Micronutrient Impact of Oysters in the Diet of Women Shellfishers Project

Fish Innovation Lab

Final Technical Report April 1, 2021 – March 31, 2023

Cooperative Agreement 7200AA18CA0030

Submission Guidelines

The technical / scientific report is designed to communicate the research process(es) that took place under the activity, and to inform USAID about the outputs and outcomes of the research effort.

The project team should prepare one technical/scientific report using this template and uploading in the Piestar module. For formatting, use no smaller than Times New Roman 12 pt, double-spaced with one-inch margins, use the American Psychological Association (APA) style and the page count should not be longer than 25 pages, excluding the following sections: the title page, partners/institutions, abbreviations and acronyms, glossary, table of contents, references or appendices. Tables longer than one page should be added to the appendices. The required reporting sections are included in this template and each section must be completed.

Micronutrient Impact of Oysters in the Diet of Women Shellfishers Project

Final Technical Report

April 1, 2021 – March 31, 2023

[

Cooperative Agreement 7200AA18CA0030

June 21, 2023

Prepared by:

Seth Adu-Afarwuah and Brietta M. Oaks

This report is made possible by the generous support of the American people provided by the Feed the Future Innovation Lab for Fish through the United States Agency for International Development (USAID). The contents are the responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government. The Feed the Future Innovation Lab for Fish is managed by Mississippi State University through an award from USAID (Award No. 7200AA18CA00030; M. Lawrence, PI) and provides support to Grant No. 193900.312455.31; S. Adu-Afarwuah, PI.

Partners/Institutions

URI Depart. of Nutrition and Food Sciences
Fogarty Hall
Kingston RI 02881 USA
Brietta Oaks

University of Ghana
Depart. of Nutrition and Food Science
P.O. Box LG 134
Legon, Ghana
Seth Adu-Afarwuah

Abbreviations and Acronyms

ADC	Average Daily oyster Consumption
ABW	Average Body Weight
DDS	Dietary Diversity Score
EDI	Estimated Daily Intake
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FCT	Food Composition Table
HFIAS	Household Food Insecurity Access Scale
HI	Hazard Index
LPG	Liquified Petroleum Gas
MDD-W	Minimum Dietary Diversity for Women
RDA	Recommended Dietary Allowance
RfD	Oral reference Dose
SD	Standard Deviation
THQ	Target Hazard Quotient
UCC	University of Cape Coast
URI	University of Rhode Island
USAID	United States Agency for International Development
WPS	Wealth-Poverty Score

Glossary

Anemia: Blood hemoglobin (Hb) concentration < 12 g/dl. Mild anemia is defined as Hb 10.0-11.9 g/dl. Moderate anemia is defined as Hb 7.0-9.9 g/dl. Severe anemia is defined as Hb < 7 g/dl.

Dietary diversity score (DDS): the number of food groups consumed over a 24-h period. A DDS <5 indicates a diet that is not diversified; A DDS ≥ 5 indicates a diversified diet.

Estimated Daily Intake (EDI) of a heavy metal: Amount of the heavy metal in food expressed as mg/kg body weight/day, which can be ingested daily over a lifetime without an appreciable health risk.

Oral reference dose (**RfD**) of a heavy metal: Amount of a heavy metal in food expressed as mg/kg/day which will not result in health issues in the course of a lifetime.

Target Hazard Quotient (**THQ**) of a metal: The ratio of Estimated Daily Intake (exposure) to the Oral Reference Dose. A THQ value <1 is used to indicate that the level of exposure to metal contamination is smaller than the oral reference dose, and therefore, it is negligible or not likely to cause any deleterious effects during lifetime. A THQ value >1 indicates that the level of exposure to metal contamination is greater than the oral reference dose, and therefore, it is likely to cause deleterious effects during lifetime.

Hazard Index (**HI**): The sum of the Target Hazard Quotients of all heavy metals in a food sample. HI <1 indicates no potential for adverse non-carcinogenic health effects from consumption of the food. HI >1 indicates potential for adverse non-carcinogenic health effects from consumption of the food. Even if the THQ values were <1 individually, the simultaneous exposure to several heavy metals from a food sample may result in adverse health effects.

Women shellfishers: Women who engage in estuarine and mangrove ecosystem-based shellfisheries activities including harvesting, processing, transporting, and/or retailing of shellfish.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

Abstract

Introduction: Oyster shellfishing offers a rich source of iron and other nutrients for women shellfishers in Ghana, where anemia prevalence and household food insecurity among women remains high. Many women engage in oyster harvesting, processing, and/or marketing across estuarine sites in Ghana, but little is known about the level of oyster consumption among these women shellfishers, and the extent to which oysters contribute to the iron and zinc intakes of the women. In addition, data on the heavy metal contamination of estuarine oysters in Ghana are limited, and it is unclear whether heavy metal contamination of oysters is a concern for women shellfishers at estuarine sites. This **M**icronutrient **I**mpact of **O**ysters in the **D**iet **o**f **W**omen **S**hellfishers (MIODOWS) Project aimed to examine nutrition outcomes including food intake, food insecurity, dietary diversity, and anemia prevalence among women shellfishers of reproductive age (15-49 years of age) in three estuarine areas in Ghana. In addition, we aimed to determine the mineral and heavy metal concentrations of oysters from each of the three sites, and to analyze the safety risks posed to the women shellfishers of reproductive age by four heavy metals (arsenic, cadmium, lead, and mercury) related to the consumption of oysters.

Methods: The MIODOWS Project was an add-on to the USAID-funded parent project entitled "Women Shellfishers and Food Security Project", which was led by the University of Rhode Island (URI), with partners including the University of Ghana (UG), University of Cape Coast (UCC), TRY Oyster Women's Association in The Gambia, and World Agroforestry (ICRAF) in Kenya. We conducted a cross-sectional study among women shellfishers 15-49 years of age living at three oyster estuarine sites selected according to three levels of mangrove ecosystem degradation (*less degraded*, *moderately degraded*, and *highly degraded*) and three categories of fisheries "health status" (*underexploited*, *fully exploited*, and *overexploited*). We collected information on the women's demographic and socioeconomic characteristics, household food insecurity, and consumption of food from different food groups by using a questionnaire. We estimated oyster consumption and nutrient

intakes per day as the mean of the intakes from repeat 24-hour dietary recalls and determined blood hemoglobin (Hb) concentration using a Hemocue photometer. We defined "any oyster consumption" as oyster consumption > 0 g in the repeat 24-hour dietary recalls, and any anemia as Hb < 12 g/dl. Across estuarine sites, we compared mean oyster consumption and nutrient intakes (including iron intake from oysters) per day, household food insecurity, percent of women achieving the MDD-W, and anemia prevalence using ANOVA (SAS PROC GLM) for continuous variables and logistic regression (SAS PROC LOGISTIC) for binary variables. Finally, we compared the mean mineral and heavy metal concentrations of oysters collected from each of the three estuarine sites (n = 315/per site; total n = 915) by ANOVA and evaluated the potential health risks of exposure to heavy metals (arsenic, cadmium, lead, and mercury) through oyster consumption among the women shellfishers. For all statistical analyses, the level of significance (α) was set at 0.05.

Results: A total of 504 women were recruited from the Densu (*highly degraded* mangrove ecosystem and *underexploited* fisheries health status, n = 200), Narkwa (*moderately degraded* mangrove ecosystem and *overexploited* fisheries health status, n = 166), and Whin (*less degraded* mangrove ecosystem and *fully exploited* fisheries health status, n=138) estuarine sites from June 8, 2021 to July 16, 2021. Mean ± SD age (32 ± 9 y) did not differ by site (P = 0.30). Mean ± SD wealth-poverty score (WPS) was higher among the Densu (5 ± 3) and Whin (5 ± 2) women than the Narkwa (3 ± 2) women (P < 0.001) and the percentage of households with high (≥ 4) WPS (indicative of higher socioeconomic status) was greater for the Densu (63%) and Whin (65%) sites than the Narkwa (37%) site (P <0.001). Only 12.5% of the women shellfishers reportedly consumed any oyster (> 0 g) day during the week after recruitment into the study. Mean ± SD oyster consumption (g/d) was significantly higher among the Densu (9.6 ± 26.0) and Narkwa (6.7 ± 25.7) women than the Whin (0.3 ± 2.2) women, (P = 0.001); iron intake from oyster (mg/d) was significantly higher (P < 0.001) among the Densu (2.4 ± 6.5) and Narkwa (1.6 ± 5.4) women contributing 11-14% of the total iron intake, compared with that for the Whin women (0.07 ± 0.55) which contributed only 0.3% of the total iron intake. Percentage of women with any household food insecurity did not differ by site (92% overall, P = 0.09), but the percentage with severe food insecurity was greater for the Narkwa site (85%) than the Densu site (72%), with the Whin site (79%) in between, (P = 0.012). Anemia prevalence (20% overall; P = 0.08) and the percentage of women who achieved the MDD-W (21% overall; P = 0.65) did not differ by site. The oysters from the three estuarine sites differed significantly in the mean concentrations of all the 17 minerals and heavy metals measured. Mean ± SD (mg/kg wet weight) iron concentration was highest in the oysters from Narkwa (147 ± 142) followed by those from Densu (126 ± 90) and Whin (103 ± 87), P < 0.001. None of the oysters from the three sites exceeded the maximum concentration limit for As, Cd, Pb, and Hg, except for one oyster sample from the Narkwa site, which exceeded the maximum concentration limit for Hg. The mean cumulative HI for oyster consumption among the women shellfishers ranged from 0.04 at the Whin site to 0.13 at the Narkwa site; none of the estuarine site had a mean HI exceeding 1. At all three sites, the primary driver of the HI values among the women shellfishers was Hg accounting for 50-57%, followed by lead accounting for 22 -29%, with Cd contributing the least (6-8%).

