolgatanga and Tamale markets. Overall, freshwater SIS were more frequently observed at the northern markets, while markets in Accra had distinct freshwater or marine profiles. As a result, not all SIS were sampled from each location and some of the composite samples consisted of less than five batches, which may have affected variability. These disparities underscore the importance of regional stratification of sampling in Ghana, where factors such as food distribution systems and local food culture largely influence fish availability and accessibility between regions (Greenfield and Southgate 2003). This issue was encountered in Bolgatanga, when upon arrival we were informed that the main fish market was only open every third day, and that fish mammies returned from southern Ghana with fish fortnightly (Figure 7).

Figure 7: Calm day at the fish market in Bolgatanga (left) and selection of processed fish at a market stall in Accra. Photos by Astrid E. Hasselberg

The fat content of fish is known to display large variations during the year (Ababouch 2005), which in turn may influence the concentration of n-3 fatty acids, fat-soluble vitamins, deposition of PAHs during smoking and post-harvest spoilage. Our sampling was conducted during November, between the two upwelling seasons, and should ideally have been repeated biannually or quarterly to capture seasonal variations. However, seasonal variations may be present at both intra-and interspecies levels in the samples, since processing can extend the shelf life of SIS up to nine months (Sakyi et al. 2019).

5.4 Sample preparation and analyses

Preparation of analytical samples and choice of analytical methods are recognized as critical points in FCD generation (Greenfield and Southgate 2003). As discussed by Stadlmayr et al. (2013), FCD generated in West Africa may be limited by financing and use of older analytical methods may compromise data validity. Thus, being able to analyze the Ghanaian fish samples at the IMR laboratories using ISO 17025:2005 accredited methods should be recognized as one of the study’s strengths.

The analytical method used for vitamin A_1 determination is reported as sum retinol, which consists of 13-, 11-, 9- cis and all-trans retinol (ROH) isomers (Moxness Reksten et al. 2020). While the distinct cause remains unknown, instrument interference led to uncertain identification and quantification of 13-cis ROH and all-trans-ROH in certain samples of smoked European anchovy. In paper II this involved two samples of whole European anchovy, while six samples (40%) were affected in paper III. On this basis we decided to exclude vitamin A_1 from the formal analyses in paper III.

Wet sample material was used for both vitamin A, D and FA-analyses, with each aliquot consisting of 0.1-1 g. The texture of the whole processed SIS was viscous and tough and made homogenization challenging, which is exceedingly important when using small aliquots from composite samples. Analytical samples for mineral, heavy metal and PAH analyses were freeze dried prior to analysis, which improved the homogenate substantially. We therefore theorize whether a more powerful food grinder or freeze drying could better facilitate homogenization of the wet sample material and recognize this as one of the study's limitations.

5.5 Risk-benefit scenario design

Available risk-benefit methodologies are primarily based on a stepwise approach, which includes a lower tier assessment where risks and benefits are qualitatively evaluated and use of increasingly sophisticated methods at higher tiers (EFSA 2010a, Hoekstra et al. 2012, Boobis et al. 2013). The initial, or step1, RBA performed in paper III is best executed by addressing two, or a series of different scenarios depicting various levels of exposure (EFSA 2010a). However, based on the multitude of nutrients and contaminants included in paper III and the lack of data on fish consumption in Ghana, we decided to perform a single-scenario RBA. As an alternative approach, we re-calculated the portion size in order to meet the safety margins of selected contaminants, thus illustrating the decrease in benefits associated with portion reduction.

While the majority of RNIs used in paper III account for differences in bioavailability for key nutrients (FAO and WHO 2004), ULs do not encompass variations in habitual dietary intakes or health status (Pike and Zlotkin 2019). Thus, its application may not be compliant with a Ghanaian setting, since diets high in phytates and infectious disease can affect nutrient absorption and utilization (Engle-Stone et al. 2019). Furthermore, no data was available to set age-specific ULs for young children. Leading institutions, including the US Institute of Medicine, extrapolate ULs from other age groups based on body weight, which may lead to overly restrictive ULs (Institute of Medicine 2000, Pike and Zlotkin 2019).

Both the joint FAO/ Expert Committee (JECFA) and European Food Safety Authorities (EFSA) have derived MOEs for BaP, PAH4 and Pb (EFSA 2008, EFSA 2010b). EFSA's approach was used to assess the MOE for BaP and PAH4 in smoked European anchovy from Ghana paper III, but yielded unrefined results. We recognize that EFSA is a European institution and that the low dietary exposure in high-income populations may lead to no further refinement of risk assessment tools. However, this is a symptom of the lack of focus on LMICs in risk assessments. Furthermore, in the case of the MOE-range for Pb, it is clearly stated that this does not include exposure from other sources (EFSA 2010b). The calculated MOE-range for Pb in a given food commodity consumed by a child living in a polluted area in Ghana, or elsewhere, would not necessarily be reflective of the true exposure. Children are a complex population group in risk assessments, both due to the higher exposure levels compared with adults, but also on account of behavioral traits such as mouthing behavior, which may increase toxicant exposure from non-food sources (Gati et al. 2014, WHO 2015).

6. Discussion of results

In this section, results from papers I, II and III are discussed collectively and presented according to the pillars of food security: availability, access, utilization and stability.

6.1 Small fish availability and access

SIS availability represents the backbone of Ghana's small-scale fish value chain. As presented in paper I, official (FAO) statistics have reported an estimated 30% reduction in Ghana's marine capture fisheries since the 1990's (FAO 2016a). A similar trend has recently been reported for pelagic SIS stocks by Asiedu et al. (2021), who estimates a 57% decline between 2000-2018. Due to the open access in Ghana's marine small-scale fisheries, the number of canoes has increased drastically in later years, which has resulted in a reduced catch per unit effort (CPUE) (Dovlo 2020). The current CPUE represents about 25% compared with the highest CPUE recorded in the early 1990s and is indicative of a declining economic profitability in Ghana's small-scale fisheries (Lazar et al. 2020).

Official and unofficial fisheries statistics in Ghana were compiled in paper I, which showed that annual fish availability ranged between 716.500-1.025.000 mt in low versus high estimates. Translated to per capita fish supply, this represents 25 or 35 kg per annum; a variation which may have substantial implications for FNS. One of the main confounders in Ghanaian marine fisheries statistics is the missing data on transshipment of illegally caught SIS from industrial bottom trawlers to artisanal canoes at sea, locally termed "saiko" (FAO 2007, Lazar et al. 2020). The volume of fish transshipped in the saiko trade was estimated at 101.000 mt in 2017 by the Environmental Justice Foundation (EJF and HenMpoano 2019), which may account for some of the reported decline in pelagic SIS catches and CPUE. Although there is a prohibition against foreign ownership and operation of trawlers in Ghana (Ghana Fisheries Commission 2002), an estimated 90% is reportedly owned by foreign nations (EJF 2021). Saiko thus represents a dual threat to Ghana's small scale-fisheries, where not only the available SIS increasingly ends up in the hands of a foreign power, but the revenues alike.

As we reported in paper I, the uncertainties in fish availability extends to Ghana's inland fisheries. In the compilation of data, we reported that official FAO estimates have remained stagnant for the last decade at 90.000 mt (FishStat 2019), while The World Bank reported that over four times the amount, 398.000 mt, was landed in 2012 (The World Bank 2012). This resonates with the notion that fish landings from lakes and rivers are largely unmonitored and underreported in LMICs and its importance is subsequently overlooked in FNS-policies (De Graaf et al. 2015, Fluet-Chouinard et al. 2018). Estimations of Ghana's artisanal inland fleet ranges between 17.500 (FAO 2007) and 24.000 in the 1998 canoe frame survey (referenced in Sarpong (2005)), surpassing the most recent marine survey counting 12.000 canoes (Dovlo et al. 2016). The number of inland fishermen was last reported in 1998 at 80.000, but both the number of fishermen and the fleet has likely increased since (referenced in FAO (2007)). Taking these factors into account, the estimated contribution of inland fisheries to total fish availability in Ghana (16%, (FAO 2016a)) may be underestimated.

Assessment of fish availability and accessibility was primarily examined through the literary revision in paper I, but the sampling for paper II and III provided some novel information. Although our sampling scheme was limited to six SIS, we registered variations in regional availability. Freshwater species were more abundant in Tamale and Bolgatanga, which may be attributed to the proximity to inland fisheries (Figure 5). Malnutrition is more frequent in northern Ghana, and assuming a higher consumption of freshwater species at the expense of marine species may be significant in terms of nutrition security. SIS are furthermore known for being retailed at a low price level in Ghana, thus enabling access for low income groups (Aheto et al. 2012, Nunoo et al. 2015). This was confirmed when sampling the SIS, with price levels ranging between US $ 0.8-1.6 for 100 g of fish. European anchovy was the most affordable fish species (0.8 US$), while round sardinella and African moonfish were retailed for twice the price (1.6 US$).

6.2 Small fish utilization

In paper I we discussed the nutritive potential of SIS, while recognizing that processing methods commonly used in Ghana may alter the nutritional composition. We reported the concentration of selected nutrients and contaminants in six processed SIS from Ghana in paper II, highlighting both the nutritive potential and potential health hazards associated with consumption. This was further elaborated in paper III, where the risks and benefits associated with consumption of different tissues of smoked European anchovy was assessed in the context of young child feeding. In this section, we present all sampled SIS and tissues in the scenario from paper III and include analytical data on nutrients and contaminants in unprocessed SIS for comparison.

6.2.1 The potential contribution of processed SIS to FNS

In paper II we determined that all marine SIS represent excellent sources of essential minerals and trace elements including Fe (43-100% RNI), Zn (29-93% RNI), I (24-44%), Se (100% RNI) and Ca (>99%) (Fig 8). The contribution of raw European anchovy and European sprat to the RNIs of these elements were on average 62% lower (Fig 8), indicating that minerals are retained in SIS despite being subjected to high temperatures, as previously reported by Polak-Juszczak (2016). Both the nutrient density and co-occurrence of key elements in our study therefore reinforces the potential processed SIS have in food-based approaches aimed at ameliorating micronutrient deficiencies (Bogard et al. 2015, Byrd et al. 2021). The different tissues of European anchovy analyzed in paper III displayed increasing concentrations of all elements in the order: samples without heads and skin> whole fish > samples of heads and skin. This is in tune with scientific literature on the distribution of elements in tissues of fish, identifying heads, bones and viscera as primary sites of deposition (Roos et al. 2007b, Kawarazuka and Béné 2011). In Ghana, SIS such as anchovy are often sold beheaded and deskinned at the markets, while the heads and skin are utilized as poultry feed (Nunoo et al. 2015). Our results thus highlight the importance of consuming the whole fish in order to utilize the full nutritive potential. Although considerably less nutrient dense than the marine SIS, a portion of salt dried tilapia could contribute >23% to the RNIs of Fe, Zn, Se, and Ca and. This is substantially higher compared with fillet of raw tilapia, where a 25 g portion contributed 3-7% to the equivalent RNIs, as reported by Bogard et al. (2015).

Marine SIS are often highlighted in food-based strategies (The World Bank 2012, McIntyre et al. 2016), but our results underscore that eating whole SIS from freshwater habitats also represent valuable sources of key nutrients. Notably, this pertains to WA pygmy herring, which displayed similar nutritional characteristics to the marine SIS and contained the overall highest concentrations of Fe and Zn (>100% RNI). In the case of both WA pygmy herring and African moonfish the Fe content would exceed the provisional maximum tolerable daily intake (PMTDI) in our reference scenario (0.8 mg/kg body weight, (JECFA 2019)), signifying potential consumer risk. For WA pygmy herring, a portion reduction to 10 g would be needed to avoid exceeding the PMTDI, which in turn would reduce the overall nutrient contribution.

As discussed in section 5.5, the lack of age-specific ULs for many nutrients, including Fe, does not enable a straightforward evaluation of risk. Furthermore, the risks associated with intakes above the UL are known to differ substantially between nutrients. For Zn, health effects of intakes above the UL appears to be minimal, while the consequences of deficiency, including increased mortality and stunting, are severe (European Commission 2003, Engle-Stone et al. 2019). On the other hand, a high intake of Fe, mainly from high-dose supplementation, is associated with increased diarrheal incidence and morbidity, which must be weighed against the detrimental health effects associated with Fe deficiency. It should, however, be noted that excessive intakes are rarely reported from food sources, and that strategies including dietary diversification are well suited to collectively address nutrient deficiencies, while avoiding excessive intakes (Engle-Stone et al. 2019). However, the ULs are intended to apply to chronic total dietary intakes and a thorough dietary assessment is needed to capture these intake distributions, which is missing in Ghana (Carriquiry and Camaño-Garcia 2006).

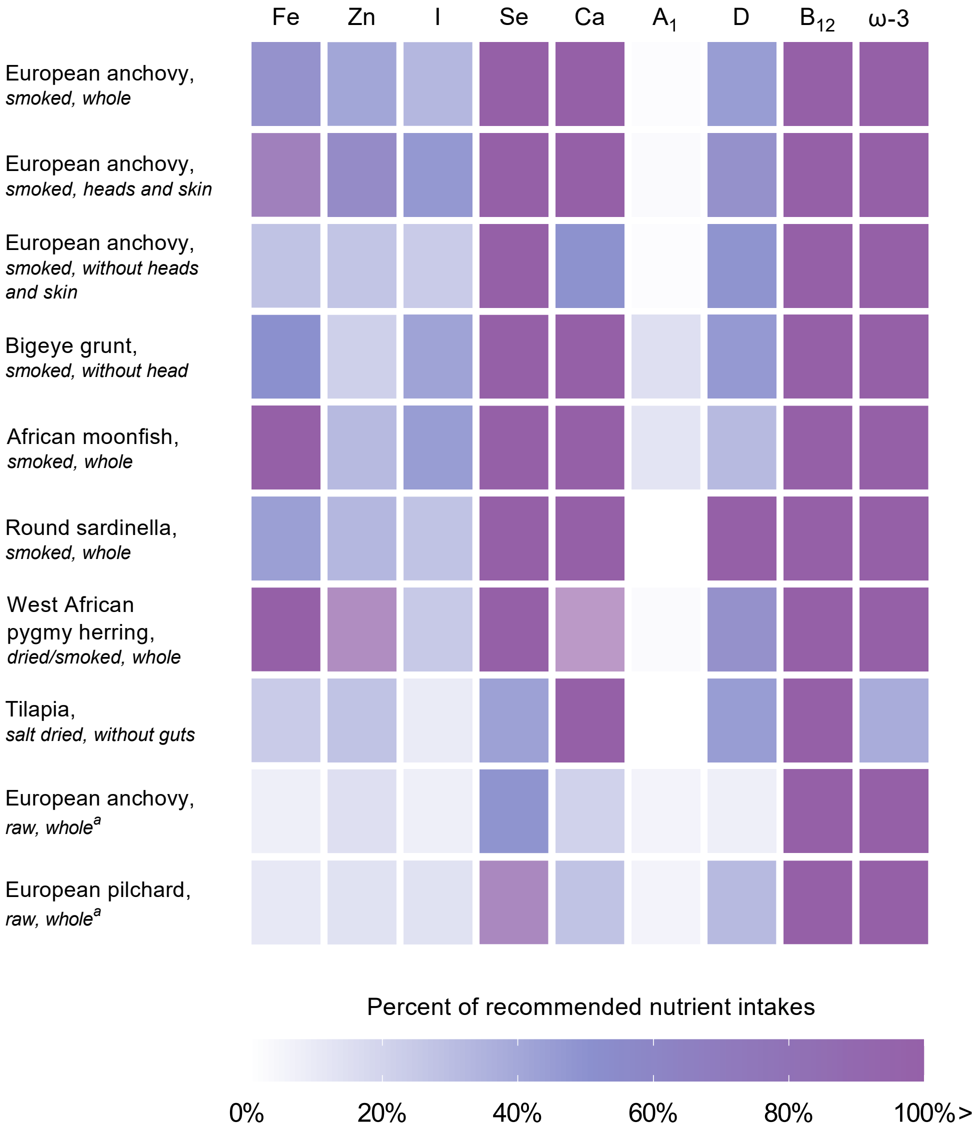

Figure 8: Contribution of a portion (17 g) of processed indigenous small fish from Ghana and raw European anchovy and European pilchard to the RNI-targets of key nutrients for infants and toddlers (6-23 months). Calculations are presented in paper III. [a] From Aakre et al. (2020).

In paper II and III (supplementary data) we reported a large variance in vitamin A content in the different SIS and tissues, from levels below the LOQ to 323 µg/100. Variations in vitamin A content has also been reported in unprocessed SIS from Bangladesh by Bogard et al. (2015), where it was determined that 25 g portions of three SIS could contribute >25% to the RNI of infants. Taking the different portion sizes into account, bigeye grunt and African moonfish could similarly be good sources of dietary vitamin A in Ghana, providing 14 % and 12% to the RNI, respectively (Fig 8). Vitamin A is known to be sensitive to both sunlight and high temperatures (Chittchang et al. 1999), and thus it remains unclear as to what degree vitamin A was retained in the smoked and dried SIS. Compared with their raw counterparts, the vitamin A content was approximately 90% lower in smoked European anchovy and round sardinella (e.g. European pilchard) (Fig 8). Yet, the natural nutrient variations in fish may mask differences arising from processing and vice versa. Conversely, vitamin A levels in bigeye grunt and African moonfish were >250% higher compared with raw European anchovy. While the dearth of analytical data on raw bigeye grunt and African moonfish inhibits a true estimation of vitamin A retention in the current study design, estimating a reduction within the range of European anchovy or round sardinella (90%) entails great nutritive potential. Supplementation remains the main strategy towards alleviating vitamin A deficiency among Ghanaian children under five years, currently estimated at 21% (Wegmüller et al. 2020), but this study supports the potential certain SIS have as a food-based alternative.

Concurrent with the large variations reported in the literature (Larsen et al. 2011, Reksten et al. 2020a), Vitamin D content ranged considerably between SIS and tissues (9-34 µg/100) in papers II and III, contributing 31-100%> to the RNI (Fig 8). Interestingly, the patterns of vitamin D retention appeared opposite to that of vitamin A, with raw European anchovy and European pilchard providing an average 26% less to the RNI in comparison with smoked samples of comparable SIS (Fig 8). Although different processing methods were not directly evaluated in this study, our results imply that vitamin D levels in fish remain stable albeit being subjected to high temperatures.

A former study on round herring (*Etrumeus teres*) concluded that vitamin B_{12} is heat sensitive, and that common cooking methods such as grilling, boiling or frying may reduce levels up to 62% (Nishioka et al. 2011). Both the temperature and duration of traditional fish smoking may exceed that of cooking (Essumang et al. 2013), still, all SIS and different tissues of European anchovy in paper II and III were consistently good sources of vitamin B_{12}, providing >100% of the RNI (Fig 8). A similar pattern was observed for the n ω-3 PUFAs, where tilapia (37% RNI) was the only species or tissue containing concentrations equivalent to less than 100% of the RNI (Fig 8). A former study showed that the variability of the FA composition in raw fish was greater than changes induced by smoking (Stołyhwo et al. 2006), which is representative to our findings. Furthermore, the deposition of antioxidative woodsmoke components during smoking, such as phenols (Arvanitoyannis and Kotsanopoulos 2012), may partly explain why all smoked SIS in our study represent good sources of ω-3 PUFAs.

6.2.2 Food safety constraints in processed SIS

The occurrence of hazardous contaminants in fish has been widely disseminated and most studies have found that the benefits far outweigh the risks in the general population (Cohen et al. 2005, Hellberg et al. 2012). In paper I we highlighted possible food safety hazards in Ghana's small-scale fish value chain, while in papers II and III we reported analytical data on heavy metals and PAHs in a range of processed SIS and tissues. By adapting the risk-benefit scenario in paper III, we illustrate that the concentration of PAHs and heavy metals range considerably between SIS and different tissues (Fig 9).

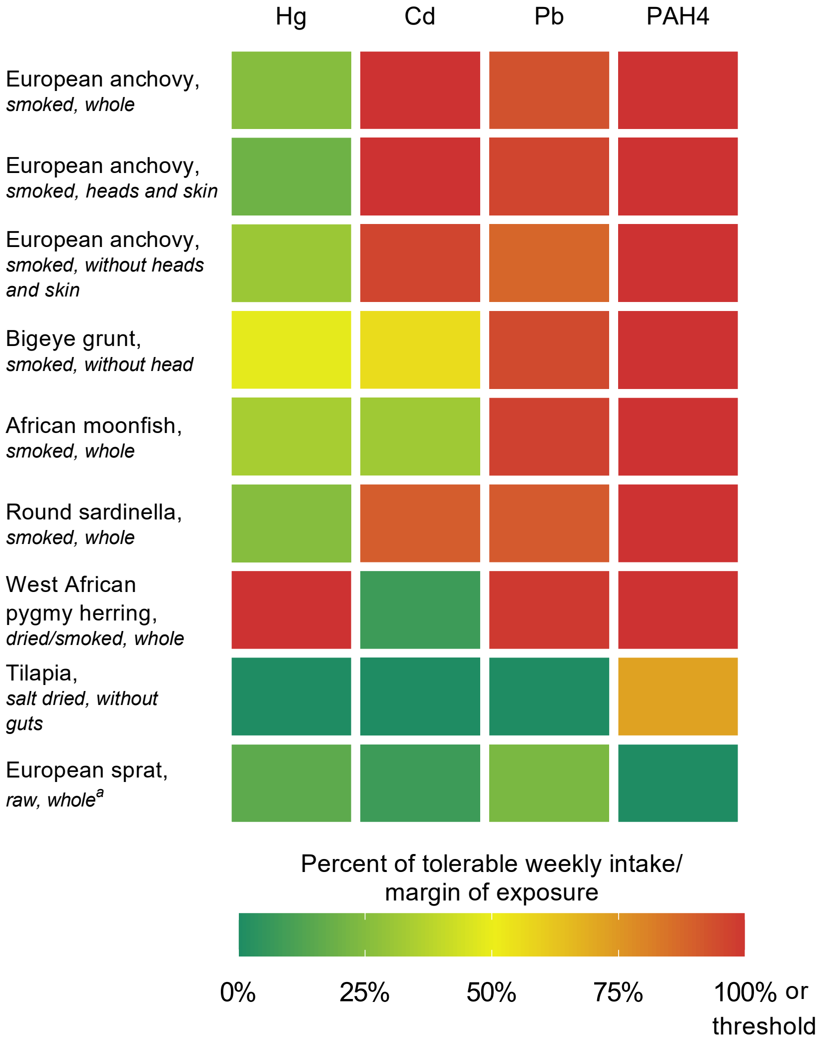

Figure 9: Contribution of daily portions (17 g) of processed SIS fish from Ghana and raw European sprat to the TWI (Cd)/PTWI (Hg) (7 days intake) and MOEs (Pb and PAH4) for key contaminants for infants and toddlers (6-23 months). Calculations are presented in paper III. [a]From "*Seafood data*", IMR (2018).

High levels of PAHs in smoked fish from Ghana have been thoroughly reported in the literature and is linked to the use of traditional kilns where fish are in direct contact with open flames (Essumang et al. 2012, Bomfeh et al. 2019, Asamoah et al. 2021). In paper

II we determined that samples of all SIS exceeded the EU limit for PAH4 in smoked fish muscle (12 µg/kg) between 33-51-fold (400-608 µg/kg) (European Commission 2011), indicating that the adoption rate for new smoking technologies is low. As an alternative method for PAH4 reduction, a previous report from Ghana concluded that removing the skin of round sardinella after smoking could reduce PAH4 levels up to 73% when a barrel oven was used, and 65 % when a chorkor oven was used (Amponsah et al. 2018). In comparison, removal of both skin and heads of European anchovy resulted in a 34% reduction of PAH4 in our study. Although this represents a substantial reduction in PAH4 levels, it was neither sufficient to lower PAH4 levels below the EU's limit for foods nor within the MOE-threshold. Thus, while salt dried tilapia was the overall least nutrient dense SIS, it represents the only species which consumption is not limited by PAH4 content. On the contrary, all species and tissues of smoked SIS would require a portion reduction down to <1 g to achieve acceptable MOEs, effectively invalidating the high nutrient content. This represents a major hindrance in the utilization of smoked SIS in food-based strategies in Ghana, and possibly other LMICs with widespread use of traditional smoking methods (Adeyeye et al. 2016a, Adeyeye and Oyewole 2016b). As discussed in paper II and III, improved kilns which generate PAH4-levels within the EU-limit are available on the Ghanaian market (Asamoah et al. 2021). However, various factors including higher cost, lower yield and organoleptic properties of the final product may explain the low adoption rate (Avega and Tibu 2017).

As discussed in paper I, anthropogenic pollution represents major routes of food contamination in Ghana. Heavy metals exist naturally in the hydrosphere where they bio magnify and bioaccumulate in fish, but environmental hotspots such as the Agbogbloshie e-waste dump in Accra also represents potential sources of contamination (Itai et al. 2014, Kyere et al. 2017). The majority of smoked marine SIS in paper II contained heavy metal concentrations below the respective provisional tolerable weekly intake (PTWI), tolerable weekly intake (TWI) and MOE, and displayed similar patterns between species and degree of retention compared with concentrations IMR (2018) found in raw European sprat (Fig 9). Concentrations of all heavy metals were below the

LOQ in salt dried tilapia, which was the only species that represented no risk in terms of heavy metal exposure to consumers. Heavy metal distribution in the different tissues of European anchovy in paper III were compliant with previous scientific publications, where the highest concentrations of Pb and Cd were determined in samples including heads and viscera (Boalt et al. 2014), and the highest concentration of Hg in muscle tissue (Amlund et al. 2007). Concentration of Cd in fish is known to accumulate with increasing age and size (Boalt et al. 2014), and our finding of Cd levels exceeding the TWI in tissues of smoked European anchovy (Fig 9) may therefore be attributed to post-harvest activity. The highest overall heavy metal load was determined in the smallest SIS, WA pygmy herring, where the concentration of Hg exceeded the PTWI and the Pb concentration was marginally below the MOE-range in our scenario (Fig 9). As discussed in paper II and III, Pb, Cd and Hg are among the main pollutants arising from burning of e-waste (Itai et al. 2014, Kyere et al. 2017). The origin of heavy metal contamination could not be distinguished in our study, however, the proximity of the Agbogbloshie fish market and e-waste dump was reckoned as a plausible source given the naturally low concentrations of heavy metals in low-trophic SIS (Gewurtz et al. 2011, Subotić et al. 2013). Nevertheless, the elevated levels of heavy metals recorded in certain samples is indicative of a larger pollution problem in Ghana, which emphasizes the need for source-identification and mitigation of anthropogenic pollution. By taking both the risks and benefits of fish into consideration in, we illustrate that including both nutrients and contaminants in FCD is essential in order to secure safe utilization of fish.

6.3 Stability

Stability is reflected by the availability and access dimensions of food security (FAO 2006). In paper I we highlighted current challenges to stability in the fish value chain in Ghana, including seasonal variations in fish availability, governmental strategies towards ensuring future fish availability and access, and the effects of climate change on stability in the fisheries.

We determined that SIS are rich in essential micronutrients, and therefore the current limitations in fish availability, access and food safety may have implications for the temporal stability of the Ghanaian diet. A former analysis has modelled the dietary effects of predicted declines in global fish stocks, reporting that 845 million people are at risk of micronutrient deficiencies due to declined availability (Golden et al. 2016). This is supported by a recent publication, estimating that a high-production scenario for seafood may improve FNS substantially and prevent 166 million cases of micronutrient deficiency (Golden et al. 2021).

In paper II we determined that while certain freshwater SIS may provide valuable micronutrients to the Ghanaian population, marine SIS were consistently more nutrient-dense. Thus, in reference to the current promotion of aquaculture described in paper I, our results indicate that promoting stability in the SIS value chain may have a greater effect on enhancing FNS in Ghana. This may also be translated to the lower climatic footprint of small-scale fisheries versus industrial harvest or expanding aquaculture (Hallström et al. 2019, Gephart et al. 2021). Harvesting low- instead of high-trophic species puts less pressure on the aquatic food web (Kolding and van Zwieten 2011, Plank et al. 2017) and small-scale fisheries require no use of land, feed or freshwater to be operated (Gephart et al. 2021). Furthermore, the small-scale fish value-chain in Ghana encompasses close to 3 million people (MoFAD 2015), who should be recognized for their essential roles in FNS by fishing, processing and distributing SIS throughout the country.

7. Conclusion

SIS represent a cornerstone in FNS in Ghana, both as an affordable and nutritious food commodity and through the small-scale fish value-chain, which provides a livelihood for up to 3 million Ghanaians. However, declining catches are currently limiting the availability, access and utilization of SIS throughout the small-scale fisheries value-chain. Strengthening the resilience of small-scale fisheries through adapting legislation may enhance the role of SIS in FNS, both in Ghana and other LMICs. However, the potential of SIS to reduce malnutrition is often underrecognized in food policies and needs to be given higher priority.

Processed SIS from Ghana are rich in vitamins, minerals and essential fatty acids, and may increase the micronutrient content in a typical plant-based Ghanaian diet. Tilapia is one of the species that could increase availability of fish through farming, but our results demonstrate that tilapia cannot substitute wild SIS in terms of micronutrient content. The high concentrations of heavy metals and coliform bacteria determined in certain SIS, and elevated PAH-levels in all smoked SIS, represent pressing food safety concerns that need to be mitigated in order to secure safe utilization of SIS.

Different tissues of smoked European anchovy contained high levels of nutrients and represent a promising food-based approach to alleviate micronutrient deficiencies in Ghana. In our scenario, consuming certain tissues of European anchovy will entail potential risk for young children due to the elevated levels of Cd and PAHs. Thus, the extent to which smoked SIS consumption needs to be regulated in order to meet food safety standards currently invalidates its nutritive potential.

8. Future perspectives

Through the course of this thesis we have demonstrated the potential role of SIS towards enhancing FNS in Ghana and highlighted current restraints limiting availability, access, utilization and stability. We also uncovered knowledge gaps which are important to address in order to explore these linkages in future research strategies.

This thesis provides novel FCD on nutrients and contaminants in commercially important fish species in Ghana, however, generation of additional FCD is needed to gain a more comprehensive overview of the role of fish in FNS. This may include analyses of SIS processed using different methods, such as sun drying, in order to assess variations in nutrient retention and identify potential food safety concerns. Furthermore, the high concentration of nutrients determined in WA pygmy herring suggests that the diversity of freshwater SIS in Ghana should be further investigated.

Food safety is a major constraint to safe utilization of SIS in Ghana, but the risk-benefit assessment in the current study was limited to a hypothetical scenario. Filling the knowledge gap on fish consumption data, especially among vulnerable population groups, could thus enable more refined risk-benefit assessments of SIS. The overall low compliance between RBAs and LMIC-settings also call for refinement in terms of establishing upper intake levels that are representative both for the age group and account for factors such as infectious disease. Further assessment of the synergetic relationship between toxicant exposure and micronutrient deficiencies should also be investigated. The effects of heavy metal and PAH exposure during early life stages have been sparsely reported, but should be further investigated, particularly in polluted areas such as Agbogbloshie.

The high levels of PAHs in smoked fish in all regions necessitate the improvement of the smoking process by implementing best practices and improved kilns (e.g. FTT-kiln and Ahotor oven) or promoting alternative processing methods. Given the current low adoption rate for alternative ovens, ensuring that future technologies are sensitive to local sociocultural and institutional factors, in addition to being cost-effective, is key.

While these suggestions for further research may be valid for many LMICs, the contribution of SIS and small-scale fisheries to FNS remains undervalued. Thus, future policies and global targets, such as the SDGs, needs to recognize the potential of SIS and small-scale fisheries towards ameliorating micronutrient deficiencies and securing sustenance for millions of people across the globe.

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Errata for
Nutrients and contaminants in processed small indigenous fish species from Ghana

-Implications for food and nutrition security

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Thesis for the degree philosophiae doctor (PhD)
at the University of Bergen

15.03.22
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Errata

Page 8 Superfluous word: “potential risk consumer risk” corrected to “potential consumer risk”

Page 14 Missing italics: “…access of all people at all times to enough food for an active, healthy life” corrected to *“…access of all people at all times to enough food for an active, healthy life”*

Page 16 Missing italics: “physical, economic and social access to a balanced diet, safe drinking water, environmental hygiene, primary health care and primary education” corrected to *“physical, economic and social access to a balanced diet, safe drinking water, environmental hygiene, primary health care and primary education”*

Page 16 Missing italics: “*..and active* life (UNICEF 2008)” corrected to “*..and active life (UNICEF 2008)”*

Page 16 Missing italics: “*..and* care (FAO 2011a)” corrected to “*..and care (FAO 2011a)”*

Page 17 Wrong use of italics: *“under five years (UNICEF et al. 2021)”* corrected to “under five years (UNICEF et al. 2021)”

Page 18 Missing word: “and post-harvest processing” corrected to “and through post-harvest processing”

Page 19 Premature use of abbreviation“terrestrial ASFs” corrected to “terrestrial animal source foods (ASFs)”

Page 19 Missing abbreviation “than terrestrial animal source foods (ASFs)” corrected to “than terrestrial ASFs”

Page 20 Missing word: “that essential minerals” corrected to “that more essential minerals”

Page 20 Missing word: “with raw” corrected to “with raw fish”

Page 26 Missing word: “characterized the use” corrected to “characterized by the use”

Page 28 Missing italics: “porridge (koko)” corrected to “porridge (*koko*)”

Page 29 Wrong use of hyphen: “nutrient-intake” corrected to “nutrient intake”

Page 29 Wrong use of hyphen: “dietary-assessments” corrected to “dietary assessments”

Page 30 Misspelled sentence: “always be feasible due to” corrected to “always feasible to obtain due to”

Page 42 Superfluous words: “EU-limits maximum limit” corrected to “EU-limits”

Page 51 Superfluous word: “in the 1998” corrected to “in 1998”

Page 51 Wrong figure reference: “to inland fisheries (Figure 6)” corrected to “to inland fisheries (Fig 5)”

Page 54 Wrong figure caption: “Figure 9” corrected to “Figure 8”.

Page 54 Missing figure: The missing figure 8 is inserted above the figure caption.

Page 54 Superfluous word: “the vitamin A deficiency” corrected to “vitamin A deficiency”

Page 59 Missing word: “freshwater be operated” corrected to “freshwater to be operated”

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Fish for food and nutrition security in Ghana: Challenges and opportunities

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ABSTRACT

Fish is an important dietary source of micronutrients, particularly in low-and middle-income countries. In Ghana, effective management of fish and the fisheries is essential for food, economic and nutrition security and is critical towards achieving many of the UN Sustainable Development Goals especially those pertaining to hunger, poverty, gender equality and life under water. Ghana has experienced significant economic growth in the last few decades, but increasing inequality, uncertainties in fish availability and unsustainable management of the fisheries are challenging local food and nutrition security. This literature review examines and evaluates the role of fish and fisheries in supporting FNS in Ghana, and highlights the lack of focus on fish in the literature with regard to regional food security and fisheries governance. Our review highlights the importance of ensuring the viability of small fish populations to enhance micronutrient availability and counteract micronutrient deficiencies in Ghana. Additionally, strengthening women's role in decision making and promoting female education and empowerment in the fisheries sector is an important strategy towards enhancing FNS in the region.