Conclusion and recommendations:

The level of oyster consumption among the women shellfishers 15-49 years at the three estuarine sites in Ghana may be too low to make any substantial impact on the women's iron and zinc intakes from oyster, food security, dietary diversity, and anemia prevalence. More research is needed to explore how well women living in estuarine areas in Ghana might use shellfishery resources to prevent anemia. Heavy metal contamination does not appear to pose a major health risk for the women shellfishers related to oyster consumption. Promoting oyster consumption may be a promising strategy to increase nutrient intakes and prevent anemia in estuarine communities. There should be regular monitoring of mercury and lead contamination of oysters and other aquatic animal foods, especially at the Narkwa area.

Introduction

In West Africa, low dietary iron intake is recognized as a major cause of anemia (Mwangi et al., 2017). Among the West African coastal countries, anemia prevalence in women of reproductive age ranges from 35% to 52% (World Health Organization (WHO), 2020). In Ghana, the national prevalence of anemia among women of child-bearing age was estimated at 42% in 2015; in the four coastal regions (Volta, Greater Accra, Central, and Western regions) the anemia prevalence ranged from 42% to 49% (GSS, 2015). A recent meta-analysis (Young et al., 2019) showed anemia during pregnancy is associated with an 84% increased risk of postpartum hemorrhage, a leading cause of maternal death in Africa. Maternal anemia is also a risk factor for poor birth outcomes including low birth weight, preterm birth, and neonatal mortality (Young et al., 2019). Reducing the prevalence of anemia in women of reproductive age is one of the WHO 2025 Global Nutrition Targets (WHO, 2014).

Coastal fisheries, including shellfish-harvesting, provide a rich source of nutrients for people living near the estuaries (Taylor, Roberts, Milligan, & Ncwadi, 2019) and shellfish are an excellent source of vitamins and minerals (USDA, 2020). Comparative data on the nutrient content of oysters from estuarine sites in Ghana are lacking, one serving (100 g) of raw U.S. eastern oysters provides 39.3 mg zinc and 4.6 mg iron (USDA, 2020), which meets 100% of the Recommended Dietary Allowance (RDA) for zinc (8 mg/d) and 26% of the RDA for iron (18 mg/d) for women of reproductive age (IOM, 2011). By contrast, one serving (100 g) of beef steak, widely recognized as a recommended source for iron and zinc, provides 10.1 mg zinc and 3.6 mg iron (USDA, 2020), which meets 100% of RDA for zinc and 20% of RDA for iron for women of reproductive age (IOM, 2011). As an affordable and accessible source of iron, shellfish could help reduce anemia among women of reproductive age in estuarine areas in Ghana and similar settings in West Africa.

Many women of reproductive age across estuarine areas in Ghana engage in oyster harvesting, processing, and/or marketing (Agbekpornu, Ennin, Issah, Pappoe, & Yeboah, 2021), but little is known about these women (Bennett, 2005; Ogden, 2017), including their level of oyster consumption and its percent contribution to iron and zinc intakes, food insecurity, dietary diversity, and anemia prevalence. For this reason, it is unclear whether there is any need to promote oyster consumption as one of potential strategies to address anemia among the women. In addition, data on the heavy metal contamination of estuarine oysters in Ghana are limited, and it is unclear whether heavy metal contamination of oysters is a concern for women shellfishers of reproductive age in estuarine areas.

This study of women shellfishers of reproductive age (15-49 years of age) in three estuarine areas in Ghana aimed to: (a) determine the level of oyster consumption and its contribution to iron and zinc intakes of the women shellfishers across the three estuarine sites; (b) determine household food insecurity, dietary diversity, and anemia prevalence among the women shellfishers across the three estuarine areas; (c) determine the mean ± SD mineral and heavy metal concentration of oysters from the three estuarine areas and whether there were any variations in the mineral and heavy metal contents of oysters across the three estuarine areas; and (d) to analyze the safety risks posed to the women shellfishers by four heavy metals (arsenic, cadmium, lead, and mercury) related to the consumption of oysters.

Research Methods

2.1. Selection of study areas

The **M**icronutrient **I**mpact of **O**ysters in the **D**iet **o**f **W**omen **S**hellfishers (MIODOWS) Project was an add-on to the USAID-funded parent project entitled "Women Shellfishers and Food Security Project", which was led by the University of Rhode Island (URI), with partners including the University of Ghana (UG), University of Cape Coast (UCC), TRY Oyster Women's Association in The Gambia, and World Agroforestry (ICRAF) in Kenya. The procedure for selecting the three estuarine sites for the study has been reported previously (E. O. Chuku et al., 2020). Briefly, the sites were selected purposively based mainly on the sites' (a) level of mangrove ecosystem degradation (i.e., *less degraded*, *moderately degraded*, and *highly degraded*) and (b) fisheries "health status" category (i.e., *underexploited*, *overexploited*, and *fully overexploited*). Our intent was to include women shellfishers or oyster samples across contrasting coastal environmental areas in the study. The other site selection considerations were having: (i) relatively large scale of existing shellfishery activities, (ii) relatively high level of involvement of women in shellfishery activities, (iii) relatively large scale of existing mangrove systems-based livelihoods, and (iv) experienced substantial changes in mangrove health condition over time.

First, a purposive sampling approach was used to identify candidate sites with significant variation in key outcome variables such as fisheries and mangrove health, and treatment variables such as governance, gender dimensions, and women's empowerment. The information needed to identify the candidate sites was derived from secondary sources, expert opinions, and local knowledge. Second, additional information on the candidate sites was collected through rapid field assessments. Lastly, the research team held discussions and decided, by consensus, the final three sites for the study. There were: the Densu Estuary (Greater Accra Region), the Ekumfi Narkwa Lagoon (Central Region), and the Whin Estuary (Western Region) (**Figure 1**).

Densu Estuary

The study was conducted at the Bortianor area, including the Tsokomey, and Tetegu communities in the Ga South Municipal District in the Greater Accra Region. The area covers about 5,892 ha of land, with an estimated population of 350,121 according to the 2021 population and housing census. The coastal mangrove area was estimated at approximately 206 ha in 2020 (E. O. Chuku et al., 2020). The main occupation there is fishing and fish processing, predominantly at a small scale level. The main shellfishing activity is oyster harvesting, with an estimated 150-200 women shellfishers. The livelihood activities connected to mangroves include culture-based fishing, firewood collection, and salt mining. The mangrove condition in the area is considered to be *highly degraded* because of factors such as mangrove-harvesting for fuel and settlement expansion. The fisheries “health status” of the area was categorized as *underexploited* based on the relatively low fishing mortality rate (0.07) and exploitation ratio (0.04) (E. O. Chuku et al., 2020).

This Densu estuarine area is a protected wetland declared as a Ramsar Site in 1992, but the enforcement of Ramsar Site regulations is weak. The Densu Delta Community-Based Fisheries Management Plan which delegates exclusive use rights to the oyster fisheries resources to the Densu Oyster Pickers Association was approved by the Ministry of Fisheries and Aquaculture Development (MOFAD), and the Fisheries Commission in December 2020 (MOFAD, 2020). Currently, the area operates a 5-month (November - April) oyster-harvesting closed season each year to allow the oyster population to replenish itself. The area is noted for fishing-dependent households with low dietary diversity (low consumption of “other vitamin A rich fruits and vegetables”, “other fruits and vegetables”, organ meat, meat and fish, legumes and nuts, and milk and milk products) and moderate-to-severe hunger, particularly in the period of the year when artisanal and inshore fishing is closed (E. O. Chuku et al., 2020).

Ekumfi Narkwa Lagoon

The main shellfishery community at the estuarine site where the study was conducted was Ekumfi Narkwa in the Ekumfi District, with an estimated population of 56,741 according to the 2021 population and housing census. The coastal mangrove area was estimated at about 110 ha in 2020 (E. O. Chuku et al., 2020). The main shellfishing activity includes oyster, cockle, and shrimp harvesting. The livelihood activities connected to mangroves include staple-food (maize, cassava, plantain) farming and salt mining. The mangrove condition in the area is considered to be moderately degraded consisting of low-density naturally occurring mangroves. The key factors affecting mangroves are mangrove-harvesting for fuel, settlement expansion (land reclamation), and mangrove dieback due to factors including environmental pollution. The fisheries “health status” of the area was categorized as overexploited based on the relatively high fishing mortality rate (1.65) and exploitation ratio (0.5) (E. O. Chuku et al., 2020). The fishing system at the Ekumfi Narkwa site is open-access, and compliance with the customary law on no-fishing on Tuesdays is low. This area does not operate the oyster-harvesting open and closed seasons as does the Bortianor area, but access to oysters becomes more limited during the rainy season (May - October) because of increased difficulty with harvesting during the period. The Central Region, where Narkwa is located, is a known food insecure region of the country, with low average dietary diversity among children 6-59 months of age (E. O. Chuku et al., 2020).