1. Introduction

World hunger is on the rise, affecting an estimated 821 million people worldwide (FAO, 2019a). The significance of this global challenge is highlighted in the United Nations Sustainable Development Goals (SDGs), where goal two is designed to address ending hunger, achieving food security and improving nutrition and to end all forms of malnutrition (UN, 2015). Malnutrition, which is an umbrella term for both excess consumption of nutrients (overnutrition), inadequate consumption of nutrients (undernutrition) or micronutrient deficiency (“hidden hunger”), is primarily caused by a suboptimal diet (UNICEF, 2019). However, underlying factors such as economy, health care and status, food culture, gender equality, education and environmental issues all play a critical part in these causal pathways (Development Initiatives, 2018). As defined by the Food and Agricultural Organization (FAO), food security encompasses a multitude of underlying factors in four key dimensions (FAO, 2006): **Food availability**, **food access**, **utilization** and **stability**. Conversely, the concept of nutrition security is not anchored in technical terminology but has evolved from UNICEF's conceptual framework on malnutrition (Jonsson, 1992); including the dimensions of food security in addition to acknowledging the importance of key nutrition concerns such as care and feeding practices, public health and sanitation issues (CFS, 2012). Nutrition security and food security are parallel and symbiotic, and “food and nutrition security” (FNS) has been acknowledged as a representative term to combine the two concepts as a unitary goal of policy and programmatic actions (CFS, 2012).

To achieve FNS, FAO recommends a food-based approach that includes food production, dietary diversification and food fortification (FAO, 2011). The importance of terrestrial agri-food systems is widely recognized in this context, but the importance of fish and fisheries with regard to FNS tends to be underrecognized (Thilsted et al., 2014; Béné et al., 2015). Even though absolute fish consumption volumes may be low in low-and middle-income countries (LMICs), it may be an

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Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAO, The Food and Agriculture Organization of the United Nations; FNS, food and nutrition security; GDP, gross domestic product; HLPE, High Level Panel of Experts; LMICs, low-and middle-income countries; MAD, minimal acceptable diet; NGO, Non-Governmental Organization; PAH, polycyclic aromatic hydrocarbons; POP, persistent organic pollutant; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PUFA, polyunsaturated fatty acid; SDG, Sustainable Development Goals

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important source of animal protein, vitamins, minerals and essential trace elements (Tacon and Metian, 2009). The West African country of Ghana is home to an estimated 29 million people with domestic fish supply originating off the 550 km continental coastline or the many inland waterbodies covering approximately 10% of the land surface, including Lake Volta (8.482 km^2) (FAO, 2016a). Fish constitutes 50–80% of consumed animal protein in Ghana (Sumberg et al., 2016; FAO, 2018a) and the yearly per capita consumption is estimated at 28 kg (FAO, 2016a), which is significantly higher than most African countries. Still, the burdens of malnutrition are a persistent and ongoing challenge in Ghana, where there is a well-documented, high prevalence of undernutrition, stunting, anemia and vitamin A deficiency among children < 5 years of age co-occurring with increasing obesity rates in the adult population (GSS et al., 2014). The fisheries sector is essential for Ghana's economy and plays a critical role in national FNS and poverty alleviation, employing approximately 10 percent of the labor force and contributing 4.5 percent of the gross domestic product (GDP) (Republic of Ghana, 2011; FAO, 2016a). The strong linkages between fish, fisheries and FNS in Ghana are steadily gaining the attention of researchers and development agencies, however, there is a lack of focus on fish in the existing fisheries governance and food security literature and a thorough review of these topics is missing in the scientific literature. This is also the case for other emerging coastal LMICs including Bangladesh, Cambodia, the Gambia, Indonesia, Sierra Leone and Sri Lanka, where fish contributes 50% or more of total animal protein intake (FAO, 2018a). Here we provide a mixed method literature review focusing on the linkages between fish, fisheries and FNS in Ghana and their contribution to selected areas of focus within the four dimensions of food security: food availability, food access, utilization and stability (Fig. 1). The objective of this article is to review and integrate the fragmented literature on fish, fisheries and FNS in Ghana in order to assess these factors in a holistic way and to examine the potential opportunities and challenges that lie ahead.

2. Methods

The selection of literature for this review adhered to the recommendations outlined for the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement (Moher et al., 2009).

2.1. Literature search strategy

A single primary systematic literature search was performed in the following databases: Embase (OVID), Ovid MEDLINE® and Web of Science (Clarivate) from inception up to April 2019. These systematic searches were conducted in collaboration with an independent university librarian for quality assurance and to reduce the risk of selection and detection bias. The complete literature search strategies are available in the supplementary materials section (Appendix 1).

2.2. Inclusion and exclusion criteria

To select topics related to the different pillars in the food security framework, key persons were consulted to ensure the topics' relevance to fish, fisheries and FNS in Ghana before the systematic literature search was conducted (Fig. 1). Quantitative and qualitative research articles fulfilling the following criteria were identified for review: 1) written in English, 2) published after year 2000, 3) presenting data from Ghana, 4) available online, and 5) relevance to one of the selected topics on fish, fisheries and FNS presented in Fig. 1. Further, specific exclusion criteria were applied in the full text review: 1) data on non-commercial fish species, 2) data from other waterbodies than the Gulf of Guinea or Lake Volta. In the section on food safety, articles on 1) rare contaminants and/or specific non-communicable diseases (NCDs), 2) studies involving migrants or 3) sensory studies were excluded.

3. Results

3.1. Study selection

Fig. 2 shows the PRISMA flow chart outlining the steps in retrieving appropriate studies for the review. The formal systematic search yielded 848 potentially relevant articles. After duplicates were removed, 573 articles remained for screening. Upon reviewing the title and abstract, 49 articles were considered potentially relevant and full text of the articles were assessed for eligibility. Eleven out of the 49 articles were excluded due to the criteria specified in the PRISMA flowchart (Fig. 2). Thirty-eight articles met the inclusion criteria and were included in the qualitative synthesis. An overview of the number

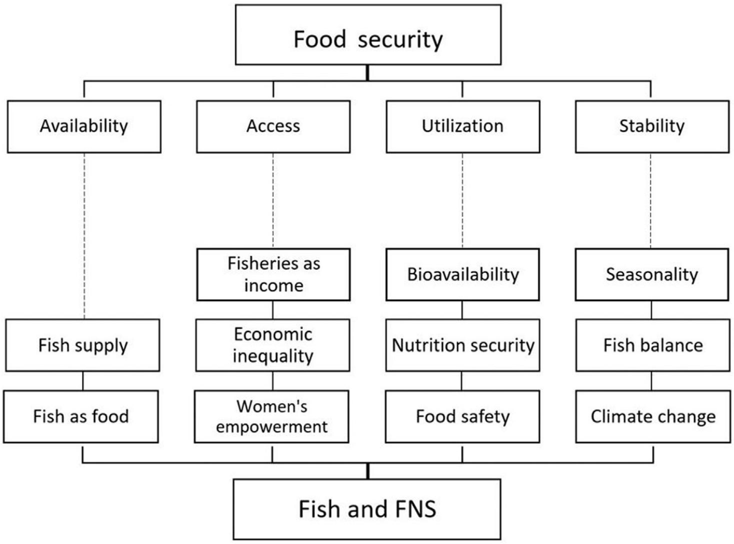

Fig. 1. Conceptual framework for the review article, the four pillars of food security (FAO, 2006) and selected focus areas related to fish and FNS in Ghana.

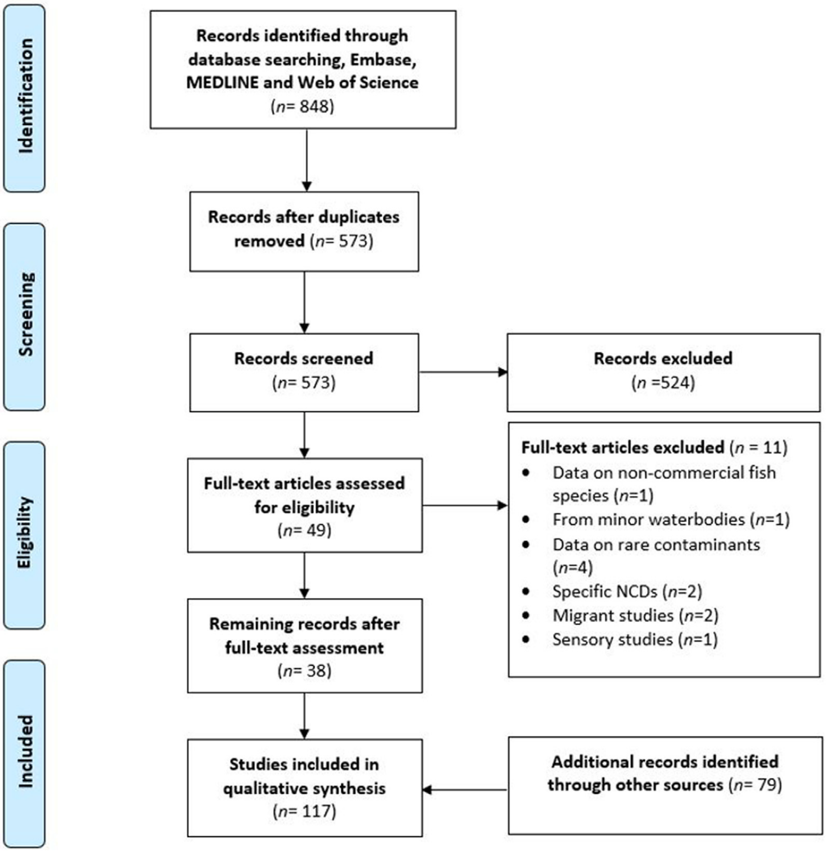

Fig. 2. PRISMA flow diagram of the study selection process (Moher et al., 2009).

of articles corresponding to each of the selected topics and pillars of food security (Fig. 1) is available in the supplementary material section (Appendix 2). The number of research articles resulting from the systematic search on each selected topic varied significantly and additional literature was needed to strengthen theoretical links in both the quantitative and qualitative research evaluations. To evaluate current legislation in the fisheries, fisheries statistics and reporting on the prevalence of stunting, wasting, underweight and other national statistics in Ghana, including official (n = 35) and NGO reports (n = 6) in the qualitative synthesis was considered essential especially given that these data are rarely provided in research articles. Furthermore, recommended research articles from associated experts (n = 38) were included to further strengthen the literature basis of this review.

3.2. *Food availability*

Food availability refers to the physical presence of food in sufficient quantities, supplied through domestic food production, import, stocks or aid (FAO, 2006; WFP, 2009). The referenced foods should also be of appropriate quality, and acceptable according to the local culture in a given population. We highlight two dimensions of availability in this section: 1) how the different sectors of the fisheries contribute to food availability, and 2) how fish enhances nutrient availability in the population.

3.2.1. *Fish supply*

The Ghanaian fisheries are diverse comprising marine, freshwater and aquaculture sectors. The marine sector is the largest and supplies approximately 80% of the recorded domestic catch (Lauria et al., 2018). The small-scale fisheries sector, using canoes of which 73% are motorized, dominates marine fisheries, and harvests approximately 70% of the coastal marine fish captures (Akyeampong et al., 2013). Sardine (*Sardinella aurita)*, Cunene horse mackerel (*Trachurus trecae*), Atlantic chub mackerel (*Scomber colias*), anchovy (*Engraulis encrasicolus*) and other small pelagic fish comprise most of the marine catch. According to FAO estimates, marine catches increased substantially with the motorization of canoes and introduction of an industrial fleet in the 1970s, peaking at nearly 500,000 mt in 1999 (FAO, 2016a). FAO statistics are currently reporting a downwards trend in marine catches in Ghana with an estimated 30% decline since the late 1990s, resulting in an annual import of up to 361,000 mt of fish and fishery products in order to satiate local demand (Table 1) (FAO, 2016a; FAO, 2019c). Data on marine fish production, however, vary considerably depending on the source consulted. For example, official FAO data estimated marine captures at 292,000 mt for 2017, while unofficial sources (SAU, 2015) reported 350,000 mt for 2014 (Table 1). There are no official estimates for the landings by small boats engaged in illegal offshore *"Saiko"* transshipments, but a recent report released by the Environmental Justice Foundation estimated that this hidden harvest equaled 101,000 mt in 2017 (Table 1) (EJF and HenMpoano, 2019) suggesting

Table 1
Fish supply estimates and utilization in Ghana (metric tons) including official and unofficial data, 2017[a].

Supply	Lower estimates	Year and source	Higher estimates	Year and source
Marine capture fisheries landings	292,000	2017 (FAO, 2019b)	350,000	2014 (SAU, 2015)
“Saiko” transshipments	0	No official data	101,000	2018 (EJF and HenMpoano, 2019)
Inland capture fisheries	90,000	2017; (FAO, 2019b)	398,000	2009 (The World Bank, 2012)
(Inland) aquaculture	57,000	2017; (FAO, 2019b)	57,000	2017 (FAO, 2019b)
Import	361,000	2017; (FAO, 2019c)	361,000	2017 (FAO, 2019c)
Total supply	800,000		1,267,000	
Utilization				
Export	72,000	2017; (FAO, 2019c)	72,000	2017 (FAO, 2019c)
Post-harvest losses	3% of landings = 11,500	FAO (2019b)	20% of landings = 170,000	FAO (2019b)
Total apparent availability[b]	716,500	Calculated from above	1,025,000	Calculated from above
Apparent per capita consumption (kg)[c]	25	Calculated from above	35	Calculated from above

[a] Data from 2017 unless not available.
[b] Apparent fish availability = (production + import – export – post-harvest losses).
[c] Apparent fish consumption per capita = (production + import – export – post-harvest losses)/population estimate (29,000,000).

significant discrepancies between official and unofficial marine fish capture estimates.

The inland fisheries are primarily centered on Lake Volta and its surrounding rivers, yielding approximately 16% of the domestic catch and includes up to 90% of the total inland fisheries production (FAO, 2016a). Reported inland fish landings are dominated by larger fish species including several species of tilapia (*Tilapia* spp.), catfish (*siluriformes*) and elephant fish (*Mormyridae*) (FAO, 2016a). Estimates of the annual yield from Lake Volta and inland waterbodies vary significantly, ranging from the official 90,000 mt presented by the FAO for 2017 (FAO, 2019b) to 398,000 mt reported by the World Bank in 2012 (The World Bank, 2012).

Aquaculture is a relatively recent addition to the Ghanaian fisheries and now operates in all ten regions of the country (FAO, 2016a; Kassam and Dorward, 2017). Nile tilapia (*Oreochromis niloticus*) represents 80% of the harvested farmed fish species, while catfish (*Clarias* spp.) make up the remaining 20%. Currently, the number of small-scale fish farms is close to 3,000 and the number of ponds and cages exceed 19,000; numbers that are expected to increase over the next years (Kassam and Dorward, 2017). Small-scale pond aquaculture has been the main production system promoted in Ghana, however, in recent years there has been a shift towards large-scale cage-aquaculture which has a higher dependency of using fish in the feed (Tacon and Metian, 2015). According to official statistics, aquaculture production has experienced rapid growth from 950 mt in 2004 to over 27,000 mt in 2012, with an average annual, overall growth rate of 73% from 2009 to 2014 (FAO, 2016a). Official sources estimate fisheries production of 57,000 mt for inland aquaculture in 2017 (Table 1) (FAO, 2019b) and although there are indications that this number may be biased low, these data currently remain unpublished.

Another increasingly important source of fish is derived from imports which have increased from ∼20,000 mt in the early 1990s to ∼∼361,000 mt in 2017 (Table 1) (FAO, 2019c). The imported fish species (mainly horse mackerel and sardinella) are relatively cheap and originate primarily from West Africa and Europe (Sumberg et al., 2016). Official data estimates that Ghana exported 72,000 mt fish in 2017 (Table 1) (FAO, 2019c), however, the full extent of trade flows in Ghana is not fully documented. Informal fish trade with neighboring countries is also prevalent which creates additional sources of uncertainty with regard to the overall accuracy of these estimates (Ayilu et al., 2016). Furthermore, improper fish handling and poor processing technologies are the main causes of post-harvest losses, which represents a significant loss (– ∼3% to – ∼20%) in total fish landings (Table 1) (FAO, 2019b).

3.2.2. *Fish as food*

Fish is the most frequently consumed animal protein source food in Ghana, irrespective of socioeconomic status or locality (Colecraft et al., 2006). According to official estimates the yearly per capita fish consumption in Ghana currently stands at 28 kg (FAO, 2016a), however, when unofficial data are included, this number ranges from 25 to 35 kg/cap/year (Table 1). Marine and freshwater fish species are available in many forms throughout the country, including smoked, dried and fried small pelagic species and larger fish that are mainly grilled, smoked, fried or fermented (Adeyeye and Oyewole, 2016). In combination with export and import data, fish landings and production data suggest that the main species consumed are imported mackerel and sardinella, locally landed herrings, anchovy, and tilapia and catfish from inland areas (SAU, 2015). However, factual data on fish consumption in Ghana are currently missing.

The Ghanaian diet largely consists of starchy staple foods cassava, yams, bananas and cereals (rice, maize) (Nti, 2008; FAO, 2010), with fish being central in the local cuisine serving as a complementary addition with its composition of other essential macro- and micronutrients (Kawarazuka and Béné, 2011; Weichselbaum et al., 2013). The lipid profile of fish is unique, including long-chain polyunsaturated fatty acids (PUFAs) arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Larsen et al., 2011; Weichselbaum et al., 2013). The concentration of PUFAs in fish is variable, ranging from high concentrations in marine fish species such as mackerel (3.3 g/100 g) and herring (2.4 g/100 g), to lower levels in freshwater species such as tilapia (0.47 g/100 g) (Kawarazuka and Béné, 2011). Larger fish are usually consumed for their fleshy parts, but removing the bones, viscera and organs reduces the micronutrient content compared with whole small fish (Thilsted et al., 2014). This effect was observed in a study by Bogard et al. (2017), where consumption of farmed freshwater fish at the expense of smaller wild species resulted in a decreased intake of iron and calcium in some populations. Thus, eating small fish whole is a particularly advantageous due to their rich content of minerals including iodine, selenium, iron, zinc, calcium, phosphorus and potassium, and vitamins A, D and B_{12} (HLPE, 2014; Thilsted et al., 2014; Abbey et al., 2017). Ensuring the availability of fish, particularly the nutrient-dense small fish, is therefore essential to enhance micronutrient availability and counteract micronutrient deficiencies in Ghana.

3.3. *Food access*

Food access refers to the ability of people to acquire adequate amounts of nutritious food by way of their own production, hunting and foraging, or purchasing of foods (FAO, 2006; WFP, 2009). We highlight three dimensions of accessibility in this section: 1) how livelihoods provided throughout the fish value chain help generate income and allow people to access nutritious foods; 2) how economic inequality affects the ability of people to access fish; 3) how gender inequality affects income generation and fish accessibility.

3.3.1. Fisheries as income

Through the livelihoods provided in the fish value chain, from boatyards and fishers to processors and market women, the fisheries sector is acornerstone of food security in Ghana (FAO, 2016a). The total value of marine fish landed in Ghana (2014, ex vessel price) was approximately 500 million USD (SAU, 2015), while fish imports in 2014 were valued at 350 million USD (FAO, 2018b). These values, in addition to the value added in the post-harvest sector, provides a significant number of livelihoods and thus allows families to access food. According to FAO, Ghana's marine sector employs 135,000 fishers, while 500,000 affiliated workers are engaged in the processing (drying, smoking, canning), distribution and marketing of fish throughout the country (FAO, 2016a). Adding the families of those gaining a livelihood in this sector, it is estimated that ~2.6 million people rely on the marine fisheries sector, and another 300,000 individuals rely on the inland fisheries sector (FAO, 2016). An increasing number of canoes as well as fishers per boat, in combination with increasing competition from trawlers (Penney et al., 2017; EJF and HenMpoano, 2019), higher input costs (Marquette et al., 2002) and declining catches (Nunoo et al., 2014a, 2014b), has resulted in apparent income reductions for small-scale fishermen and subsequently fish processors and traders. Moreover, concerns have recently been voiced that significant amounts, up to 90% of fish value landed by the industrial fleet, is concentrated in the hands of a small number of Chinese shadow owners (EJF and HenMpoano, 2019). Given the high levels of unemployment in Ghana and limited availability of alternative livelihood opportunities, a sizeable small-scale fishing fleet with high employment and diverse ownership is desirable over a scenario with a highly consolidated fleet. With the high level of capital flight associated with the industrial fleet and increasing concentration of ownership in the hands of a small group, a significant proportion of Ghanaian fish revenues subsequently does not end up benefiting the Ghanaian economy.

Due to the projected high levels of stress experienced in capture fisheries, the Ghanaian government is currently focused on furthering aquaculture development to create job opportunities and enhance fish production (MoFAD, 2019). However, studies have estimated that the costs and resources needed to adopt various forms of aquaculture are likely to be too high for the average farmer and seems to disproportionally benefit the wealthiest owners (Bénéand Obirih-Opareh, 2009; Nunoo et al., 2014a, 2014b; Kassam and Dorward, 2017). Hence, although small-scale aquaculture has the potential to increase food access and act as a source of supplementary income for some, the impact on improved FNS for poor fish farmers is currently limited (Kassam, 2014; Kassam and Dorward, 2017).

3.3.2. Economic inequality

Ghana has recently experienced significant economic growth and has reduced the poverty rate between 1992 and 2006 by ~50% while attaining middle-income country status in 2010 (UNDP, 2014; Cooke et al., 2016). However, these gains have not been evenly distributed, with increasing wealth in southern regions and high poverty rates in northern regions (UNDP, 2014). The growing economic inequality has serious implications for FNS and emphasizes the importance of having access to affordable, nutritious foods (Cooke et al., 2016). The role of fish is crucial in this regard, as it is available throughout the country at relatively low prices. Smoked sardines are particularly accessible and affordable, not only because they are relatively cheap (0.85 USD/kg) (Aheto et al., 2012), but also because they can be purchased in small quantities. According to estimates by Sumberg et al. (2016), Ghanaians spend 61% of their expenditures on animal protein source foods on fish, while fish provides 70% of the total animal protein intake, suggesting that fish is largely a relatively affordable source of nutritious food. Low-income consumers purchase less expensive fish species, however, their expenditure on fish (25.7%) exceeds the national average of 22.4%, which emphasizes their stark dependence on fish in the diet (FAO, 2016a). Conversely, more wealthy consumers have developed a preference for larger and more expensive fish such as tilapia (Asiedu et al., 2016) and are steering towards a more westernized diet with increased intake of poultry and beef at the expense of fish (Sumberg et al., 2016). Combined with other energy-dense foods, this dietary shift has increased the prevalence of obesity and related non-communicable diseases to epidemic proportions among the urban populace (Pereko et al., 2013; Ofori-Asenso et al., 2016). Thus, while securing the access to affordable fish is essential for low-income consumers, making food choices based on nutritional quality rather than ease of access could benefit wealthier consumers.

3.3.3. Women's empowerment

The Ghanaian fisheries has a distinct gender division, with female fish traders, commonly known as *konkofo,* dominating the onshore activities of the fish value chain (Gordon et al., 2011). Female fish processors, small-scale retailers and large-scale wholesalers, many of whom are wealthy and invest in canoes hiring men to fish for them, are involved in multiple sectors of the fish value chain, from landing site to consumers throughout the country (Walker, 2001; Gordon et al., 2011). Women also play a vital role in FNS, making fish available in urban and rural markets at relatively affordable prices in smoked and dried forms that can be prepared and stored in homes frequently lacking electricity, refrigerators and freezers (Overå, 2007). Although their roles in the fisheries are considered essential, Ghanaian women have limited access to funding, education and institutional support compared with their male counterparts (FAO, 2016b; Forkuor et al., 2018), which limits the role of women in decision making and their opportunities for enterprise expansion in the fisheries sector (FAO, 2016b). Most women working as fish processors and traders in the informal economy have small incomes, which represents an obstacle in terms of having sufficient funds to access fish, subsequently affecting household FNS (Overå, 2007). Despite these limitations, Kawarazuka and Béné (2010) reported that the purchasing power from selling fish (i.e. increased access) resulted in a greater proportion of income being spent on food when women were engaged in these activities. Strengthening women's role in decision making and promoting female education and empowerment in the fisheries sector is therefore an important strategy towards enhancing both food access and household FNS in Ghana.

3.4. Utilization

Utilization refers to both household and individual utilization of accessible foods, and includes several relevant themes and spheres including nutrient bioavailability, nutrition security, sanitation, feeding practices and food safety (FAO, 2006; WFP, 2009). We highlight three dimensions of food utilization in this section: 1) bioavailability of essential nutrients in fish, 2) the contribution of fish to nutrition security for children in Ghana and, 3) food safety concerns regarding fish handling and consumption.

3.4.1. Bioavailability

Fish contains numerous nutrients in highly bioavailable forms and is a valuable addition to the mainly vegetarian diets of many households affected by food insecurity (FAO, 2010; WFP, 2016). While Ghanaian staple foods contain low amounts of the essential amino acid lysine, which limits protein synthesis, fish contains particularly high levels of lysine and thereby enhances the overall protein quality from other foods when included in a meal (Kawarazuka and Béné, 2011). Iron in fish is present as readily available heme iron, in contrast to non-heme iron plant-source foods which has lower bioavailability (Béné et al., 2015; Wheal et al., 2016). Fish also counteracts the effect of inhibitors, such as phytate, found in typical Ghanaian staple foods and thus co-ingestion enhances absorption of non-heme iron and zinc from plant foods (Thilsted et al., 2014). High levels of vitamin A have been reported in small fish, but both the form and bioavailability of vitamin A varies among fish species (Roos et al., 2002). Despite containing high levels of retinol activity equivalents, some freshwater species contain vitamin A in the form of 3,4-dehydroretinol, which may not have the ability to convert to bioavailable retinol (Kongsbak et al., 2009). Vitamin A is sensitive to sunlight and heat, but the effect of processing methods on micronutrient levels in fish is yet to be thoroughly elucidated. Sun-drying is a common processing method in Ghana, which has reduced vitamin A content in fish up to 90% (Chittchang et al., 1999). To maximize the utilization of fish in Ghana, selection of both fish species and processing method are thus important factors with regard to nutrient density and preservation, but this research is still not yet well established.

3.5. Nutrition security

Food security and nutrition security are closely interlinked but food availability and access are not always synonymous with FNS. Optimal nutrition during the first 1,000 days of life (from conception through the first two years) plays a foundational role in child development, and how well or how poorly mothers and children are nourished and cared for during this time have implications for brain and cognitive development, immune systems and growth (WHO, 2013). Although progress is being made, the most recent Ghana Demographic Health Survey reported that the prevalence of stunting, wasting, underweight, anemia (Fig. 3), vitamin A supplementation-coverage and limited access to iodized salt among children under five, are all issues of great concern (GSS et al., 2014). The critical transition from exclusive breastfeeding to solid foods has been identified as one of the main causes of child malnutrition in Ghana, where the frequency and diversity of complementary feeding (minimum acceptable diet, MAD) (Fig. 3) meet recommendations in only 13% of cases (Issaka et al., 2015; Agbadi et al., 2017). Studies have identified the attitudes of caregivers and nutrition knowledge gaps as potential barriers to optimal child feeding regimes in rural Ghana (Armar-Klemesu et al., 2018) and across different agro-ecological zones (Christian et al., 2016). Inclusion of small fish as a complimentary food during the first 1,000 days of life have been found to significantly contribute to both macro- and micronutrient intakes in infants and young children and represents a promising food-based strategy towards improving nutrition (Bogard et al., 2015). Dietary fortification of toddler food with fish powder is common practice in Ghana, however, scientific literature on the topic remains scarce. In a study by Egbi et al. (2015) the effect of adding small amounts (3%) of fish powder and vitamin C to school meals proved beneficial, resulting inthe prevalence of anemia being reduced among study participants. Fish powder is commonly made of anchovy or sardine but replacing it with cheap commodities such as underutilized fish species and byproducts is a proposed low cost strategy toward alleviating micronutrient deficiencies (Nunoo et al., 2009; Glover-Amengor et al., 2012; Abbey et al., 2017). However, due to the custom of smoking and drying fish in Ghana, it remains uncertain as to what degree micronutrients are degraded by these processes and the potential consequences this represents for nutrition security. In sum, utilizing fish in the diet of young children is essential to counteract the multiple forms of malnutrition that are currently prevalent in Ghana. Furthermore, both the choice of fish species and utilization of byproducts should be considered in order to optimize the benefits of FNS in young children.

Fig. 3. Prevalence of wasting, stunting, underweight, overweight, anemia and minimum acceptable diet (MAD) of children 6–59 months in Ghana, 2014. Data source (GSS et al., 2014). ^{a}MAD = Breastfed children 6–23 months of age who had at least the minimum dietary diversity and the minimum meal frequency during the previous day or non-breastfed children 6–23 months of age who received at least two milk feedings and had at least the minimum dietary diversity not including milk feeds and the minimum meal frequency during the previous day (UNICEF, 2018).

3.5.1. Food safety

While fish containa wide array of nutrients, they are also a source of contaminants. Depending on habitat, trophic position, energy flow, and proximity to anthropogenic activities, heavy metals, persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs) and microorganisms are present in varying concentrations in fish (van der Oost et al., 2003). In Ghana, fish are also affected by high post-harvest losses and freshly captured fish can undergo rapid quality deterioration and perishability as a result of the intrinsic properties such as high moisture content, near neutral pH and highly digestible protein (Ghaly et al., 2010). These conditions permit for microbial proliferation resulting in microbial contamination, and bacterial species belonging to genuses *Escherichia coli, Salmonella* spp, *Shigella*, *Streptococcus,* amongst others, have been isolated from different Ghanaian fishes (Takyi et al., 2012; Kombat et al., 2013; Kwenin et al., 2013; Antwi-Agyei and Maalekuu, 2014). Contaminated fish find their way to the markets and subsequently consumers, which raises public health concerns due to the health hazards associated with consuming contaminated fish (Scott et al., 2007). Fish from waterbodies with nearby mining activity also represents a significant potential health hazard, where mercury used for gold extraction is discharged into the hydrosphere where inorganic mercury is converted to the highly neurotoxic form, methylmercury, and subsequently bioaccumulates in fish (Hilson, 2002; Aryee et al., 2003). Variable levels of mercury have been registered in fish from areas with nearby mining-activity (Kwaansa-Ansah et al., 2011; Voegborlo et al., 2011; Gbogbo et al. 2017, 2018), and elevated blood- and urinary levels of mercury have been detected in both miners and residents of mining-communities (Rajaee et al., 2015; Henriquez-Hernandez et al., 2017). A maiden study on halogenated contaminants in tilapia from lakes and lagoons in Ghana reported low levels of selected POPs, however, the continuous discharge of untreated effluents is expected to increase their presence (Asante et al., 2013). Marine fish inhabit waters far from anthropogenic activities and point sources, but certain coastal areas in Ghana are severely polluted due to burning of e-waste and dumping of raw sewage, particularly the Agbogbloshie district in Accra (Wittsiepe et al., 2017). Analyses of pelagic fish off the Ghanaian coast show low levels of mercury (Voegborlo et al. 2004, 2011), insignificant levels of PAHs and thus poses a minimal health risk through direct consumption of fresh fish (Essumang et al., 2012). However, smoking fish in kilns operating on fuelwood causes formation of PAHs, which are known carcinogens (Nti et al., 2002). Elevated levels of PAHs have been registered in smoked sardinella from Ghana, particularly during the dry season when fat content in fish is at its highest (Essumang et al., 2012). Although the levels of various contaminants are currently low in many fish species in Ghana, implementing strategies for monitoring levels of contaminants, safe management of toxic discharge from industrial activities and continued exploration of alternative processing techniques are key actions to ensure safe utilization of fish as food.

3.6. Stability

Stability is reflected by the availability and access dimensions of food security, where a household or an individual have access to nutritious foods at all times and is resilient in adapting to economic or environmental crisis, or cyclical events such as seasonal food insecurity (FAO, 2006; WFP, 2009). We highlight three dimensions of stability in this section: 1) seasonal variations and its effect on fish availability, 2) fish balance, and governmental strategies towards ensuring future fish availability and access, 3) climate change and its effects on fish availability and the fish industry.

3.6.1. Seasonality

While fish are readily available in Ghana during the two upwelling seasons, the minor from December–February and the major from July–September, marine fish catches are less accessible in the leaner months (FAO, 2016a). The nutrient composition of marine fish also varies seasonally, with higher water content in fish during the wet season (June–September) and higher fat content in the dry season (November–May) (Essumang et al., 2012). To compensate for reduced catches of pelagic fish in the lean season, fishermen respond by fishing further from the coast and often targeting other fish species (Lenselink, 2002; Perry and Sumaila, 2007). Seasonal fishing migration within Ghana as well as to nearby countries serves as an income generation strategy and has been practiced by Ghanaian fishermen for more than a century (Marquette et al., 2002; Mensah and Antwi, 2002). Ghanaian fisheries migrants are periodically being constrained by political actions from neighboring countries, with the intent of excluding them from their waters (Duffy-Tumasz, 2012), but the high degree of mobility continues to characterize fish harvesting patterns and is crucial for the resilience of the small-scale fisheries. In Lake Volta seasonal fluctuations in water level affect both catch rates and use of fishing gears, with fish migrating to deeper waters during recession and spawning in shallow submerged vegetation at high tide (van Zwieten et al., 2011; Mensah et al., 2019). Seasonal variations in fish availability also lead to price fluctuations, which has the greatest impact on poor inland inhabitants. During the lean season most consumers adapt by eating more imported fish while some resort to increased bushmeat hunting, which adds increased pressure on Ghana's already vulnerable wildlife (Brashares et al., 2004; Rowcliffe et al., 2005). Thus, seasonal fluctuations in fish availability have a considerable, cascading effect on economic stability and the livelihoods of fisherfolk in the marine and inland sectors. Furthermore, the seasonal variations in fish access combined with increased intake of other foods such as bushmeat during the lean season, have important implications for nutrition stability.