Whin Estuary

The study was conducted at the New Amanful-Apremdo-Beahu area in the Ahanta West District (Western Region), with a population of about 153,140 according to the 2021 population and housing census. The coastal mangroves was estimated at approximately 178 ha in 2020 (E. O. Chuku et al., 2020). The main shellfishing activity includes oyster, shrimp, and periwinkle harvesting involving more than 80 women shellfishers. The livelihood activities connected to mangroves include firewood collection and a low level of bivalve shell trade. The mangrove condition in the area is considered to be less degraded/more stable. The fisheries health status of the area was categorized as fully exploited based on the fishing mortality rate of 0.8 and exploitation ratio of 0.29 (E. O. Chuku et al., 2020). The key factors affecting mangroves in the area are harvesting for fuel, settlement expansion (land reclamation), tourism, and mangrove dieback due to factors including pollution from sewage. The fishing system is open-access, and compliance with the customary law on no-fishing on Tuesdays is low. This area is also noted for fishing-dependent households with low dietary diversity and moderate-to-severe hunger in the period of the year when artisanal and inshore fishing is closed (E. O. Chuku et al., 2020).

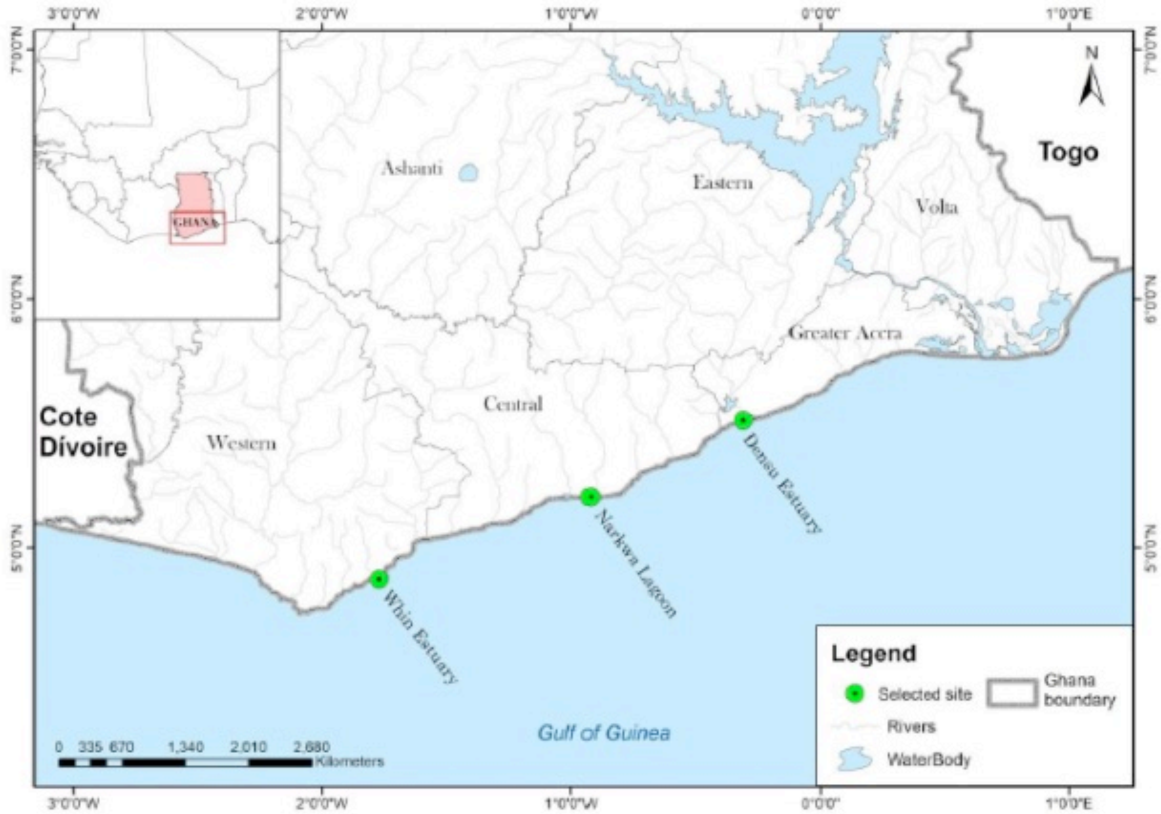

Figure 1: Estuary sites/Communities selected for the study (E. O. Chuku et al., 2020): Densu, Narkwa, and Whin.

2.2. Study design and sampling

This was a cross-sectional study among non-pregnant women shellfishers of reproductive age (15-49 years) in three estuarine sites in Ghana. We used a combination of random and snowballing sampling techniques to select women for the study. Where the estuarine site had a women shellfishers' association, the leaders of the association were contacted for the list of their members, so that the target sample size was selected randomly from the list if there were more women shellfishers on the list than the target sample size.

The Development Action Association provided the list of shellfishers' association members for the Densu estuary site. This list, however, had a smaller number of women shellfishers of reproductive age than the target sample size. Thus, the study team made efforts to contact all the women on each list and, in addition, used the snowballing technique to identify other unlisted women shellfishers, with the aim of meeting the target sample size. For the other two estuarine sites (Narkwa Lagoon and Whin Estuary) where no women shellfishers' association was identified, field workers used the snowballing technique to find women shellfishers for the study, often with assistance from opinion leaders and community members. Only one woman shellfisher per household was recruited into the study.

2.3. Data collection

On the day of recruitment, field workers collected women's information including demographic and socioeconomic characteristics, household food insecurity (Coates, Swindale, & Bilinsky, 2007; FAO, 2021), weight (Seca 874), height (Seca 217), and blood hemoglobin by finger-prick (HemoCue AG, Switzerland). The HemoCue device was calibrated daily using control cuvettes (Sigma Chemical Co., St. Louis, MO). Field workers conducted two non-consecutive 24-hour dietary recalls, the first on the day of recruitment and the second, within seven days after recruitment, by which we collected detailed data on the type of foods and quantities of foods the women shellfishers consumed.

A dedicated team collected oyster samples from each of the three estuarine sites for the purpose of determining the mineral contents and heavy metal contamination. The sample size for the individual oyster samples (*n* = 305 per study site; total *n* = 915) was calculated using procedures described by the Food and Agriculture Organization (Greenfield & Southgate, 2003). At each study site, the team identified the main oyster harvesting locations and proportioned the 305 oysters per study site to the number of known harvesting locations at the site. At each oyster harvesting location, the team earmarked an estimated quadrat of 20 m^2 (Baggett, 2014) from where a local guide randomly collected the oysters. To ensure that the samples were representative from each study site, the team collected more samples from each oyster harvesting location than the number needed to meet the quota for the location. The samples from each location were mixed thoroughly before the quota sample was selected randomly. The oysters were shucked, and the meat was extracted and hand-cleaned with a soft brush. The meat samples were weighed and packed individually in air-tight dispensing polystyrene bags, labelled, and transported on ice to the laboratory, where they were stored at -86 ºC until analysis. The data collection was conducted from 8 June 2021 to 16 July 2021 (over 6 weeks).

2.4. Ethics approval

We obtained ethics approval for the study from the Ghana Health Service Ethics Review Committee and informed consent from all women who participated in the study.

2.5. Laboratory analysis of oyster samples

Digestion of Oyster Samples

The full muscle tissue of each oyster sample was digested in a mixture of 10 ml HNO_3 and 5 ml H_2SO_4 and heated on a hot plate at 95^0C for 1-3 hours. The sample was cooled to room temperature, after which 2 ml of 30% H_2O_2 was added and the sample was reheated for 20 minutes. The mineralized sample was subsequently topped up with distilled water to 100 ml before reading the mineral content.

Determination of Phosphorus Concentration

After mineralization, 1 ml of sample was diluted to 50 ml by the addition of one drop of *p*-nitrophenol NH_4OH, 5 ml of a mixture of ascorbic acid and reagent A [$(NH_4)_6Mo_7O_{24}$, H_2SO_4 and $K_2Sb_2(C_4H_2O_6)_2$], and distilled water (Cho & Nielsen, 2017; Nielsen, 2017). Total P was determined using Spectroquant Pharo 300 UV-visible spectrophotometer (Chew et al., 2018; Jastrzębska, 2009). Phosphorus content in each sample was calculated as:

$$Phosphorus\ content, mg/kg\ = \frac{(Sampling\ reading - Blank\ reading)\ x\ Volume\ of\ extract}{weight\ of\ oyster\ x\ Volume\ of\ aliquot}$$

Determination of other minerals mineral concentrations

The concentrations of other minerals (besides P) were determined using Atomic Absorption Spectrometry (PinAAcle 900T Perkin Elmer, KY, USA). Most of the metals (Ca, Mg, K, Na, Cr, Co, Cu, Fe, Mn, Ni, Se, Zn, Cd, and Pb) were determined by flow injection analysis-flame atomic absorption spectrometry using air-acetylene. As (Hydride Generation Technique) and Hg (Cold Vapour Technique) were measured using argon gas as fuel (Agemian, Sturtevant, & Austen, 1980; Edgell, 1989).