3.6.2. Fish balance

To bridge the gap between fish supply and demand, Ghana has increased its import of fish and the seafood trade balance has shifted from a USD 33 million surplus in 1997 to a USD 319 million deficit in 2013 (FAO, 2016a). Marine fish stocks are currently uncertain and the Ghana Fisheries Management Plan (2015–2019) aims to guide conservation of fish stocks and has implemented periodical bans on artisanal and industrial fishing (MoFAD, 2015). Moreover, fisheries regulations prevent legal access to many small species, particularly in freshwater systems (Kolding et al., 2019). The current strategy for increasing fish availability in Ghana is to stimulate aquaculture growth by prohibiting import of farmed fish and initiating The Ghana National Aquaculture Development Plan with an ambitious production target of 100,000 mt (MoFAD, 2012). While some propose that increased aquaculture production will lead to reduced poverty (Asiedu et al., 2016) and improve FNS in Ghana (Asiedu et al., 2017; Chan et al., 2019), others argue that the higher purchase price of farmed fish and the resultant changes in species and thus nutrient composition of the fish eaten will be detrimental to FNS (Kawarazuka and Béné, 2010). The nutritional value of farmed fish species can be improved by including fish-derived products such as fish oil from small pelagic fish in the feed, however, with a large group of the Ghanaian population depending on these fish for food, the sustainability of this practice is debatable (Beveridge et al., 2013; Béné et al., 2015). Although fish species such as tilapia naturally feeds on organisms lower in the aquatic food chain and are less reliant on fish-derived products in the feed than marine fish (Tacon and Metian, 2015), Fry et al. (2018) estimate that farmed tilapia only has a 15–20% protein retention compared with 35–40% for poultry. Thus, how the scale is balanced in terms of fish supply, feed requirements and nutritive quality is an issue that will have substantial implications for future FNS in Ghana.

3.6.3. Climate change

West Africa has been identified as one of the most vulnerable regions to climate change, and models predict that climate change may cause a substantial reduction in marine fish landings and lead to extensive economic losses in Ghana by 2050 (Lam et al., 2012). The effects of climate change are already evident in Ghana, and fishermen have reported increased coastal erosion, oxygen minimum zones, changes in upwelling events, stronger waves and more frequent storms as key-stressors (Freduah et al., 2017; Ankrah, 2018). The resultant interplay of climate and non-climate stressors is profound for the livelihood of coastal fishermen, who are taking greater risks in more treacherous waters to adapt and compensate for decreasing catches, increasing fuel prices and competition from industrial trawlers (Freduah et al. 2017, 2019; Ankrah, 2018). The situation is further aggravated by conflicting interests between fishermen and the offshore petroleum industry regarding the use of ocean space (Adjei and Overå, 2019). With the projected changes in performance and dynamics of aquatic ecosystems (Lam et al., 2012), it is vital that those engaged in the fisheries sector are able to have the flexibility and resilience to sustain these shocks without compromising their commitments to sustainability. However, with the current legislation periodically banning all artisanal fishing (MoFAD, 2015) and sanctioning common fishing methods targeting small fish such as light fishing and use of small mesh sizes (Kolding et al., 2019), the economic viability of fisherfolk is not taken into account. Restricting only trawl fishing periodically and allowing artisanal harvesting of small fish species could increase the resilience of fishermen substantially and lead to a more balanced harvest causing less disturbance to the ecosystem.

4. Future perspectives

4.1. Putting fish on the agenda

Fish has the potential to significantly reduce food and nutritional insecurity in Ghana and other LMICs, but its potential has repeatedly been overlooked by policymakers. The separation of fisheries from other agri-food systems was addressed by the High-Level Panel of Experts (HLPE) on FNS, underscoring the importance of incorporating fisheries as an integral element in FNS strategies, policy making and debates (HLPE, 2014). The Committee on World Food Security recognized the importance of sustainable fisheries and aquaculture for FNS and included several recommendations in its Global Strategic Framework for Food Security and Nutrition (CFS, 2014). This was partially adopted in the agenda of the FAO International Conference on Nutrition (ICN2) in 2014, where fish was recognized as having "*… a special role in nutrition and health*" (FAO, 2014). In September 2015, the 17 Sustainable Development Goals (SDGs) of the 2030 Agenda for Sustainable Development was adopted by the United Nations (UN) (UN, 2015). In theory, fish, fisheries and FNS are interlinked with several SDGs, including SDG 1 (no poverty), 2 (zero hunger), 3 (good health), 8 (decent work and economic growth), 14 (life below water), 16 (peace, justice and strong institutions) and 17 (partnerships for the goals) (UN, 2015). However, many of the SDGs are not adapted nor conducive to small scale-fisheries which are essential to Ghana and other coastal LMICs. In 2016, the UN Decade of Action on Nutrition (2016–2025) was declared, which aims to achieve the global nutrition targets by 2025 and contribute to the realization of the Sustainable Development Goals (SDGs) by 2030 (UN, 2016). The United Nations has proclaimed a Decade of Ocean Science for Sustainable Development (2021–2030) with a focus on reversing the cycle of decline in ocean health and supporting countries in sustainable ocean development (UNESCO, 2018). In 2021–2025, the Decade of Nutrition and the Decade of Ocean Science will coincide, providing a unique opportunity for fish and the fisheries sectors to be recognized as vital towards achieving a food and nutrition secure future.

5. Conclusion

The available literature on fish, fisheries and FNS in Ghana is fragmented, and the objective of the current article was to review and synthesize the literature in order to assess these factors in a holistic way and examine the potential opportunities and challenges that lie ahead. This review primarily contributes to filling two gaps in the literature: first, the food security and nutrition literature lacks a focus on the role of fish, and secondly that the fisheries (governance) literature lacks a focus on fish as food, and its nutritional importance. By bringing these two perspectives together through this review paper, we demonstrate that the importance of fish for food security needs to be given greater priority and inform policy at all stages of the value chain.

Fish availability is a rising challenge for FNS and the millions of Ghanaians who depend on fisheries for their livelihoods, and access to the nutrient-dense small fish species is inherently linked to the activities of small-scale fisheries rather than to the industrial or aquaculture sectors. Currently, the incursion of international industrial trawlers not only threatens the fish supply and sustainability of fish stocks but results in declining incomes of already vulnerable small-scale fishers, processors and traders, subsequently affecting their access to food. Policy makers should make sure that legislation and governance practices ensure the interests of small-scale fisherfolk, and sustainable harvests of small fish should be advocated in order to realize the potential of these species to reduce micronutrient deficiencies. This includes increasing the focus on small fish in health policy (e.g. maternal and young child feeding and school feeding programs) and evaluating the impact of declining fish and seasonal availability through local data on fish consumption patterns.

To secure fish access, investments should be made in the fish value chain by carefully supporting the import of inexpensive fish to compensate and buffer the seasonality in fish landings, and to support low-tech smoking facilities that allow for affordable yet effective preservation of fish. Moreover, an expansion of affordable electricity in the region will allow for development of cold storage and may prevent future scenarios where small and low-cost fish could be purchased by aquafeed companies to supply the growing aquaculture industry and would be highly beneficial for FNS in West Africa.

With the Decade of Nutrition and the Decade of Ocean Science coinciding between 2021 and 2025, there is a unique opportunity for policymakers to recognize the vital role of fish and fisheries towards achieving the SDGs and secure future FNS in Ghana and other LMICs.

Declaration of competing interest

All authors declare no competing financial interests nor any other conflicts of interest.

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Appendix A. Supplementary data

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II

PLOS ONE

RESEARCH ARTICLE

Composition of nutrients, heavy metals, polycyclic aromatic hydrocarbons and microbiological quality in processed small indigenous fish species from Ghana: Implications for food security

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Abstract

The triple burden of malnutrition is an incessant issue in low- and middle-income countries, and fish has the potential to mitigate this burden. In Ghana fish is a central part of the diet, but data on nutrients and contaminants in processed indigenous fish species, that are often eaten whole, are missing. Samples of smoked, dried or salted *Engraulis encrasicolus* (European anchovy), *Brachydeuterus auritus* (bigeye grunt), *Sardinella aurita* (round sardinella), *Selene dorsalis* (African moonfish), *Sierrathrissa leonensis* (West African (WA) pygmy herring) and *Tilapia spp.* (tilapia) were collected from five different regions in Ghana. Samples were analyzed for nutrients (crude protein, fat, fatty acids, several vitamins, minerals, and trace elements), microbiological quality (microbial loads of total colony counts, *E. coli*, coliforms, and Salmonella), and contaminants (PAH4 and heavy metals). Except for tilapia, the processed small fish species had the potential to significantly contribute to the nutrient intakes of vitamins, minerals, and essential fatty acids. High levels of iron, mercury and lead were detected in certain fish samples, which calls for further research and identification of anthropogenic sources along the value chains. The total cell counts in all samples were acceptable; *Salmonella* was not detected in any sample and *E. coli* only in one sample. However, high numbers of coliform bacteria were found. PAH4 in smoked samples reached high concentrations up to 1,300 μg/kg, but in contrast salted tilapia samples had a range of PAH4 concentration of 1 μg/kg to 24 μg/kg. This endpoint oriented study provides data for the nutritional value of small processed fish as food in Ghana and also provides information about potential food safety hazards. Future research is needed to determine potential sources of contamination along the value chains in different regions, identify critical points,

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and develop applicable mitigation strategies to improve the quality and safety of processed small fish in Ghana.

Introduction

With a growing global population and an increasing number of undernourished people, having access to sufficient amounts of safe and nutritious food is crucial [1]. The significance of food and nutrition security (FNS) is anchored in the United Nations Sustainable Development Goals (SDGs), specifically SDG 2 “Zero Hunger” and SDG 3 “Good Health and Well-Being” [2]. Fish is a source of many essential nutrients [3–7], and can substantially improve FNS especially in a diet that largely consists of starchy staple foods such as cassava, yam, rice, maize and millet, which is the case in Ghana [8–11]. Compared with animal source foods, starchy foods contain low amounts of micronutrients, have poorer protein quality and may be a source of phytate which further aggravates the severity of undernutrition by inhibiting the absorption of essential minerals and limiting protein and lipid utilization [12, 13]. Fish is an essential part of the Ghanaian diet and is accessible at a low cost, with an apparent per capita consumption of 28 kg/year and constituting 50–80% of consumed animal protein [14–16]. In Ghana small fish is usually eaten whole [3], which represents the most beneficial form of consuming fish in terms of nutrient density, providing vitamins A, D and B_{12}, minerals such as calcium and trace elements iodine, selenium, iron and zinc [17, 18]. In contrast, when consuming larger fish, the micronutrient-rich organs, viscera and bones are usually removed, which limits the potential nutrient content [19].

Different processing techniques, such as smoking, drying and salting enables the availability of marine and freshwater fish throughout the country [20]. To prevent spoilage and prolong shelf life, smoking is the leading technique of fish processing in Ghana constituting 70–80% of locally consumed fish, while the remaining percentage is consumed fresh or subjected to drying, salting, frying or fermentation [21]. Commonly, a metal drum kiln or “Chorkor smoker” is used [22–24] in which fat and other fluids from fish may drip into the open flames and form harmful substances, such as polycyclic aromatic hydrocarbons (PAHs), which have reported carcinogenic and mutagenic properties [23, 25, 26]. Another potential hazard for consumers is microbial contamination, especially in countries where cold storage is not widely available to inhibit microbial growth. Here, the management and way of processing, like smoking or drying of the fish, plays an essential role [24, 27]. Further, improper handling and hygiene practices, storage, and insufficient smoking or drying can increase the likelihood of recontamination and growth of microorganisms including pathogenic bacteria from fecal sources [28–31]. Additionally, fish may be a source of heavy metals e.g. mercury, arsenic, lead and cadmium [32, 33]. The majority of fish consumed in Ghana is of marine origin and low levels of mercury have been registered in fillet of fresh marine fish from the Gulf of Guinea [34, 35]. Nonetheless, one of the largest e-waste recycling sites in Africa is located in Accra, resulting in large-scale environmental contamination with many toxicants, particularly lead, at high concentrations through smoke emission [36, 37]. The elevated ambient burden of heavy metals in surrounding areas contributes to the toxic exposure of the general population, and quite possibly, to the fish markets situated nearby [36]. In the freshwater lakes and rivers, discharge of effluents from gold mines and other industries increases the presence of mercury and other contaminants [38, 39].

The current study is part of the SmallFishFood project (https://smallfishfood.org/), an international consortium collaborating to understand how socio-cultural, economic and institutional transformations of the fish food and value chain—from ecosystem to consumer—can contribute to improved, sustainable utilization of small fish resources for Africa's low-income population. Access to local, reliable and up-to-date food composition data (FCD), particularly on foods that are essential to vulnerable population groups, is scares but imperative to improve FNS. Although the main body of FCD comprises nutrients, data on potentially harmful food components such as heavy metals, polycyclic aromatic hydrocarbons (PAHs) and pathogenic microorganisms are also important for nutritional recommendations, as it provides the basis for the estimation of exposure, risk assessments and risk-benefit analysis [40]. Combined, this knowledge may also be used to develop programs and policies to improve FNS [41, 42]. In most published data on nutritional quality and safety parameters in fish only the flesh of raw fish has been analyzed, and the analytical data on nutritional quality and safety of processed whole small fish are scarce.

The aim of the present study was to generate analytical data on selected nutrients, as well as microbial, heavy metal and PAH contamination in six commercially important processed small fish species sampled from five regions in Ghana.

Materials and method

Sampling

Selection of fish species sampled from Ghana were based on a market survey conducted in February 2018, where open markets in Accra, Techiman and Kumasi were visited to determine the most frequent fish species, fish sizes and the most common processing method used for each species. During November 2018, samples of processed small marine fish species *Engraulis encrasicolus* (European anchovy), *Brachydeuterus auritus* (bigeye grunt), *Sardinella aurita* (round sardinella), *Selene dorsalis* (African moonfish) and small freshwater species *Sierrathrissa leonensis* (West African (WA) pygmy herring), and *Tilapia spp*. (tilapia) were sampled at selected fish markets throughout Ghana. The identification details of the sampled species, including scientific and Ghanaian names, are presented in Table 1. The species were sampled from six markets in five cities, representing five different regions in Ghana; Accra Agbogbloshie and Adabraka Market (Accra, Greater Accra region, near coast), Kumasi Central Market (Kumasi, Ashanti region, about 215 km from coastline), Techiman Market (Techiman, Brong-Ahafo region, about 335 km from coastline), Tamale Market (Tamale, Northern region, about 600 km from coastline) and Bolgatanga Market (Bolgatanga, Upper East region, about 760 km from coastline).

Sample size. The sampling was conducted according to Greenfield and Southgate's requirements for retail sampling [43]. Sample size (number of specimens) was adapted from Öhrvik, von Malmborg [44], who estimated that each composite sample of fish should consist of minimum 12 specimens. The calculation was based on a formula for sample size by Greenfield and Southgate [43] using the variability of key nutrients in fish including docosahexaenoic acid (DHA) [45], vitamin D and selenium [46]. This would require that for each fish species, more than 60 individuals in total and 12 individuals from each selected location should be collected. However, approximately 500 g of organic material was estimated to be needed to perform the targeted analyses, and more than 12 individuals of each fish species were thus collected from each location.

Sampling procedure. Upon arrival at the fish markets, the sampling was started in one section of the market and the selected fish species were sampled from every third market stall. The selected species were identified by a taxonomist, and two separate batches of approximately 100 g of fish was weighed and collected in zip lock plastic bags. Depending on availability of the selected fish species at each fish market, which was influenced by the unpredictable

Table 1. Species, habitat, weight and length of fish sampled from five different locations in Ghana.

Common name	Scientific name	Local name	Habitat	Tissue analyzed	Fish length (cm)[a]	Processing method	Batches	Composite samples (n)	Specimens in each composite sample	Batch/ composite weight (g)
European anchovy	*Engraulis encrasicolus*	Amoni, Bonu, Abobi, Saskawesi, Ablobi[d]	Marine, pelagic	Whole fish[b,c]	6.3 (5–7.6)	Smoked	25	5	>250	100/500
Bigeye grunt	*Brachydeuterus auritus*	Boeboe, Moe, Hawui, Eboe, Ano kpetei[d]	Marine, benthopelagic	Whole fish without head[b]/ whole fish[c]	12.4 (11.8–12.9)	Smoked	15	3	45 (42–50)	100/500
African moonfish	*Selene dorsalis*	Antele-wawaa, Ngogba lolotor, Epo edwire, Tantemire ansoradze, Epo edwile, Ndademire[d]	Marine, demersal	Whole fish[b,c]	9 (6.6–10.8)	Smoked	12	3	75 (35–100)	100/500
Round Sardinella	*Sardinella aurita*	Kankama, Man, Vetsimu, Eban[d]	Marine, pelagic	Whole fish[b,c]	13.3 (12.7–15.4)	Smoked	24	5	49 (33–73)	100/500
Tilapia	*Tilapia spp.*	Apatire Tidie, Akpaa, Apataa, Koobi[e]	Freshwater, benthopelagic	Whole fish without guts[b]/ whole fish[c]	11.3 (10.2–12.8)	Salted	23	5	43 (22–73)	100/500
West African pygmy herring	*Sierrathrissa leonensis*	One man thousand, Woevi[e]	Freshwater, pelagic	Whole fish[b,c]	4.6 (3.4–6)	Dried or smoked	7	3	230 (>100–500)	100/500

[a] Numbers presented as mean and range (min-max).

[b] Tissue analyzed for nutrients, heavy metals and PAHs.

[c] Tissue analyzed for microbiological contamination.

[d] Local names from [59].

[e] Local names.

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nature of fish supply from small-scale fishermen and fishmongers, between 1–5 batches of each processed fish species were collected from different market stalls at each location. After sampling, the samples were stored in Styrofoam boxes and kept at room temperature along the sampling route from Bolgatanga to Accra. The sampling was conducted between the 2nd and 24th of November 2018. In Accra, the batches of each sampled fish species were pooled into composite samples for analysis of nutrients, heavy metals and PAHs, with each composite sample representing one species from one location. Subsequently, the pooled samples were shipped in boxes containing cooling elements via express airmail to Norway on November 29th, 2018. Un-pooled batch samples for microbial analysis were shipped via express airmail to Germany on December 17th, 2018.

Chemical analyses of nutrients and contaminants

Depending on the fish species, the tissue for analysis was prepared according to Ghanaian food customs, and may or may not include head, viscera, bones, scales or other parts (Table 1). The composite samples were homogenized as per edible parts prior to analysis in a food processor (Braun 3210, Neu-Isenburg, Germany), and subsamples of the homogenate were distributed into tubes (Thermo Scientific Nunc A/S, Roskilde, Denmark) and stored at -20˚C or -80˚C pending analysis. Samples for analyses of metals, minerals, trace elements, and PAHs were freeze dried in accordance with the AOAC 930.15 method as previously described [47]. All composite samples were analyzed for total protein, total fat and fatty acid (FA) content including saturated fatty acids (SFA), sum monounsaturated fatty acids (MUFA), sum polyunsaturated fatty acids (PUFA), n-3/n-6 ratio, eicosapentaenoic acid (EPA), and DHA. Furthermore, the concentration of vitamins A (A_1 and A_2) and D_3, calcium and trace elements iodine, selenium, zinc, and iron were analyzed. The concentration of heavy metals arsenic (total arsenic), mercury (total mercury), lead, and cadmium was analyzed in all composite samples in addition to levels of PAHs. All analyses were performed in singular parallels at the IMR laboratories using accredited methods with NS-EN ISO/IEC 17025 standards, except for iron. The analytical methods including corresponding limits of quantification (LOQ) and measurement uncertainties are described in detail elsewhere [48].

Determination of crude protein, crude fat and fatty acids. The crude protein content was determined by burning the material in a combustion tube containing pure oxygen at 830˚C. Nitrogen (N) was detected with a thermal conductivity detector (TCD), and the content of N was calculated from an estimated average of 16% N per 100 g protein using the following formula

$$\mathrm{N\,g/100\,g} \times 6.25 = \mathrm{protein\,g/100\,g}.$$

The method is accredited according to AOAC Official Methods of Analysis [49].

For determination of crude fat, the fat content was extracted with ethyl acetate and filtered before the solvent was evaporated and the residual fat was weighed. The method is accredited in accordance with ISO-EN 17025 and standardized as a Norwegian Standard, NS 9402 [50].

The fatty acid content was determined using gas liquid chromatography (GLC). Lipids in the samples were extracted according to Folch, Lees [51], and the fatty acid composition of total lipids was analyzed as previously described in Fauske, Bernhard [52]. Methyl esters were separated on a capillary gas column (CP-sil-88, 50 m WCOT, ID:0.32) and peaks were identified by retention time using standard mixtures of methyl esters (Nu-Check, Eylian, USA). Content of fatty acids per gram sample was subsequently calculated using 19:0 methyl ester as an internal standard.

Determination of vitamins. To determine vitamin A_1 (sum all trans-retinol and 13-, 11-, 9 cis retinol) and A_2 (4,4 didehydro-all-trans retinol) content, the samples were saponified and the unsaponifiable material was extracted. Vitamin A_1 and A_2 was determined by high-performance liquid chromatography (HPLC) (normal phase) using a PDA detector (Photo Diode Array) and the retinol content was calculated by external calibration (standard curve) [53].

For determination of vitamin D_3, the samples were saponified, and the unsaponifiable material was extracted and subsequently purified in a preparative HPLC column. The fractions containing D_2 (ergocalciferol) and D_3 (cholecalciferol) were pooled (normal phase) and injected on an analytic HPLC column (reverse phase). Vitamin D_3 and D_2 was determined by an ultraviolet (UV) detector and the content of vitamin D_3 was calculated using vitamin D_2 as an internal standard [54].

Vitamin B_{12} (Cobalamin) was released from the sample by extraction (autoclaving in acetate buffer) and mixed with growth medium before the microorganism (*Lactobacillus delbruecki* -ATCC 4797) was added and subsequently incubated at 37˚C for 22 hours. The concentration of vitamin B12 was calculated by comparing the growth of the organism in the unknown samples with the growth of the organism in known standard concentrations by turbidimetric reading (Optical Density, OD, v / 575 nm) [55].

Determination of metals, minerals and trace elements. The concentration of calcium, heavy metals cadmium, arsenic (total), mercury (total), and lead as well as trace elements selenium, zinc, and iron was determined by Inductively Coupled Plasma-Mass Spectrometry (iCapQ ICP-MS, ThermoFisher Scientific, Waltham, MA, USA) equipped with an autosampler (FAST SC-4Q DX, Elemental Scientific, Omaha, NE, USA) after wet digestion in a microwave oven as previously described by Julshamn, Maage [56]. For determination of iodine, the sample was extracted with tetramethylammonium hydroxide (TMAH) before ICP-MS analysis. The elements were quantified using an external standard curve in addition to different internal standards for specific elements; scandium (Sc) was used as internal standard for calcium, rhodium (Rh) was used as internal standard for zinc and selenium, tellurium (Te) was used for iodine and either Rh, germanium (Ge), indium (In) or thulium (Tm) was used for the heavy metals.

Determination of PAHs. The concentration of the PAHs benz(a)anthracene (BaA), benzo (a)pyrene (BaP), benzo(b)fluoranthene (BbF) and Chrysene (Chr), collectively referred to as PAH4, were determined by Gas Chromatography Mass Spectrometry (GC-MS/MS) after extraction with dichloridemethane (DCM): cyclohexane (1:3) using an US EPA 16 PAH cocktail (CIL ES-4087) internal standard. The extract was evaporated and rinsed on an SPA column (Envichrom) before adding recovery standard and analyzing the samples by GC-MS/MS [57, 58].

Microbial analysis

Sample preparation. After arrival at the German Federal Institute for Risk Assessment, the samples were stored at 4˚C until further processing. Samples were homogenized batchwise using a GRINDOMIX GM200 (Retsch GmbH, Haan, Germany) for 20 seconds at 4.000 rpm and stored in plastic cups with a screw-on lid (WMC Medical Consulting, Pulheim, Germany) at 4˚C until microbiological analysis.

Rehydration of fish and preparation of pooled initial suspension. The batchwise samples were processed as described in ISO 6887–3:2003. A batch sample aliquot of 11 g was mixed with 22 g buffered peptone water (Mast Group, Bootle, United Kingdom) in a peristaltic lab blender (Bag Mixer CC, Interscience, Saint Nom, France) for 10 seconds and rehydrated at room temperature for 1 h. The batchwise initial suspensions were prepared as a tenfold dilution of the rehydrated fish by addition of 297 g of buffered peptone water and mixing it for 90 seconds. For microbial analysis, initial suspensions of batch samples were pooled per species and market, as described for the analysis of nutrients and minerals.

Analysis of total colony count, *E. coli*, coliform bacteria and *Salmonella* spp.. The total colony count (TCC) analysis was based on ISO 4833:2015, however, instead of plate count agar, Columbia blood agar plates (Oxoid Deutschland GmbH, Wesel, Germany) were used. The pooled initial suspension was further diluted 1:10 in maximum recovery dilution (Oxoid Deutschland GmbH, Wesel, Germany) and 100 μL per dilution step were spread on agar plates. One set of plates were incubated at 30˚C for 72 h aerobically, and an additional set of plates were incubated at 30˚C for 72 h under anaerobic conditions in anaerobic jars with AnaeroGen™ sachets (Oxoid Deutschland GmbH, Wesel, Germany). The limit of detection (LOD) based on the detection of one colony per plate was calculated as 2.5 log CFU/g of processed fish.

For quantification of *E. coli* and coliform bacteria, Brilliance™ *E. coli*/coliform agar plates (CM1046, Oxoid Deutschland GmbH, Wesel, Germany) were inoculated with 100 μL of the pooled initial suspension and their further dilutions. In addition, 1 mL of the pooled initial suspension was spread on three plates to increase the level of detection 10-fold (LOD 1.5 log CFU/g). The plates were incubated at 37˚C for 24 h, aerobically.

The detection of *Salmonella* spp. was performed according to ISO 6579–1:2017. The 300 mL initial suspensions per batch were incubated at 37˚C for 18 h, aerobically. After incubation, enriched batch samples were pooled as described above. An aliquot of 1 mL of the pooled enrichment culture was added to 10 mL Mueller Kauffman Tetrathionate Novobiocin broth (MKTTn; Oxoid Deutschland GmbH, Wesel, Germany) and incubated at 37˚C for 24 h, aerobically. Further, 100 μL was dripped as three droplets on modified semi-solid Rappaport Vassiliadis agar (MSRV; Oxoid Deutschland GmbH, Wesel, Germany) and incubated at 41.5˚C for 24 h, aerobically. After incubation, 10 μL of MKTTn selective culture or a loop full of spreading growth from MSRV were spread to Xylose Lysine Deoxycholate agar plates (XLD, Merck, Darmstadt, Germany) and Gassner agar plates (GAS, sifin, Berlin, Germany) both incubated at 37˚C for 24 h aerobically to achieve single colonies. After incubation, plates were checked for presumptive colonies.

Data management

The analytical data was exported from Laboratory Information Management Systems (LIMS) and processed using Microsoft Excel 2013. When calculating mean and standard deviation, analyses below LOQ were included in the calculations as the respective LOQ value divided by two. Statistical analyses was performed using IBM SPSS version 26 (IBM Corp., Armonk, NY, USA) and for all tests a significance level of $\alpha = 0.05$ was used. Normal distribution was tested using Shapiro-Wilk test. For normally distributed data, the homogeneity of variances was tested using Levene-test. If homogeneity of variances was given, the differences between the fish species was analyzed using one-way analysis of variance (ANOVA). As a post hoc test to discriminate different pairs, a Tukey-test was performed. If the homogeneity of variances was not given, a Welch-ANOVA was used to determine the differences between the fish species, followed by a Dunnet-T3-test. If the values were not normally distributed, a Kruskal-Wallis-test was performed to determine differences between the species followed by pairwise comparison of the fish species with a Bonferroni correction.

Results and discussion

In the present study, analytical data on 24 composite samples of processed, small fish (total length 4.6–13.3 cm) from several fish markets covering a large geographic range in Ghana are reported (Table 1). Based on the nutritional analyses all the processed fish samples, except tilapia, had the potential to significantly contribute with essential nutrient to the intakes of vitamins, minerals, and essential fatty acids. In all samples, the microbiological total cell counts were acceptable (Table 2). High levels of PAHs, mercury and lead were detected in some samples (Table 2), which calls for further research and identification of anthropogenic sources in different regions and along the value chains.

Nutrient content

Proximate and fatty acid composition. The content of protein and fat as well as fatty acid composition of the processed fish species are presented in Table 2 and in S3 Table. Protein content ranged from 31.80 g/100 g in salted tilapia to 71.80 g/100 g in smoked European anchovy, thus constituting a significant source of complete proteins in the otherwise starchy-staple-dominant Ghanaian diet. The stability of proteins in fish have previously been examined, and analyses of sardine (*Sardinella* spp.) from Ghana reported minimal variation in protein content between fresh and smoked specimens [60]. Furthermore, Usydus, Szlinder-Richert [61] found that in terms of both quality and quantity, the amino acids in smoked and salted fish remain stable and highly digestible according to WHOs protein standard [61]. Despite being subjected to a variety of processing techniques, dried, salted and smoked fish from Ghana can thus be regarded as a high-quality protein source [62].

Table 2. Analytical values (means ± standard deviation, wet product weight) for selected nutrients, microbial quality and contaminants of processed whole fish from Ghana.

		Unit	Anchovy	Bigeye grunt	Round sardinella	African moonfish	WA pygmy herring	Tilapia	p-value
Moisture content		%	8.45±1.31^{AB}	8.22±1.77^{AB}	7.92±1.75^{A}	10.36±1.66^{AB}	7.87±2.04^{AB}	28.50±7.96^{B}	0.019
Nutrients	**Protein**	g/100g	71.80±2.05^{A}	64.33±1.53^{B}	64.80±3.19^{B}	67.67±1.53^{AB}	67.67±3.06^{AB}	31.80±3.90^{C}	<0.001
	Total Fat	g/100g	6.44±0.77^{A}	15.03±3.80^{AB}	13.84±1.29^{B}	6.37±0.97^{A}	12.23±3.38^{AB}	7.02±2.11^{A}	0.001
	Sum SFA	g/100g (%)	2.00±0.47^{A} (37)	5.16±1.44^{B} (38)	4.94±0.77^{B} (33)	1.99±0.21^{A} (38)	4.02±0.78^{BC} (39)	2.93±0.38^{AC} (41)	<0.001
	Sum MUFA	g/100g (%)	0.75±0.13^{A} (14)	3.20±1.01^{B} (24)	2.48±0.52^{AB} (26)	0.92±0.10^{AB} (18)	2.86±0.97^{B} (27)	2.06±0.36^{AB} (28)	0.003
	Sum PUFA	g/100g (%)	2.33±0.58^{AB} (43)	4.25±1.04^{C} (32)	3.98±0.58^{C} (35)	1.92±0.20^{AB} (37)	3.12±0.63^{AC} (30)	1.68±0.17^{B} (23)	<0.001
	EPA	g/100g (%)	0.41±0.13^{AB} (7)	0.77±0.26^{B} (6)	0.82±0.17^{B} (7)	0.34±0.07^{AB} (6)	0.29±0.06^{AB} (3)	0.04±0.01^{A} (1)	0.001
	DHA	g/100g (%)	1.41±0.32^{A} (26)	2.21±0.47^{B} (16)	2.01±0.30^{B} (16)	1.04±0.12^{AC} (20)	0.77±0.22^{CD} (8)	0.18±0.06^{D} (3)	<0.001
	Vitamin B_{12}	μg/100g	14±3^{AB}	9±1^{A}	23±1^{B}	14±1^{A}	16±6^{AB}	11±3^{A}	0.014
	Vitamin D_3	μg/100g	12±3^{AB}	15±6^{AB}	34±9^{A}	9±3^{B}	17±10^{AB}	13±4^{AB}	0.024
	Vitamin A_1	μg/100g	14±15^{AB}	323±153^{B}	10±3^{AB}	290±46^{B}	39±28^{AB}	4±8^{A}	0.004
	Calcium	mg/100g	2940±207^{AB}	3533±577^{ABC}	3040±207^{B}	5467±153^{C}	2633±379^{AB}	2900±436^{AB}	0.026
	Iron	mg/100g	25±6^{A}	22±9^{AB}	19±3^{A}	50±12^{AB}	78±89^{AB}	10±3^{B}	0.010
	Zinc	mg/100g	6.3±0.9^{ABC}	3.4±0.4^{BC}	5.1±0.2^{ABC}	4.9±1.0^{ABC}	14.7±2.9^{A}	4.3±0.6^{BC}	0.003
	Selenium	μg/100g	192±28^{A}	113±12^{B}	242±37^{A}	173±15^{A}	94±15^{BC}	33±7^{C}	<0.001
	Iodine	μg/100g	170±48^{A}	219±122^{A}	142±31^{AB}	233±25^{A}	129±74^{AB}	49±11^{B}	0.002
Microbial Quality	**TCC aerob**	log CFU/g	5.06±0.74^{A}	4.58±0.23^{A}	4.62±0.52^{A}	4.93±0.30^{A}	6.15±0.74^{A}	4.23±0.64^{A}	0.077
	TCC anaerob	log CFU/g	4.36±0.92^{A}	3.67±0.42^{A}	4.05±0.92^{A}	3.98±0.27^{A}	NA^{A}	4.43±1.01^{A}	0.505
	Coliform	log CFU/g	2.73±0.85^{A}	2.86±1.05^{A}	2.93±1.19^{A}	2.69±0.07^{A}	3.12±1.28^{A}	<LOD^{A}	0.639
Contaminants	**PAH4**	μg/kg	478±164^{AB}	553±155^{A}	418±103^{AB}	443±91^{AB}	600±666^{AB}	7±10^{B}	0.034
	Cadmium	mg/kg	0.306±0.053^{A}	0.116±0.016^{BC}	0.186±0.031^{B}	0.065±0.019^{CD}	0.015±0.004^{D}	<LOQ^{D}	<0.001
	Lead	mg/kg	0.13±0.06^{A}	0.16±0.11^{A}	0.10±0.04^{A}	0.24±0.10^{A}	0.64±0.61^{A}	<LOQ^{A}	0.059
	Mercury	mg/kg	0.034±0.006^{AB}	0.065±0.014^{A}	0.034±0.009^{AB}	0.045±0.013^{AB}	0.223±0.127^{A}	<LOQ^{B}	0.003
	Arsenic	mg/kg	7.8±1.9^{A}	4.9±0.6^{A}	9.8±3.0^{A}	5.7±1.4^{AB}	0.9±0.6^{B}	0.1±0.0^{B}	<0.001

Different superscript capital letters indicate statistical significance with p-value below 0.05. <LOD—Mean value below limit of detection; <LOQ—Mean value below limit of quantification; NA- Not available; SFA—saturated fatty acids; MUFA—monounsaturated fatty acids; PUFA—polyunsaturated fatty acids; EPA—eicosapentaenoic acid; DHA—docosahexaenoic acid; TCC—total colony count; PAH4 –sum of benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene and Chrysene.