The concentration of each metal in an oyster sample was calculated as:

$$Concentration\ of\ metal = \frac{concentration\ in\ extract\ (\mu g/ml)\ x\ volume\ of\ extract (ml)}{Weight\ of\ oyster\ sample\ (g)}$$

2.6. Assessment of the concentrations of heavy metals in the oyster samples

We evaluated the concentrations of 4 heavy metals (As, Cd, Pd, and Hg) in the oyster samples by calculating the percentage of oyster samples which had concentrations above the maximum concentration limit per kg of oyster meat according to international regulatory guidelines. Because As is toxic to human health in its inorganic form (Bjorklund, Tippairote, Rahaman, & Aaseth, 2020), we estimated the average percent inorganic As (i-As) in the oyster samples using a conservative 25% of the total As (Lorenzana, Yeow, Colman, Chappell, & Choudhury, 2009), before calculating the percentage of oyster samples which had i-As concentrations above the maximum 1.0 mg/kg oyster meat set by the Food Standards Australia and New Zealand (FSANZ, 2020). We used the European Union/Food and Agriculture Organization's maximum concentration limits per kg wet weight of oyster meat for Cd (1.0 mg), Pb (1.5 mg), and Hg (1.0 mg) (EU/FAO, 2006; Montojo et al., 2021; Romero-Estévez et al., 2020; Wang & Lu, 2017).

2.7. Assessment of potential health risks of heavy metal exposure from oyster consumption among women shellfishers

We evaluated the potential health risks of heavy metal (i-As, Cd, Pb, and Hg) exposure from oyster consumption among women shellfishers based on the Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Hazard Index (HI) (Bristy et al., 2021; Joseph, Iwok, & Ekanem, 2021). The EDI for each of the 4 heavy metals (amount of the heavy metal in oysters expressed as mg/kg body weight/day, which can be ingested daily over a lifetime without appreciable health risk) was calculated as (Bristy et al., 2021; Yap et al., 2020):

$$Estimated\ Daily\ Intakes, EDI\ = \frac{Metal\ conc\ in\ oyster\ x\ Av.daily\ oyster\ consumption}{Av.body\ weight\ of\ women\ shellfishers}$$

We estimated the average daily oyster consumption from the repeat 24-hr recalls as the overall mean consumption across the three sites, which was equivalent to 0.006 kg/person/d. We could not use the median consumption value because it was zero. The average body weight of the women shellfishers was based on the 50th percentile of the overall weight distribution of the women across the three sites, which was 61 kg.

The THQ method estimating the health risk of oyster consumption based on non-carcinogenic effects (Bristy et al., 2021) was calculated for each of the 4 heavy metals as the ratio of EDI (exposure) to the oral reference dose (RfD) (Joseph et al., 2021; Moslen & Miebaka, 2017; USEPA, 2018):

$$Target\ Hazard\ Quotient, THQ = \frac{EDI}{RfD}$$

The RfD values (mg/kg/day dose which will not result in health issues in the course of a lifetime) used were 0.0003 for i-As (USEPA, 2018), 0.001 for Cd (USEPA, 2018), 0.0002 for Pd (given reference value of 12.5 µg/day (FDA, 2018; Wong, Roberts, & Saab, 2022) for women of reproductive age with average weight of 61 kg) and 0.0001 for Hg (Holloman & Newman, 2012; Wong et al., 2022). A THQ value <1 was used to indicate that the level of exposure to metal contamination was smaller than the oral reference dose, and therefore, it was negligible or not likely to cause any deleterious effects during lifetime in human population (Chien et al., 2002).

We calculated the HI as the sum of the individual THQ of i-As, Cd, Pb, and Hg of the oyster samples (Antoine, Fung, & Grant, 2017; Joseph et al., 2021; Vieira et al., 2021):

$$HI = THQ_{\text{inorganic arsenic}} + THQ_{\text{cadmium}} + THQ_{\text{lead}} + THQ_{\text{mercury}}$$

The Hazard Index assesses the cumulative potential health risk posed by the four metals related to the average consumption and body weight of the women shellfishers in the study. The HI calculation assumed that oyster consumption would result in simultaneous exposure As, Cd, Pb, and Hg. HI >1 was used to indicate a potential for adverse non-carcinogenic health effects from oyster consumption. Even if the THQ values were < 1 individually, the simultaneous exposure to the 4 heavy metals from oyster consumption may result in adverse health effects (Antoine et al., 2017).

2.8. Outcome variables

The three main outcome variables in this study were: (a) household food insecurity (yes/no), (b) achievement of MDD-W (yes/no), and (c) any anemia status (yes/no). Secondary outcome variables were (i) oyster consumption and nutrient (including iron and zinc) intakes from the repeat 24-hour dietary recall, (ii) mild, moderate, and severe food insecurity at the time of enrollment, (iii) percentages of women who consumed food from different food groups and mean food group dietary diversity score at enrolment, (iv) hemoglobin concentration and mild, moderate and severe anemia status at enrollment, (v) minerals and heavy metal concentrations of oysters collected from three estuarine sites, and (vi) health risk variables (EDI, THQ, and HI) for four heavy metals (inorganic-As, Cd, Pb, and Hg) related to the consumption of oysters.

The women's nutrient intakes from the repeat 24 hour recalls were analyzed based on a food composition database used in a previous study in Ghana (Lartey et al., 2014). We supplemented this database with data from the West African Food Composition Table (FAO, June, 2012) and the United States Department of Agriculture Nutrient Database (USDA, February, 2011). For the iron and zinc contents of oyster, we used the average values obtained from the mineral analysis of the 915 oyster samples collected from across the three estuaries site and reported herein. Women's average daily intakes of oyster and specific nutrients were estimated as the mean of the intakes from the two dietary recalls (Zhou et al., 2021). We defined "any oyster consumption" as average daily oyster consumption greater than 0 g in the two dietary recalls.

Household food insecurity was assessed using the Household Food Insecurity Access Scale (HFIAS) (Coates et al., 2007). This scale measures the HFIAS score, which is a continuous measure of the degree of food insecurity (access) in the household in the past four weeks, calculated by summing the codes for each frequency-of-occurrence question. The score ranges from 0 to 27 and women are categorized into mild, moderate, and severe food insecurity, with the higher the score, the more food insecurity the household has experienced. We created the binary variable "household food insecurity" (yes/no) by combining mild, moderate, and severe food insecurity.

Women's consumption of food from different food groups, 10-food group dietary diversity score (DDS), and minimum dietary diversity for women (MDD-W: DDS ≥ 5) were determined based on the first 24 hour recall conducted on the day of enrolment using procedures described by the FAO (FAO, 2021). The 10-food groups were: (i) Grains/roots/tubers, (ii) Pulses, (iii) Nuts/seeds, (iv) Milk/milk products, (v) Meat/poult/fish, (vi) Eggs, (vii) Dark green leafy vegetables, (viii) Other vitamin A-rich fruits and vegetables, (ix) Other vegetables, and (x) Other fruits (FAO, 2021).

Any anemia was defined as hemoglobin, Hb < 12 g/dl, mild anemia as Hb 10.0-11.9 g/dl, moderate anemia as Hb 7.0-9.9 g/dl, and severe anemia Hb < 7 g/d (GSS/GHS/ICF, 2015).

2.9. Sample size and data analysis

The sample size for the study was based on detecting Cohen's moderate effect size *d* of 0.3 in mean blood hemoglobin concentration between any pair of the three estuarine sites with a two-sided 5% test and 80% power. This gave an estimated target sample of 200 participants per site and 600 for each country.

To assess women shellfishers' socioeconomic status, we first created the wealth-poverty score (WPS) level (high or low) for each woman by assigning a value of 1 (yes) or 0 (no) to each of 10 items, including some items used for the Poverty Probability Index for Ghana (IPA, 2017), namely: (i) purchased any chicken eggs (fresh or single) in past month, (ii) purchased any raw or corned beef in past month, (iii) main construction material used for the outer wall of house is cement, (iv) main fuel used by the household for cooking is LPG, (v) any household member owns a gas stove, (vi) any household member owns a refrigerator, (vii) any household member owns a fan, (viii) any household member owns a television, (ix) any household member owns a mobile phone, and (x) any household member owns a canoe. The scores were summed to give a total possible score of 10. No score was calculated if any of the 10 summative variables was missing. A higher wealth-poverty score indicated a richer household. Next, we used each country's median WPS value as the cut-off for defining "high" or "low" WPS. Thus, WPS values were classified as high if the score was ≥ the country median value and low (poorer household) if < the country median value.

We performed all statistical analyses using SAS for Windows version 9.4 (Cary, NC). By estuarine site and for the overall sample, we summarized continuous variables as mean ± SD and categorical variables as number of participants and percentages. Subsequently, we compared the three estuarine sites by using ANOVA (SAS PROC GLM) for continuous variables and logistic regression (SAS PROC LOGISTIC) for binary variables. For all comparisons, the level of significance (α) between groups was set at $P < 0.05$. One participant with an extremely large average daily oyster consumption (> 400 g/day) was excluded from the analysis of daily oyster and nutrient intakes to avoid the possible distorting-effect of an outlier observation. Finally, by estuarine site and for the overall sample, we summarized the (i) percentage of oysters with heavy metal concentration above maximum regulatory limit, (ii) mean EDI, (iii) mean (minimum, maximum) THQ, (iv) mean (minimum, maximum) HI, and (v) mean percent contribution of each heavy metal to the total HI.

Research Results

In total, 504 women shellfishers of reproductive age were recruited from the three estuarine sites, including 200 from the Densu estuary (Bortianor, Tsokomey, and Tetegu communities), 166 from the Narkwa Lagoon (Ekumfi Narkwa), and 138 from the Whin estuary (New Amanful, Apremdo, and Beaho communities). These women comprised nearly all women shellfishers in the target age group (women of reproductive age) available for enrollment at the three sites.

3.1. Demographic and socioeconomic characteristics of women shellfishers

The summary results of the women's demographic and socioeconomic characteristics are presented in **Table 1.** The mean ± SD age of women in the overall sample was 32 ± 9 years. Across the three sites, the women shellfishers differed significantly ($P < 0.05$) in most of the variables measured. In general, the women at the Densu and Whin sites had more years of schooling and indicators suggestive of higher socioeconomic status (e.g., more women living in households possessing various household items), compared with the Narkwa women. Mean ± SD WPS was significantly greater among the Densu (5 ± 3) and Whin (5 ± 2) women than the Narkwa (3 ± 2) women ($P < 0.001$). Similarly, the percentage of women from households with high WPS was greater for the Densu (63%) and Whin (65%) sites than the Narkwa (37%) site ($P < 0.001$).

Table 1 Demographic and socioeconomic characteristics of women shellfishers 15-49 years of age who participated in the study, by estuary site, in Ghana[1].

	Estuary sites in Ghana				
	Densu	Narkwa	Whin	P[2]	Total
Age, y	32 ± 9 (200)	34± 10 (166)	32 ± 10 (138)	0.30	33 ± 9 [504]
Ever attended school	162/200 (81.0)[a]	93/166 (56.0)[b]	98/137 (71.5)[a]	<0.001	353/504 (70.0)
Total years of schooling	6 ± 4 (200)[a]	3 ± 4 (166)[b]	6 ± 4 (138)[a]	<.0001	4.9 ± 4.0 [504]
Able to read (literacy)	70/162 (43.2)	37/92 (40.2)	53/97 (54.6)	0.10	160/351 (45.6)
Woman is married	99/200 (49.5)[b]	108/166 (65.1)[a]	61/138 (44.2)[b]	<0.001	268/504 (53.2)
Worked in the last 7 days	121/200 (60.5)[a]	62/166 (37.3)[b]	89/138 (64.5)[a]	<0.001	272/504 (54.0)
Number of household members	6 ± 2 (200)[b]	7 ± 2 (166)[a]	7 ± 3 (138)[a]	<0.001	6 ± 3 [504]
Wealth-poverty score (WPS)[3]	5 ± 3 (198)[a]	3 ± 2 (165)[b]	5 ± 2 (138)[a]	<0.001	4.1 ± 2.4 [501]
High WPS[4]	124/198 (62.6)[a]	61/165 (37.0)[b]	89/138 (64.5)[a]	<.0001	274/501 (54.7)

[1]Within country, values in a row with a differing letter statistically differ from each other at α ≤ 0.05 by ANOVA and Tukey-Kramer tests for means and by Chi squared test for percentages.

[2]P-value compares within country the three group means by ANOVA or percentages by Chi squared test.

[3]Wealth-poverty score for individual women were calculated as an adaptation of the procedure described by the Innovations for Poverty Action (IPA, 2017) by assigning a value of 1 (yes) or 0 (no) to each of 10 household wealth items and summing the scores to give the total score which ranged 0-10. A higher WPS indicates higher socioeconomic status.

[4]WPS values ≥ the country median value were classified as high (richer households). Median WPS was 4 for Ghana.

3.2. Oyster consumption, iron and zinc intakes, and household food insecurity

The summary results of oyster consumption (g), total iron and zinc intakes (mg), iron and zinc intakes from oyster, and household food insecurity of the women shellfishers are presented in **Table 2**.

Table 2 Total oyster consumption, iron and zinc intakes, percent iron and zinc contribution from oyster, and household food insecurity among women shellfishers 15-49 years of age who participated in the study[1]

	Densu	Narkwa	Whin	P[2]	Total
Total oyster consumed, g	9.6 ± 26.0 (199)[a]	6.7 ± 25.7 (166)[a]	0.3 ± 2.2 (138)[b]	0.001	6.1 ± 22.4 (503)
Any oyster consumption[3]	37/199 (18.6)[a]	23/166 (13.9)[a]	2/138 (1.4)[b]	0.001	62/503 (12.3)
Total iron intake, mg	17.4 ± 11.0 (199)	14.6 ± 8.4 (166)	26.7 ± 110 (138)	0.18	19.0 ± 58.2 (503)
Total zinc intake, mg	8.7 ± 6.8 (199)	7.3 ± 4.8 (166)	12.7 ± 45.8 (138)	0.14	9.3 ± 24.6 (503)
Iron intake from oyster, mg	2.4 ± 6.5 (199)[a]	1.6 ± 5.4 (166)[a]	0.07 ± 0.55 (138)[b]	<0.001	1.5 ± 5.2 (503)
Zinc intake from oyster, mg	1.6 ± 4.3 (199)[a]	1.0 ± 3.5 (166)[a]	0.04 ± 0.36 (138)[b]	<0.001	1.0 ± 3.4 (503)
HFIAS score[4]	11.7 ± 5.9 (200)[ba]	12.8 ± 6.5 (166)[a]	10.7 ± 6.6 (138)[b]	0.017	11.8 ± 6.3 (504)
Food secure[4]	13/200 (6.5)	10/166 (6.0)	17/138 (12.3)	0.09	40/504 (7.9)
Food insecure[4]	187/200 (93.5)	156/166 (94.0)	121/138 (87.7)	0.09	464/504 (92.1)
Mildly food insecure[4]	20/200 (10.0)[a]	3/166 (1.8)[b]	4/138 (2.9)[b]	0.003	27/504 (5.4)
Moderately food insecure[4]	23/200 (11.5)	12/166 (7.2)	8/138 (5.8)	0.147	43/504 (8.5)
Severely food insecure[4]	144/200 (72.0)[b]	141/166 (84.9)[a]	109/138 (79.0)[ba]	0.012	394/504 (78.2)

[1]Values in a row with different letters statistically differ at α ≤ 0.05 by ANOVA and Tukey-Kramer tests for means and Chi-squared test for percentages.

[2]P-value compares the three site means by ANOVA or percentages by Chi squared test.

[3]Any oyster consumption was defined as oyster consumption > 0 g in the repeat 24-hour dietary recalls.

[4]Household food insecurity was assessed using the Household Food Insecurity Access Scale (HFIAS) (Coates et al., 2007). “Food insecure” households include mildly food insecure, moderately food insecure, and severely food insecure households.

Overall, only 12.5% of the women across the three estuarine areas consumed any oyster in the repeat 24-hour dietary recalls, with the site percentages being significantly greater ($P < 0.001$) among the Densu (18.6%) and Narkwa (13.9%) women than the Whin (1.4%) women. Mean ± SD daily oyster consumption (g) was significantly higher among the Densu (9.6 ± 26.0) and Narkwa women (6.7 ± 25.7) than the Whin women (0.3 ± 2.2) ($P = 0.001$). Mean total daily iron and zinc intakes did not differ across sites. However mean daily iron intake from oyster (mg) was significantly higher among the Densu (2.4 ± 6.5) and Narkwa (1.6 ± 5.4) women than the Whin women (0.07 ± 0.55), ($P < 0.001$); likewise, mean daily zinc intake from oyster (mg) was significantly higher among the Densu (1.6 ± 4.3) and Narkwa (1.0 ± 3.5) women than the Whin women (0.04 ± 0.36), ($P < 0.001$). Thus, the average percentage contribution of oyster consumption to iron intake amounted to 11-14% for the Densu and Narkwa women and only 0.3% for the Whin women, while the average percentage contribution of oyster consumption to zinc intake amounted to 14-18% for the Densu and Narkwa women and only 0.3% for the Whin women.

The women at Narkwa had a significantly higher mean ± SD HFIAS score (12.8 ± 6.5) than those at the Whin site (10.7 ± 6.6) but not those at the Densu site (11.7 ± 5.9), $P = 0.017$. Women shellfishers at the three estuarine sites did not differ in percentage of women who were food secure, but a vast majority of them (between 72% at the Densu site and 85% at the Narkwa site) reported severe food insecurity.

3.3. Percentages of women who consumed food from different food groups, mean food group dietary diversity score, and minimum dietary diversity for women

The summary results of the percentages of women who consumed food from different food groups, mean food group dietary diversity score, and minimum dietary diversity for women are presented in **Table 3**. Across the three sites, 97-99% of the women shellfishers reportedly consumed grains/white roots/tubers and plantains, > 86% reportedly consumed meat/poultry/fish, and > 85% reportedly consumed other vegetables (than green leafy vegetables and vitamin A-rich vegetables) during the 24 hours preceding enrollment into the study. Compared with the above food groups, the percentages of women shellfishers across the three sites consuming the other food groups were lower.