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Fat content in fish is more variable than other proximate components and may reflect a natural variance in different fish species along with seasonal variations in feed sources and availability for the given species [63]. A considerable range in fat content was thus expected and found (from 6.37 g/100 g in smoked African moonfish to 15.03 g/100 g in smoked bigeye grunt). A noteworthy variation in the fat content of WA pygmy herring was observed between the different markets (8.4–14.8 g/100 g), which consisted of both dried and smoked composite samples. It has been reported that smoking may have a modifying effect on the concentration of fat in fish [64, 65]. However, by taking the FA compositions into consideration, we theorize that the variance observed in WA pygmy herring may be attributed to the use of plant-based cooking oil during processing.

The marine products smoked bigeye grunt and smoked round sardinella had the highest mean values of the n-3 fatty acids EPA and DHA, while smoked European anchovy had the highest content of EPA and DHA in terms of percentage. Salted tilapia and dried/smoked WA pygmy herring had the lowest content of EPA and DHA, which was expected given their freshwater origin. However, the difference to most marine water species was only significant for DHA. Interestingly, African moonfish had a similar content of EPA and DHA to the freshwater species WA pygmy herring. Further, the freshwater species WA pygmy herring showed high levels of PUFAs being more comparable to the sampled marine species and significantly higher than the levels of the freshwater species tilapia. Fatty acids are highly susceptible to oxidation; however, previous studies have documented that automated smoking of Atlantic mackerel (*Scomber scombrus*) and smoking of sardines (*Sardinella* spp.) and tilapia (*Tilapia* spp.*)* in a Ghanaian chorkor oven did not produce significant changes in fatty acid composition [64, 66]. However, the specific effects of different processing methods on fatty acid composition may not be concretized in the current study and is in need of further exploration.

Vitamin composition. The analytical values for vitamins D, A_1 and B_{12} in the different processed fish species are presented in Table 2 and S2A–S2F Table. Vitamin D-levels were determined, ranging from 9.0 μg/100 g in African moonfish to 34.2 μg/100 g in round sardinella. It has been documented that the reduced water content from smoking increases the concentration of several nutrients [65], but heating processes have also proven detrimental to vitamin D retention [67]. Yet, the highest concentration of vitamin D in the current study from smoked round sardinella (34.0 μg/100 g) is similar to vitamin D levels in raw summer herring (11.5 μg/100 g) from the north Atlantic [68] when the difference in water content is adjusted for. Fish is one of few foods which naturally contain vitamin D and can therefore help to prevent vitamin D deficiency [69]. Deficiency in Vitamin D can lead to maldevelopment in the skeletal system and is presumed to also have a negative influence on the cardiovascular system [69–72]. Despite being subjected to different processing techniques, the analyzed fish may be considered valuable dietary sources of vitamin D.

Vitamin A_1 content ranged considerably between species, from mean levels below the limit of quantification in salted tilapia to 323 μg/100 g in smoked bigeye grunt. To our knowledge, the impact of smoking on vitamin A degradation in fish has not been studied yet, but it has been established that sunlight and heat reduces vitamin A activity in foods up to 90% [73]. Vitamin A_1 content could not be linked to fat content in the current study, introducing exposure to sunlight and high temperatures during processing and storage at fish markets as possible variables. Furthermore, Roos, Leth [74] documented that certain small fish species are valuable sources of vitamin A, with up to 50% of vitamin A concentrated in the eyes or in the viscera. It is worth mentioning that the fish with the highest vitamin A_1 content, bigeye grunt, was the only fish analyzed without head. Analyses of vitamin A_2's characteristics are still in its inception, but current findings show that the bioavailability of vitamin A_2 is higher (119–127%) than for A_1 [75] and that some freshwater species consumed whole are a good dietary source [76]. In the present study, quantifiable levels of vitamin A_2 were only detected in smoked marine fish species bigeye grunt (8.9 μg/100 g), round sardinella (5.2 μg/100 g) and African moonfish (3.4 μg/100 g). The ratio of vitamin A_2 to total vitamin A content was low for bigeye grunt and African moonfish but constituted a considerable share ($> 30\%$) in round sardinella, thus indicating that some processed marine fish species are also a potential source of vitamin A_2. Collectively, our results suggest that including smoked bigeye grunt or smoked African moonfish in the diet may contribute towards alleviating the persistent burden of vitamin A deficiency in Ghana [77].

All fish species contained considerable amounts of vitamin B_{12}, ranging from 8.9 μg/100 g in processed bigeye grunt to 23.0 μg/100 g in round sardinella. Animal source foods are known as the major dietary source of vitamin B_{12} [78] and previous studies have documented that the concentration of vitamin B_{12} is up to three times higher in the viscera of fish compared to fillet [79]. This indicates that consuming small fish whole may be suitable, particularly for population groups with limited access to animal source foods. A study on round herring *(Etrumeus teres)* demonstrated that heating processes may decrease vitamin B_{12} levels by up to 62% [79], but the final concentration of vitamin B_{12} in smoked, dried or salted fish could nevertheless be higher than in fresh fish. Still, the cumulative effects of high temperatures during both processing and cooking on vitamin levels are yet to be thoroughly investigated.

Mineral composition. Of the analyzed processed fish species, tilapia was the least mineral dense, containing the lowest concentrations of selenium (33 μg/100 g), iodine (49 μg/100 g) and iron (10 mg/100 g) compared with all other analyzed species, and significantly lower levels of selenium and iodine than the marine species. Round sardinella had the highest levels of selenium (242 μg/100 g) while African moonfish contained significantly higher concentrations of calcium (5467 mg/100 g) and iodine (233 μg/100 g). The smallest fish analyzed, WA pygmy herring, had the highest concentrations of both iron (78 mg/100 g) and zinc (15 mg/100 g). Even though mean iron values of WA pygmy herring were not significantly higher than for other species (Table 2), high levels of iron were detected in the composite sample of dried WA pygmy herring from Accra (180 mg/100 g; S2E Table), which exceeds previous findings of iron in smoked-dried tilapia from Ghana twentyfold [64]. The high values of iron measured in the specific composite sample of WA pygmy herring from Accra may be linked to the Agbogbloshie area in Accra where the ambient metal contamination is high due to burning of e-waste. But further studies are needed to verify this potential regional contamination pathway. Still, with a high prevalence of iron deficiency anemia [80], the inclusion of processed small fish could be a valuable addition to the staple-dominant Ghanaian diet by providing bioavailable iron [3, 9, 81, 82].

Zinc is essential for optimal growth, and is closely interlinked with aggravated symptoms of iron deficiency anemia with its role as a catalyst in iron metabolism [83]. Although the prevalence of zinc deficiency has not yet been evaluated in Ghana, it can be assumed that a diet comprising limited animal-source foods is inherently low in terms of both zinc content and bioavailability [13]. Zinc levels in the current study were similar to previously determined levels in smoked sardine (S*ardinella* spp) from Ghana [64] and notably higher than the concentration in small raw freshwater fish analyzed whole [84]. These findings also correspond with the reported stability and high retention of zinc documented in cooked foods [85, 86]. Incorporating all processed fish species, but primarily WA pygmy herring, in the diet could thus be a valuable contribution towards increasing dietary zinc and simultaneously alleviating iron deficiency anemia in Ghana.

Adequate iodine intake is essential for synthesis of thyroid hormones and neurodevelopment, however, the magnitude of iodine deficiency among children and other vulnerable population groups in Ghana is not thoroughly mapped. Marine fish are regarded as the superior dietary source to iodine [87]. However, iodine content largely depends on environmental conditions, and large variations are expected both between and within different fish species [88, 89]. This great variation was confirmed in the present study, with iodine content in bigeye grunt ranging from 96–340 μg/100 g. As a freshwater fish, processed tilapia showed significantly lower iodine levels than most of the marine species. Interestingly, the freshwater species WA pygmy herring showed relatively high iodine levels which were comparable to the processed marine species. In line with our results, former analyses of iodine in whole smoked European sprat (*Sprattus sprattus*) have yielded high average levels of iodine (148 μg/100 g) and iodine retention ranged from 48–93% in a study on different cooking methods [90], thus underlining that processed and cooked fish may be considered a good source of dietary iodine.

Selenium plays a key role in ameliorating the toxic effects of mercury [91] which is present in variable concentrations in fish [92, 93]. Previous analyses of smoked European sprat (*Sprattus sprattus*) reported a selenium content of 20 μg/100 g [94], which is markedly lower than for all fish species analyzed in the current study. With the high levels of selenium determined in our study we conclude that along with fresh seafood [95], processed fish may be considered an excellent dietary source of selenium.

Food safety

Microbiological quality. A total of 24 pooled samples were analyzed for bacteria related to microbiological quality. Total colony count (TCC) was used to describe the general bacterial load which can be used as an indicator for poor hygiene conditions and control along the value chain. Coliform bacteria and *E. coli* were used as indicator bacteria for fecal contamination along the value chain and *Salmonella* was analyzed as their presence is associated with food-borne disease. The microbial quality parameters of the processed fish species are presented in Fig 1 and Table 2.

As shown in Fig 1, the highest aerobic TCC were found in WA pygmy herring from Bolgatanga (6.67 log CFU/g) while the lowest counts were detected in tilapia from Tamale (3.85 log CFU/g). The two sampled freshwater fish species differed in two orders of magnitude in mean aerobic TCC with 6.15±0.74 log CFU/g in WA pygmy herring and 4.23±0.64 log CFU/g in tilapia. While in marine species, mean aerobic TCC ranged between 5.06±0.74 log CFU/g (European anchovy) and 4.58±0.23 log CFU/g (bigeye grunt). The Ghana Standards Authority provides limits for aerobic TCC in hot smoked fish with an acceptable limit (m) for TCC at 5.7 log CFU/g and a rejection limit (M) for TCC at 7 log CFU/g (GS 95:2013). None of the here

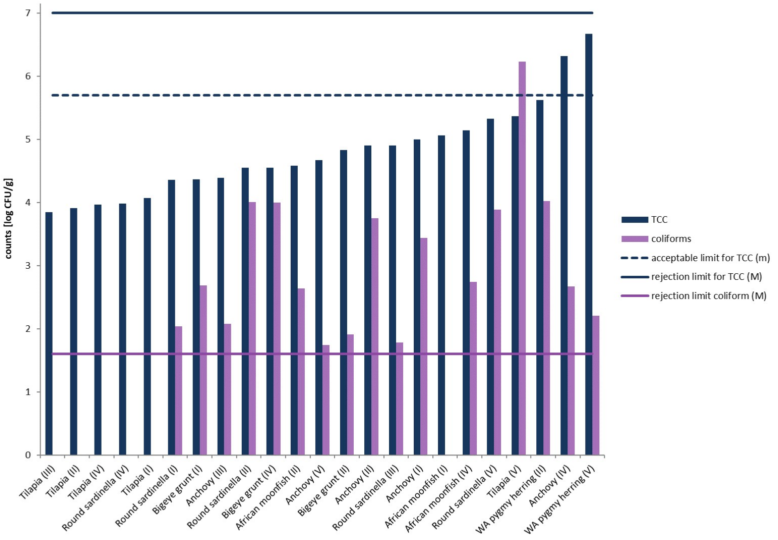

Fig 1. Total Colony Counts (TCC), counts of coliform bacteria (coliforms) and the acceptable limit (m, 5.7 log CFU/g) for TCC, the rejection limit for TCC (M, 7 log CFU/g) and the rejection limit for coliforms (M, 1.6 log CFU/g) by the Ghana Standards Authority (GS 95:2013). I: Accra; II: Techiman; III: Tamale; IV: Kumasi; V: Bolgatanga.

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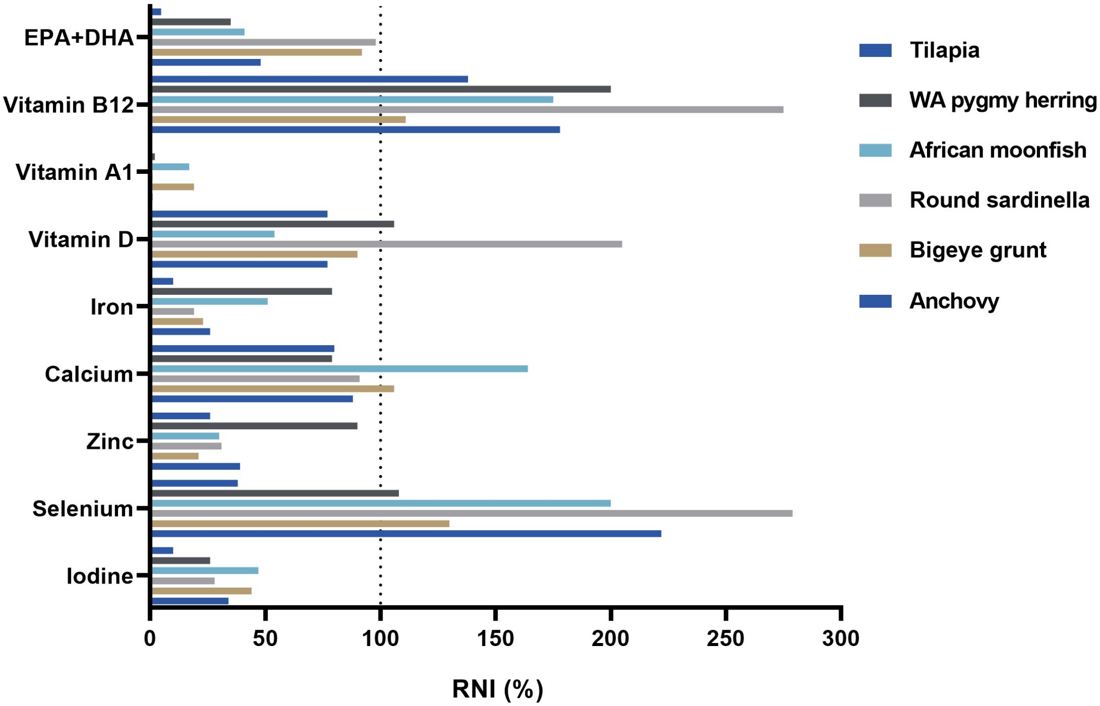

Fig 2.

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analyzed samples had an aerobic TCC above the rejection limit (Fig 2), but European anchovy samples from Kumasi (6.32 log CFU/g) and WA pygmy herring from Bolgatanga (6.67 log CFU/g) had TCCs above the acceptable limit. This means that more than 90% of all samples were within the Ghanaian acceptable limit for aerobic TCC. There are no scientific reports available on aerobic TCC in processed fish from Ghanaian markets and only few reports are available from other African countries. In Nigeria, Adegunwa, Adebowale [96] analyzed smoked herring (*Sardinella eba*) samples from three different markets and found aerobic TCC ranging from 6.36 log CFU/g to 7.47 log CFU/g, thus being more than one log-cycle higher than in the here presented samples in general. Another study from Nigeria by Akinwumi and Adegbehingbe [97] reported mean aerobic TCC ranging between 4.11 log CFU/g and 5.46 log CFU/g in freshwater fish species (tilapia and African mud catfish) and 4.70 log CFU/g and 5.46 log CFU/g in herring from three different markets. In smoked catfish from Malawi, high aerobic TCC (6.75 log CFU/g) were found by Likongwe, Kasapila [24], who compared two different hot smoking techniques. In view of the few published market studies from other African countries, the here analyzed samples of Ghanaian processed fish showed lower aerobic TCC mainly within the acceptable limit. However, aerobic TCC can only be used as an indicator for general hygiene conditions along the value chain and should be combined with more specific microbial identification.

In the sampled fish products, anaerobic TCC were showing low levels being one order of magnitude below the levels of aerobic TCC (Table 2). Consequently, the microbiota on these processed fish products were dominated by aerobic and facultative anaerobic organisms. For anaerobic TCC, no Ghanaian reference values are available. The presence of anaerobic growing bacteria was low in the analyzed samples, but to exclude the presence of hygienically relevant bacteria, further identification is needed.

The analysis of *E. coli* in sampled processed fish from Ghana revealed that only one composite sample (WA pygmy herring from Techiman) was positive for *E. coli* with a concentration of 2.51 log CFU/g). Higher levels were found for coliforms in 70.8% of the fish samples. There was a strong variance in contamination of single fish species with coliforms between market samples, where the highest coliform counts were found in tilapia from Bolgatanga (6.23 log CFU/g, Fig 1) while tilapia samples from other markets were negative for coliform bacteria, which is calling for identification of sources within the affected value chains. The mean concentrations of coliform bacteria in European anchovy, bigeye grunt, and African moonfish were 2.73±0.85 log CFU/g, 2.86±1.05 log CFU/g, and 2.69±0.07 log CFU/g, respectively. European anchovy, bigeye grunt, WA pygmy herring tested positive for coliforms in all samples whereas round sardinella from two markets (Tamale and Kumasi) and African moonfish from Accra tested negative for coliforms. The guidelines by the Ghana Standards Authority set the limit for coliform bacteria in hot smoked fish to 1.6 log CFU/g (GS 95:2013). Based on the LOD in the present study, all samples that tested positive for coliform bacteria thus exceeded the legal limit in Ghana. The high concentrations of coliform bacteria in most of the samples might indicate fecal origin, therefore contamination from a dusty environment, hygienic conditions, contact surfaces, or handling practices are likely sources. The large variations in coliform counts, both between different products and between markets, indicate the potential to mitigate the bacterial load by improving hygienic conditions along the value chains. Critical points along the value chains might differ locally, which necessitates deeper investigation in future studies.