The three sites did not differ in the percentage of women who consumed grains/white roots/tubers/plantains (P = 0.26), pulses (P = 0.97), meat/poultry/fish (P = 0.93), eggs (P = 0.12), dark green leafy vegetables (P = 0.41), and “Other fruits” (P = 0.15). The percentages of women consuming Vitamin A-rich fruits and vegetables were higher for the Narkwa (33.1%) and Whin (23.4%) women than the Densu (11.5%) women. The percentages of women who consumed “Other vegetables” (besides dark green leafy vegetables and vitamin A-rich vegetables) lower for the Whin site (85%) than the Densu (97%) and Narkwa (94%) sites, P = 0.001). The mean 10-food group dietary diversity score for women across the three sites was low (3.6 – 3.8). Only 20-23% of the women achieved the minimum dietary diversity for women or had a food group dietary diversity score ≥ 5. These indices did not differ significantly by estuary site.

Table 3 Percentages of women who consumed food from different food groups, mean food group dietary diversity score, and minimum dietary diversity for women shellfishers 15-49 years of age who participated in the study, by estuarine site[1]

	Densu	Narkwa	Whin	P[2]	Total
Grains/white roots/tubers and plantains	193/200 (96.5)	163/166 (98.2)	136/137 (99.3)	0.26	492/503 (97.8)
Pulses	22/200 (11.0)	18/166 (10.8)	14/137 (10.2)	0.97	54/503 (10.7)
Nuts and seeds	40/200 (20.0)[ba]	41/166 (24.7)[a]	16/137 (11.7)[b]	0.018	97/503 (19.3)
Milk and milk products	25/200 (12.5)[a]	4/166 (2.4)[b]	15/137 (10.9)[a]	0.006	44/503 (8.7)
Meat, poultry and fish	175/200 (87.5)	145/166 (87.3)	118/137 (86.1)	0.93	438/503 (87.1)
Eggs	33/200 (16.5)	16/166 (9.6)	23/137 (16.8)	0.12	72/503 (14.3)
Dark green, leafy vegetables	26/200 (13.0)	22/166 (13.3)	12/137 (8.8)	0.41	60/503 (11.9)
Vitamin A-rich fruits and vegetables	23/200 (11.5)[b]	55/166 (33.1)[a]	32/137 (23.4)[a]	<0.001	110/503 (21.9)
Other vegetables	194/200 (97.0)[a]	156/166 (94.0)[a]	117/137 (85.4)[b]	0.001	467/503 (92.8)
Other fruits	11/200 (5.5)	15/166 (9.0)	5/137 (3.6)	0.15	31/503 (6.2)
MDD-W 10-food group diversity score[3]	3.7 ± 1.0 (200)	3.8 ± 1.0 (166)	3.6 ± 1.3 (137)	0.12	3.7 ± 1.1 [503]
Achieved MDD-W (DDS ≥ 5)	40/200 (20.0)	39/166 (23.5)	27/137 (19.7)	0.65	106/503 (21.1)

[1]Values in a row with a differing letter statistically differ from each other at α ≤ 0.05 by ANOVA and Tukey-Kramer tests for means and by Chi squared test for percentages.

[2]P-value compares within country the three group means by ANOVA or percentages by Chi squared test.

[3]10-food group dietary diversity score (DDS) was calculated based on procedures described by the Food and Agricultural Organization (FAO, 2021); MDD-W: minimum dietary diversity for women.

3.4. Hb concentration and anemia status

The mean blood hemoglobin concentrations and anemia status of the women shellfishers by estuary site are presented in **Table 4**. Mean ± SD blood hemoglobin concentration (g/dl) was significantly higher (P <0.001) in women shellfishers at the Whin site (13.4 ± 1.5) than those at the Densu (12.7 ± 1.7) and Narkwa (12.9 ± 1.4) sites. The percent of women shellfishers with anemia (Hb < 12 g/dl) ranged from 15% at the Whin site to 25% at the Densu site, with the Narkwa site in between at 19%; these percentages did not differ significantly across the sites (P = 0.08). Similar trends were found for mild anemia (Whin, 14%; Narkwa, 16%; Densu, 19%: P = 0.51), moderate anemia (Whin, 1%; Narkwa, 2%; Densu, 5%: P = 0.17), and severe anemia (Whin, 0%; Narkwa, 1%; Densu, 2%: P = 0.73).

Table 4 Mean blood hemoglobin concentration and anemia status of women shellfishers 15-49 years of age who participated in the study, by estuary site, in Ghana[1].

	Densu	Narkwa	Whin	P[2]	Total
Hemoglobin concentration, g/dl	12.7 ± 1.7 (200)[b]	12.9 ± 1.4 (166)[b]	13.4 ± 1.5 (138)[a]	<0.001	12.9 ± 1.6 [504]
Any anemia, Hb < 12 g/dl	50/200 (25.0)	32/166 (19.3)	21/138 (15.2)	0.08	103/504 (20.4)
Mild anemia, Hb 10.0-11.9 g/dl	37/200 (18.5)	27/166 (16.3)	19/138 (13.8)	0.51	83/504 (16.5)
Moderate anemia, Hb 7.0-9.9 g/dl	10/200 (5.0)	4/166 (2.4)	2/138 (1.4)	0.17	16/504 (3.2)
Severe anemia, Hb b < 7 g/dl	3/200 (1.5)	1/166 (0.6)	0	0.73	4/504 (0.8)

[1]Values in a row without a common superscript letter differ significantly, P < 0.05 by ANOVA and Tukey-Kramer tests for means and by Chi squared test for percentages.

[2]P-value compares within country the three group means by ANOVA or percentages by Chi squared test.

3.6. Mineral and heavy metal concentrations of oysters from the three estuary sites

The mineral and heavy metal concentrations (mg/kg wet weight) of the oyster samples from the three estuarine sites are summarized in **Table 5**. The oysters from the three sites differed significantly (P < 0.05) in the mean concentrations of all the minerals and heavy metals measured. There was no consistent pattern of higher or lower mean macromineral (Ca, Mg, P, K, and Na) concentrations of the oyster samples, by estuarine site. However, the mean trace mineral and heavy metal concentrations were consistently higher in the Narkwa samples than these from the Densu and Whin sites. For those minerals most importance for hemoglobin concentration and anemia status, for example, the mean ± SD iron concentration of the oysters from the Narkwa site was 147 ± 142, compared with 125 ± 91 for the Densu site and 103 ± 87) for the Whin site, P < 0.001. Likewise, the mean zinc concentration was higher for oysters from Narkwa (118 ± 79) than those from Densu (64.0 ± 56.4) and Whin (65.5 ± 62.1), P < 0.001.

The mean heavy metal concentrations of oysters from across the three sites ranged from 0.043 – 0.189 for total arsenic, 0.011 – 0.047 for inorganic arsenic (estimated at an average of 25% of total arsenic), 0.023 – 0.065 for cadmium, 0.016 –0.057 for lead, and 0.022 – 0.065 for mercury, with oysters from the Narkwa site having significantly higher mean concentration all four heavy metals compared with the other two sites.

Table 5 Macrominerals, trace minerals, and heavy metal concentrations (mg/kg wet weight) of oysters collected from three estuarine sites in Ghana[1]

Mineral, mg/kg wet weight	Densu (n = 305)	Narkwa (n = 305)	Whin (n = 305)	P	All (n = 915)
Macrominerals					
Calcium	3811 ± 2878[a]	840 ± 940[b]	3445 ± 4081[a]	<0.001	2698 ± 3216
Magnesium	1321 ± 453[a]	1116 ± 409[b]	374 ± 264[c]	<0.001	937 ± 559
Phosphorus	2882 ± 2219[b]	5016 ± 8367[a]	2513 ± 3101[b]	<0.001	3470 ± 5416
Potassium	8314 ± 5620[a]	3090 ± 2181[b]	1443 ± 755[c]	<0.001	4282 ± 4567
Sodium	8051 ± 5315[b]	9689 ± 2990[a]	3031 ± 2929[c]	<0.001	6924 ± 4822
Trace minerals					
Chromium	0.331 ± 0.246[b]	0.737 ± 0.512[a]	0.294 ± 0.269[b]	<0.001	0.453 ± 0.413
Cobalt	0.043 ± 0.043[c]	0.086 ± 0.078[a]	0.065 ± 0.166[b]	<0.001	0.064 ± 0.110
Copper	1.5 ± 1.2[b]	3.2 ± 2.1[a]	1.4 ± 1.3[b]	<0.001	2.0 ± 1.8
Iron	126 ± 90[b]	147 ± 142[a]	103 ± 87[c]	<0.001	126 ± 111
Manganese	1.2 ± 1.1[b]	2.8 ± 2.1[a]	1.1 ± 1.3[b]	<0.001	1.7 ± 1.7
Nickel	0.539 ± 0.446[b]	1.16 ± 1.07[a]	0.631 ± 0.807[b]	<0.001	0.776 ± 0.858
Selenium	4.6 ± 3.6[b]	5.9 ± 7.4[a]	5.7 ± 6.2[a]	0.014	5.4 ± 6.0
Zinc, mg/kg	64.0 ± 56.3[b]	118 ± 79[a]	65.5 ± 62.1[b]	<0.001	82 ± 71
Heavy metals					
Arsenic (total)	0.043 ± 0.032[c]	0.189 ± 0.167[a]	0.079 ± 0.116[b]	<0.001	0.102 ± 0.132
Arsenic (inorganic)	0.011 ± 0.008[c]	0.047 ± 0.042[a]	0.020 ± 0.029[b]	<0.001	0.025 ± 0.033
Cadmium	0.023 ± 0.017[b]	0.065 ± 0.051[a]	0.025 ± 0.027[b]	<0.001	0.037 ± 0.039
Lead	0.021 ± 0.021[b]	0.057 ± 0.046[a]	0.016 ± 0.022[b]	0.002	0.032 ± 0.037
Mercury	0.027 ± 0.038[b]	0.065 ± 0.132[a]	0.022 ± 0.025[b]	<0.001	0.038 ± 0.083

[1]Values are mean ± SD. Row means without a common superscript letter differ significantly, P < 0.05 by ANOVA and Tukey-Kramer tests.

[3]Average percent inorganic arsenic was conservatively estimated at 25% of total arsenic concentration (Lorenzana et al., 2009).

Table 6 shows the percentage of oyster samples from each site that exceeded the maximum concentration limits for four heavy metals according to international guidelines, as well as the health risk assessment of heavy metal contamination through oyster consumption among women shellfishers at the three sites. The table shows that none of the oysters collected from the three sites exceeded the maximum concentration limit for As (based on average inorganic arsenic concentration) according the Food Standards Australia and New Zealand (FSANZ, 2020) guidelines. Furthermore, none of the oysters exceeded the maximum concentration limit for Cd, Pb, and Hg according to EU/FAO guidelines (EU/FAO, 2006), except for one oyster sample from the Narkwa site, which exceeded the maximum concentration limit for Hg.

The mean EDIs (mg/kg body weight/day) of the four heavy metals in oysters among the women shellfishers ranged from 1.2×10^{-6} to 5.4×10^{-6} for inorganic As, 2.5×10^{-6} to 7.3×10^{-6} for Cd, 1.2×10^{-6} to 6.5×10^{-6} for Pd, and 2.5×10^{-6} to 7.3×10^{-6} for Hg. Across the three estuarine sites, Narkwa had the highest mean EDI for all four heavy metals.

Table 6 Estimated Daily Intake (EDI), Target Hazard Quotient (THQ) and Hazard Index (HI) of heavy metals in oysters collected from three estuary sites in Ghana[1].

Heavy metal	% of oysters with metal concentration above maximum regulatory limit[2]	Mean (Min, Max) EDI (mg/kg BW /day)[3]	Mean (Min, Max) THQ[4]	Mean (Min, Max) HI[5]	Mean % contribution to HI
Arsenic (inorganic)[6]					
All	0	2.9×10^{-6} (3.4×10^{-8}, $3.4\ 10^{-5}$)	9.6×10^{-3} (1.0×10^{-4}, 1.1×10^{-1})		15.3
Densu	0	1.2×10^{-6} (2.6×10^{-7}, 7.3×10^{-6})	4.1×10^{-3} (9.0×10^{-4}, 2.4×10^{-2})		10.5
Narkwa	0	5.4×10^{-6} (3.4×10^{-8}, 3.2×10^{-5})	1.8×10^{-2} (1.0×10^{-4}, 1.1×10^{-2})		17.0
Whin	0	2.2×10^{-6} (3.4×10^{-8}, 3.4×10^{-5})	7.5×10^{-3} (1.0×10^{-4}, 1.1×10^{-2})		18.6
Cadmium					
All	0	4.2×10^{-6} (3.4×10^{-7}, 3.8×10^{-5})	4.2×10^{-3} (3.0×10^{-4}, 3.8×10^{-2})		7.2
Densu	0	2.6×10^{-6} (5.7×10^{-7}, 1.7×10^{-5})	2.5×10^{-3} (6.0×10^{-4}, 1.7×10^{-2})		6.4
Narkwa	0	7.3×10^{-6} (3.4×10^{-7}, 3.8×10^{-5})	7.3×10^{-3} (3.0×10^{-4}, 3.8×10^{-2})		6.9
Whin	0	2.9×10^{-6} (3.4×10^{-7}, 2.4×10^{-5})	2.9×10^{-3} (3.0×10^{-4}, 2.4×10^{-2})		8.2

Heavy metal	% of oysters with metal concentration above maximum regulatory limit[2]	Mean (Min, Max) EDI (mg/kg BW /day)[3]	Mean (Min, Max) THQ[4]	Mean (Min, Max) HI[5]	Mean % contribution to HI
Lead					
All	0	3.6×10^{-6} (1.1×10^{-7}, 3.1×10^{-5})	1.8×10^{-2} (6.0×10^{-4}, 1.6×10^{-1})		26.1
Densu	0	2.4×10^{-6} (1.1×10^{-7}, 1.7×10^{-5})	1.2×10^{-2} (6.0×10^{-4}, 8.3×10^{-2})		27.3
Narkwa	0	6.5×10^{-6} (1.1×10^{-7}, 3.1×10^{-5})	3.3×10^{-2} ($6.0 \times 10\text{-}^{-4}$, 1.6×10^{-1})		29.0
Whin	0	1.8×10^{-6} (1.1×10^{-7}, 1.8×10^{-5})	9.0×10^{-3} (6.0×10^{-4}, 8.9×10^{-2})		21.7
Mercury					
All	1	4.3×10^{-6} (0, 1.5×10^{-4})	4.3×10^{-2} (0, 1.5)		54.1
Densu	0	3.1×10^{-6} (0, 6.4×10^{-5})	3.1×10^{-2} (0, 6.4×10^{-1})		57.3
Narkwa	1	7.3×10^{-6} (4.5×10^{-7}, 1.5×10^{-4})	7.3×10^{-2} (4.5×10^{-3}, 1.5)		50.2
Whin	0	2.5×10^{-6} (0, 2.2×10^{-5})	2.5×10^{-2} (0, 2.2×10^{-1})		54.7
Cumulative potential health risk					
All				0.07 (0.01, 1.55)	
Densu				0.05 (0.01, 0.72)	
Narkwa				0.13 (0.01, 1.55)	
Whin				0.04 (0.01, 0.22)	

[1]Oyster samples were *n* = 305 for each of the Densu, Narkwa Whin sites (total n = 915).

[2]Maximum regulatory limit (mg/kg wet wt) were specified by the Food Standards Australia and New Zealand (FSANZ, 2020) for inorganic arsenic (1.0) and by the EU/FAO for Cd (1.0), Pb (1.5), and Hg (1.0) (EU/FAO, 2006).

[3]EDI: Estimated Daily Intake of metals expressed as mg/kg body weight/day was calculated by dividing the product of the oyster metal concentration (mg/kg wet weight) and average daily oyster consumption of women shellfishers (kg) by the average body weight of women shellfishers (kg) (Bristy et al., 2021; Yap et al., 2020)

[4]THQ, Target Hazard Quotient, is the ratio of exposure (Estimated Daily Intake, EDI) to the oral reference dose (RfD) (Joseph et al., 2021). The RfD (mg/kg BW/day) values for As (0.0003) and Cd (0.001) were reported by the USEPA (Moslen & Miebaka, 2017; USEPA, 2018) ; RfD values for lead (0.0002) (Lim, Aris, & Zakaria, 2012) and mercury (0.0001) (Holloman & Newman, 2012) were not available from USEPA (USEPA, 2018).

[5]HI, Hazard Index, is the sum of the THQ of all the heavy metals (Joseph et al., 2021; Moslen & Miebaka, 2017), i.e., HI = $THQ_{arsenic}$ $THQ_{cadmium}$ + THQ_{lead} + $THQ_{mercury}$. HI > 1 indicates potential health risk.

[6]Values refer to the average inorganic contents of arsenic in the oyster samples estimated conservatively at 25% of the total As concentration (Lorenzana et al., 2009).

Mean THQ for oyster consumption among women shellfishers across the three estuarine sites ranged from 4.1 x 10^{-3} to 1.8 x 10^{-2} for inorganic As, 2.5 x 10^{-3} to 7.3 x 10^{-3} for Cd, 9.0 x 10^{-3} to 3.3 x 10^{-2} for Pd, and 2.5 x 10^{-2} to 7.3 x 10^{-2} for Hg. The Narkwa site had the highest mean THQ for all four heavy metals when comparing with the two sites. All three sites had a mean THQ value that was well below 1 for each of the four heavy metals indicating that the average level of each heavy metal in oysters at each of the three sites was lower than the oral reference dose.

Across the three sites, mean HI for oyster consumption among the women shellfishers ranged from 0.04 at the Whin site to 0.13 at the Narkwa site; none of the estuarine site had a mean HI exceeding 1. At all three sites, the primary driver of the HI values among the women shellfishers was Hg accounting for 50-57%, followed by lead accounting for 22 -29%, with Cd contributing the least (6-8%).