Salmonella was analyzed in this study as a food-borne pathogen, but was not detected in any of the sampled processed fish. Similar to our results, Plahar, Nerquaye-Tetteh [29] did not detect the presence of *Salmonella* in two different processed marine species in Ghana and Adegunwa, Adebowale [96] did not detect *Salmonella* in smoked herring samples from three different markets in Nigeria. In contrast, Likongwe, Kasapila [24] analyzed smoked catfish from Malawi and detected *Salmonella* with counts up to 4 log CFU/g in fresh and smoked fish. Further, *Salmonella/Shigella* was found in catfish, tilapia, and herrings collected from different markets in Nigeria [97]. Based on our results in combination with published data, *Salmonella* was generally not present in processed fish samples, but might be a possible food-borne hazard in certain fish value chains.

The sampling approach in this study was endpoint oriented and showed that aerobic TCCs were in the acceptable range, while only one sample tested positive for *E. coli* and no *Salmonella* were detected. But the counts of coliform bacteria were exceeding the limit given by the Ghana Standards Authority (Fig 1) in the majority of the samples and might indicate fecal contamination. As coliform bacteria and *E. coli* are foremost fecal bacteria and are classified as indicator bacteria for fecal contamination of food that harbors an increased risk to contain pathogenic bacteria [97, 98], several points along the value chains are possible critical points where contamination could have taken place. Either, the fish was primarily populated from its habitat and the smoking and drying of the fish was not sufficient to reduce coliform counts, or the smoked products were re-contaminated along the value chain due to improper handling or storage. Distinct conclusions on the effect of different preservation techniques (e.g. smoking, salting, and drying) or the different habitats cannot be drawn from this study design and need further research.

To develop suitable, locally applicable strategies to minimize the contamination by coliforms, critical points along the specific value chains need to be identified in further studies. Such strategies might include training in best practice procedures and process control to improve hygienic conditions, processing techniques, storage conditions and transportation. At consumer level, it is recommended to thoroughly cook fish before consumption to minimize the risk of food-borne illnesses.

Polycyclic aromatic hydrocarbons (PAHs). Different PAHs are known to have carcinogenic and mutagenic properties [23, 25, 26]. To determine the amount of the most important PAHs in food, the European Commission recommends an analysis for benzo[a]anthracene, chrysene, benzo[b]fluoranthene, and benzo[a]pyrene, summarized as PAH4 [25, 99, 100]. All processed marine fish products analyzed in this study were smoked. Processed European anchovy, bigeye grunt, round sardinella, and African moonfish showed high mean PAH4 concentrations of 478±164 μg/kg, 553±155 μg/kg, 418±103 μg/kg, and 443±91 μg/kg, respectively (Table 2). The freshwater fish in this study were either smoked (WA pygmy herring from Accra and Bolgatanga), dried (WA pygmy herring from Techiman), or salted (all tilapia samples) (Table 1). The PAH4 concentration of WA pygmy herring differed between the pooled market samples, probably because the pooled samples were differently composed of smoked and not-smoked batches. In salted tilapia, the mean PAH4 level (7.4±9.6 μg/kg) was lower than all composition samples of smoked fish species (Table 1). There are no legal limits available from the Ghana Standards Authority for PAHs in fish products and the European limit value of 12.0 μg PAH4/kg of the product was therefore used as reference [101]. PAH4 levels exceeded the EU limit considerably in all market samples of processed European anchovy, bigeye grunt, round sardinella, and African moonfish as well as in WA pygmy herring from the markets in Techiman and Bolgatanga (Table 2). Only one composite sample of smoked fish did not exceed the EU limit for PAH4 (smoked WA pygmy herring from Accra, S2E Table). Generally, salted tilapia samples were below the EU limit value (except the samples from Bolgatanga containing 24 μg PAH4/kg, S2F Table). PAHs are known to be produced during the smoking process, and therefore levels in non-smoked fish species are expected to be low. Based on the literature, levels of PAH4 are highly influenced by the type of smoking kiln, type of burning material, duration of smoking, re-smoking of fish products and fat content of the fish [23, 26, 102, 103]. Consequently, the PAH4 levels in fish products can be minimized by applying best practices within the smoking process. Given the high concentration of PAH4 in the majority of smoked fish samples in this study, applied smoking techniques and smoking practices in Ghana should generally be improved. The United Nations (UN) in cooperation with the Food and Agriculture Organization (FAO) developed an improved kiln called the FAO-Thiaroye Processing Technique (FTT) [23, 26]. In this improved kiln, fat and other fluids from the fish are not able to drip into the fire. Further, the fish are not in direct contact with flames and the smoking process can be performed in a more controlled way, achieving up to a 100-fold reduction in PAH4 concentration in smoked fish when using FTT compared to traditional smoking methods [23, 26]. However, the FTT is more expensive, and the Ahotor oven [104–106] was therefore developed in cooperation with the United States Agency for International Development (USAID) as a more affordable option. This oven is equipped with a fat collector, requires less burning material compared to the chorkor and is assumed to produce less PAHs. The awareness of the negative effect of PAHs on human health should be widely communicated to the public and the use of safer ovens such as the FTT and the Ahotor oven and best smoking practices should be advocated to limit the health hazard for the consumer to a minimum.

Heavy metals. Heavy metals can have neurodegenerative as well as nephro-, and immunotoxic effects on humans and are important contaminants regarding food safety. Fish are known to be a source of heavy metals, given their ability to bioaccumulate heavy metals from the surrounding water and feed.

Maximum levels for fish as food are available from Ghanaian regulations, the Codex Alimentarius Commission and the European Commission. As all these regulatory limits are given only for fresh fish or fresh fillet with a high moisture content, an application of these limits to processed/preserved fish products with lower moisture content requires a normalization of the analytical levels to a moisture content comparable with fresh fish [107]. EU legislation has established maximum permissible levels for cadmium (0.05 mg/kg wet weight (w.w.)), mercury (0.5 mg/kg w.w.), and lead (0.3 mg/kg w.w.), however, no value is available for arsenic [107]. The maximum level for lead in raw fish is in line with the guidelines provided by the Codex Alimentarius [108] but for arsenic compounds in fish, no maximum concentration has yet been established.

The concentration of cadmium varied from unquantifiable levels (<LOQ) in tilapia to 0.31 mg/kg in processed European anchovy (Table 2), which was significantly higher than for all other species analyzed. Overall, the cadmium concentrations were significantly lower in freshwater species compared with marine species, with the exception of African moonfish. The Ghanaian regulation does not give limit values for processed fish and aligns with the limit values set by the European Commission for cadmium concentration in raw fish at 0.05 mg/kg wet weight and 0.25 mg/kg wet weight for *Engraulis species* [107, 109]; thus, a reduction in water content due to processing needs to be taken into account [109]. When normalizing the analytical levels to a 75% moisture content as in fresh fish [110–113], the concentration of heavy metals in the sampled fish species were below their respective limit values given by the European Commission. After normalizing to a 75% moisture level, the cadmium levels in marine species ranged from approximately 0.009 mg/kg in African moonfish to 0.034 mg/kg in European anchovy. Kwaansa-Ansah, Nti [114] reported cadmium concentrations in marine fish fillets from Ghana ranging from 0.007 to 0.019 mg/kg, which are in accordance with the findings in this study, with only anchovy having higher concentrations. Kortei, Heymann [115] reported cadmium concentrations in fillet of two freshwater species from below detection limit up to 0.08 mg/kg, which is higher compared to the samples analyzed in this study. Analytical data on heavy metal content including cadmium concentration in whole small fish for human consumption is scarce. Previous analyses have shown that cadmium primarily accumulates in the gills, liver, and kidneys and therefore fish species consumed whole might contain higher concentrations compared with consuming fillets [116, 117]. For cadmium, the EFSA recommends a Tolerable Weekly Intake of 2.5 μg/kg body weight [118], which would be reached by weekly consumption of 570 g of processed anchovy for a 70 kg person. However, risk assessment cannot be completed given the limited availability of food consumption data, data on smoked fish analyzed whole and overall exposure to cadmium. Further, regional differences reported in the present study should be further investigated in future studies.

Lead content in the analyzed processed fish ranged from levels below quantification in tilapia (<LOQ) to 0.64 mg/kg in WA pygmy herring. No statistically significant difference was detected between the different fish species, and consequently, no difference was detected between marine or freshwater habitats. However, WA pygmy herring from Accra were contaminated with lead at 1.3 mg/kg (S2E Table) and also had a high iron-content (see chapter on minerals). The high concentration in one species from one specific market might be caused by metal pollution from the Agbogbloshie e-waste recycling site close by, where lead is reported as the main toxicant [37, 119]. Nonetheless, it is unknown why only WA pygmy herring was affected, given that other species from this market did not contain notably higher levels and that WA pygmy herring from other markets did not show high levels of contamination. Consequently, future studies need to investigate sources of contamination including regional

difference. No scientific papers analyzing the lead content of whole fish in Ghana were found for direct comparison, but studies of lead in muscle tissue of several freshwater species ranged from 0.04 to 0.42 mg/kg [115], 0.060–0.085 mg/kg in fillet of marine species [114] and levels lower than 0.2 mg/kg in fillet of catfish and bigeye grunt [120]. The lead concentration in dried fish products from our study was higher than levels reported for fresh fish, which is mainly caused by the reduced water content in the fish due to processing [121]. It is not yet known whether further contamination by lead takes place during processing. Nonetheless, with a concentration of 0.64 mg/kg processed fish, WA pygmy herring showed highest lead content within the sampled species, but after normalizing for water content it did not exceed the limit of 0.3 mg/kg flesh given by the European Commission [107]. However, given the overall high consumption of fish in Ghana and the neurodegenerative properties of lead, consumption pattern and exposure should be further investigated, especially in vulnerable population groups. The mechanism of lead absorption is similar to that of iron, and a diet deficient in iron can thus result in excess absorption of lead, particularly in young children [122, 123]. Given the high prevalence of iron deficiency among Ghanaian children and women of childbearing age [80], further analyses and monitoring of lead-levels in processed fish is essential including regional differences.

The mean concentration of mercury in most processed small fish was low, ranging from below quantification levels (<LOQ) in processed tilapia to 0.065 mg/kg in processed bigeye grunt. WA pygmy herring was an exception with a comparably high concentration of 0.223 mg/kg in all market samples (see S2E Table). It has been reported that freshwater species might contain higher concentrations of mercury compared to marine species [124]; however, this was not observed in this study, as the second freshwater species (tilapia) had the lowest concentration of all species. Total mercury concentration in fish depends on age, size and feeding habits combined with the general mercury concentration in the habitat, with the highest concentrations of mercury usually found in large predatory fish at peak trophic levels [125, 126]. The comparably high levels of mercury in WA pygmy herring thus contradict this rationale given its small size, short lifespan and herbivorous feeding habits. Data on mercury concentration in whole small fish as food for humans is scarce in the scientific literature, which limits the basis for comparison. However, scientific reports on mercury concentrations in fish fillet from the Gulf of Guinea and Ghana have reported mercury concentrations of 0.19 mg/kg up to 0.61 mg/kg in fillet of local marine and freshwater fish [34, 115, 120, 127]. The aforementioned studies found higher concentrations of mercury than in the present study, even though the water content was reduced by processing. Due to illegal gold mining, freshwater fish from surrounding watersheds are likely to be contaminated by mercury at higher levels [115, 127]. All samples analyzed in this study were below the limit given by the Ghanaian Standards Authority and European Commission of 0.5 mg/kg [107, 109]. For methylmercury, the JECFA set the PTWI to 1.6 μg/kg body weight [128]. Assuming all mercury in the samples is methylmercury, a 70 kg person would reach the PTWI by consuming 500 g of WA pygmy herring, which was the fish species with the highest concentration of mercury in this study. In marine fish, the highest concentration was found in bigeye grunt (0.065 mg/kg) and consumption of 1.7 kg of processed bigeye grunt would reach the PTWI, without taking any other sources of exposure into account. With regard to human health, fetuses, infants and children in early life stages are particularly susceptible to the neuro-, nephro-, and immunotoxic health hazards associated with mercury exposure [129], which emphasizes the importance of further studies on mercury in processed fish from Ghana including regional differences.

The concentration of total arsenic in all processed fish species had the highest variation in metal concentration with means ranging from 0.1 mg/kg in tilapia to 9.8 mg/kg in round sardinella. The sampled freshwater species, WA pygmy herring and tilapia, had significantly lower concentrations of arsenic compared with all marine species except African moonfish. To our knowledge, arsenic concentrations in whole fish have not been reported for Ghana thus far. Arsenic levels of 0.37 mg/kg have been reported in fillet of bigeye grunt, however, the samples were collected from only one landing site [120]. The current samples of whole processed bigeye grunt contained higher concentrations of arsenic in fish from all markets (S2B Table) and the other processed marine species showed similarly high concentrations of arsenic. In freshwater, Gbogbo, Arthur-Yartel [120] sampled Bagrid catfish (*Chrysichthys nigrodigitatus*) at one landing site and found arsenic concentrations of 0.21 mg/kg in the fillet. The WA pygmy herring analyzed in the current study displayed similar arsenic concentrations (after normalizing to 75% moisture content), while the sampled whole tilapia contained markedly lower arsenic levels. The low levels of arsenic in processed whole tilapia could not be explained with the current study-design, as it is unknown whether the tilapia were harvested from a freshwater system or if they originated from aquaculture. In this study, only total arsenic was analyzed, but it can be assumed that the less toxic organic arsenic is the dominant compound [130]. In Ghana and the EU no maximum limit for arsenic is provided for fish as food, and WHO/JECFA withdrew the PTWI for arsenic in 2011 [131]. The exposure to total arsenic for the Ghanaian population through consumption of processed whole fish is unknown, and the resulting health effects cannot be estimated without including local consumption data, which is currently missing.

Nti [10] have reported that 95% of households in rural Ghana consume fish on a daily basis. In combination with the challenges resulting from expanding anthropogenic activities, it is important to gain more knowledge on consumption habits to estimate the public risk resulting from heavy metal contamination in the different fish species including regional differences and other food commodities. As the limit values by the European Commission are only available for fresh fish and fillets, the change in concentration due to drying is an important factor to be considered for further risk assessment. For fish which are consumed whole, there is a need for additional food composition data on nutritional quality and contamination levels, given that there is a plausible difference between the consumption of whole fish and their fillets.

Strengths and limitations of the study

The results provide novel data from Ghana on food quality and food safety including nutrient composition, heavy metal concentration, polycyclic aromatic hydrocarbon content and microbiological hygienic indicators in processed, i.e. smoked or dried whole small fish. As small fish are often consumed whole, the here presented data are highly valuable for future risk benefit analyses, given that existing data on fish is mostly limited to the content in the fillets of fresh fish. Still, some limitations are recognized. In the present study only pooled samples were analyzed, which would to some degree mask the presence of variance between the separate batches from the same sampling location. Further, the market sampling did not enable to trace the source and location of contamination as it was not known where and when the single fish batches were harvested, processed and stored. However, the advantage of this approach is that it gives a cross sectional outline on the nutrient composition, microbial concentration, and contamination with heavy metals and PAHs in Ghana. Origin and source distribution of the different contaminants, especially heavy metals, in the processed fish are relevant topics for future research. Especially heavy metal concentration is influenced by geographical origin of the unprocessed fish, but it might also be influenced by the location of processing, storage and marketing. Another aspect is the nutrient composition of processed fish which can vary and is affected by a multitude of pre-harvest and post-harvest factors. Pre-harvest factors include species, habitat, feed resources, life stage, seasonality, and changes in climatic patterns, while post-harvest factors include processing methods, storage conditions and shelf-life. Since this study gives an insight into the differences of the sampled processed fish, it is necessary to further investigate the aforementioned factors to display differences in nutrient composition and food safety issues throughout the year including different regions. Future research would need to take bioaccessibility of contaminants into consideration. Based on the here presented data, conclusions on the critical points along the value chains and geographical origin of the different contaminants cannot be drawn directly and need further investigation.

Conclusion

Processed fish eaten whole are rich in vitamins, minerals and essential fatty acids and can therefore contribute with high-quality proteins and essential micronutrients in order achieve a balanced Ghanaian diet, which consists of mostly starchy staples. Of the analyzed fish species, tilapia stood out as markedly less nutrient dense. As tilapia is one of the species that could increase availability of fish through farming, the present results demonstrate that tilapia cannot substitute wild species in terms of micronutrient content. On the other side, the freshwater fish WA pygmy herring showed several nutritional characteristics of marine fishes. High concentrations of mercury and lead were detected in some fish samples, while elevated levels of PAHs were detected in all smoked samples. Heavy metal concentrations determined in this study call for further analyses and identification of geographical origin and contamination sources along the value chains to mitigate the health hazards associated with heavy metals. The high levels of PAHs in smoked fish in all regions necessitate the improvement of the smoking process by implementing best practices and improved kilns (e.g. FTT-kiln and Ahonto oven). Based on microbiological analysis, the overall quality of processed fish samples was acceptable. Nonetheless, the majority of sampled processed fish showed elevated coliform counts, but no *Salmonella* was found. The cause of contamination with coliform bacteria cannot be determined by this study and should be further investigated. Data from this study contributes to building reliable food composition data for Ghana on processed small fish. Further research is needed to analyze composition of prepared meals including preparation methods of small fish on consumer level. Having local consumption data is necessary to perform any risk benefit analysis and risk assessments and should be advocated in future studies.

Supporting information

S1 Table.
(PDF)

S2 Table.
(PDF)

S3 Table.
(XLSX)

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