Research Discussion

We found that on average, only 12.5% of the women shellfishers across the three estuarine sites consumed oyster per day during the week after recruitment into the study. The average amount of oyster consumed in a day was only 6.1 g, contributing an average of 8% of the total daily iron intake, and 11% of the total daily zinc intake. The women shellfishers from the Densu and Narkwa sites generally consumed more oyster and thus had a larger iron and zinc contribution from oyster than those from the Whin site. Across the three sites, the percentage of women reporting household food insecurity was high (92.1% overall), with nearly 85% of the Narkwa women reporting severe household food insecurity. Women shellfishers across three sites did not differ in mean 10-food group dietary diversity score (3.7 overall) or percentage who achieved the minimum (≥ 5) dietary diversity for women during the 24 hours before recruitment (21.1% overall) or prevalence of anemia (20.4% overall).

The oysters from the three estuarine sites differed significantly in the mean concentrations of all the 17 minerals and heavy metals measured. The heavy metal (i-As, Cd, Pd, and Hg) concentrations of oysters from the three sites were within international regulatory limits. All three sites had a mean THQ value that was well below 1 for each of the four heavy metals suggesting that the intake of the heavy metals from oysters among the women shellfishers is unlikely to cause adverse health effects during lifetime. The mean HI (non-carcinogenic exposure) for oyster consumption among the women shellfishers across the three estuarine sites ranged from 0.04 to 0.13, with mercury and lead being the primary drivers.

This study had several strengths, including a relatively large sample size comprising nearly all the women shellfishers in the target age group at each of the estuarine sites. We used a repeat 24-hr dietary recall to assess oyster consumption and iron and zinc intakes, which provided better estimates of daily nutrient intakes than what could have been achieved with either a food frequency questionnaire or a single 24-hr recall (Olafsdottir, Thorsdottir, Gunnarsdottir, Thorgeirsdottir, &

Steingrimsdottir, 2006). To our knowledge, this is the first study in Ghana in which such a large number of oyster samples (>900) were analyzed for a total of 13 minerals and 4 heavy metals. One weakness of the 24-hour dietary recall was that the women may have had difficulty remembering what they consumed in the 24-hr before the interview, which could have led to less accurate estimates of intakes. Another weakness was the lack of specific or reliable food composition table for Ghana. For some food items consumed, which were not found on the food composition table previously used in Ghana, we chose similar or identical foods from the West African Food Composition Table (FAO, June, 2012) or the United States Department of Agriculture (USDA, February, 2011) database. These similar or identical foods may not have the same nutrient contents as the foods actually consumed, and therefore, some of the nutrient intakes may be over- or under-estimated. Those similar or identical foods were, however, relatively few. Lastly, as we tested many hypotheses in this study, it is possible that some of the observed differences may be due to chance (Li et al., 2017).

Several possible reasons many accounts for the low oyster consumption among the women shellfishers regardless of estuarine site where they lived. It appears that the women shellfishers mainly sold the oysters they harvested or processed to generate income, rather than consumed the oysters. In a study at Narkwa (B. Asare, Obodai, & Acheampong, 2019), 75% of the participants said they harvested oysters for sale and consumption; the proportion of harvested oysters actually consumed by the participants may be small compared with the amount sold. In recent times, oyster harvesting, processing, and marketing have become important livelihood activities in Ghana such that, much of the oysters harvested or processed by the women shellfishers may have been for sale (Atindana, Fagbola, Ajani, Alhassan, & Ampofo-Yeboah, 2020).

Another possible explanation may be declining oyster catch in the coastal communities, especially at the Whin site, although that may not be true for the Densu site (Osei, Chuku, Effah, Kent, & Crawford, 2021). Oyster stocks in estuarine sites may be depleted because of several factors, including population increases, mangrove degradation, and more women taking up year-round oyster harvesting. Thus, the amount of oysters available for picking and consumption at the estuarine sites may be small. Communities at the Densu site instituted an annual 5-month (December to April) closure of oyster-picking (Agbekpornu et al., 2021) to allow oyster stocks to replenish themselves; it is unclear whether this strategy increases oyster consumption among the women shellfishers. Lastly, the women shellfishers may not be interested to consume much oysters themselves because of concerns about heavy metal contamination. It is likely the women consume other aquatic foods such as pelagic fishes (Iannotti et al., 2022).

Women in fishing communities in West Africa have often been reported to be food insecure due to factors such as depleting fish stocks (World Economic Forum, 2021), but the high prevalence of household food insecurity among those in our sample seems extraordinary. A possible explanation was that we collected data (June - July 2021) when the COVID-19 pandemic was still active, which may have disrupted food supply and rendered more households food insecurity or households more food insecure (Bukari et al., 2022). In Ghana, about two-thirds of the population (~ 22 million people) reportedly experienced a decrease in household income, while 52% of households reduced food consumption due to the pandemic (UNICEF-World Bank, 2022).

The prevalence of anemia in the study sample (15%-25%) is consistent with the 22% reported for non-pregnant women 15-49 years by the Ghana Micronutrient Survey 2017 (UG/GroundWork/UWisconsin-Madison/KEMRI/UNICEF, 2017). In Ghana, anemia rate has been consistently high among women of child-bearing age for decades (GSS/GHS/ICF, 2015), primarily as a result of inadequate dietary iron intakes (Jamil et al., 2008; Stoltzfus, 2003). Results from the mineral analysis of the oysters suggest that these local resources could provide an excellent source of several dietary minerals to women shellfishers and help address anemia due to low mineral intakes. Of particular importance are the high mean concentrations (mg/kg wt) of iron (125), zinc (82), and selenium (5.4) which plan key roles in anemia prevention (Domellöf, 2021).

The concentrations of heavy metals (As, Cd, Pb, and Hg) in the oyster samples are generally consistent with those reported previously after pooling a small number of oysters samples collected from the same estuarine sites (E. O Chuku et al., 2022). The relatively large mean values of the four heavy metals for the Narkwa oysters suggest a high level of pollution in that area, given previous reports (Otchere, 2019). Potential sources of the heavy metal contamination of the oysters from the estuarine sites in Ghana include artisanal small scale gold mining, which involves panning soil with elemental mercury (Gyamfi et al., 2021; World Bank Group, 2020), e-waste (Ackah, 2017; World Bank Group, 2020), and lead battery manufacturing and recycling (World Bank Group, 2020).

The low mean HI values for oyster samples from the three estuarine sites suggests that there is less likelihood of potentially substantial health hazard as a result of oyster consumption among the women shellfishers. In addition, there are reports that selenium counteracts the toxic effects of As, Cd, and Hb poisoning (Reilly, 2002). For example, selenium may offer protection from mercury and methylmercury toxicity by preventing cellular damage from free radicals through counteracting the mercury-induced depression in glutathione-synthesizing enzymes and the formation of inactive selenium-mercury complexes (Goyer, 1997). Thus, interactions between heavy metal ions may offer protection against heavy metal poisoning related to oyster consumption among women in the sample.

We conclude that efforts to promote oyster consumption in Ghana might be a good strategy to increase iron and zinc intakes and reduce anemic among women living at estuarine sites in Ghana.

Outputs and Conclusions

1. Our results show that the current level of oyster consumption among the women shellfishers 15-49 years at the three estuarine sites in Ghana may be too low to make any substantial impact on the women's iron and zinc intakes from oyster, food security, dietary diversity, and anemia prevalence. The main explanation to this low oyster consumption might be that the women shellfishers sell a much larger proportion of their oysters than the proportion they consume. It is likely that the women consume more of other aquatic animal source foods (e.g., small pelagic fishes) than they do oysters.
2. Heavy metal contamination does not appear to pose a major health risk for the women shellfishers related to oyster consumption. However, there should be regular monitoring of mercury and lead contamination of oysters and other aquatic animal foods, especially at the Narkwa area.
3. We propose the following recommendations for further research:
    a. Explore how well women living in estuarine areas might use shellfishery resources to increase nutrient intakes and prevent anemia.
    b. Use biomarkers to assess selenium and heavy metal toxicity among women of reproductive age (15-49 years of age) and children 6-59 mo of age living in estuarine areas.
4. We propose the following recommendations for programmatic action:
    a. Increase efforts to promote oyster consumption as a promising strategy to increase nutrient intakes and prevent anemia in estuarine communities.
    b. Periodically and regularly monitor water bodies and aquatic animal source foods for heavy metal contamination; disseminate such findings to inform consumers' food selection.

Technologies/Innovations developed, and what phase was achieved

This project investigated the dietary intakes (including oyster consumption) and household food insecurity among vulnerable women shellfishers at three estuarine sites along the coastline of Ghana. We gathered evidence of the nutritional benefits of oysters and the potential health risks of heavy metal contamination via their consumption to women shellfishers. We did not develop a technology or an innovation, but the results from the study may be used as a foundation to develop an innovation to promote oyster consumption as a low-cost strategy to increase nutrient intakes and prevent anemic in estuarine communities. Results from this project may also be used in advocacy to combat potential pollution of estuarine waters in Ghana.

Key Beneficiaries

A key beneficiary of this research was Mr. Francis Z. Taabia who enrolled in the Ph.D. in Nutrition program in the University of Ghana Department of Nutrition and Food Science in 2021. This Fish Innovation Lab activity supported Taabia's dissertation research with funding for field work, laboratory analysis of the oyster samples for minerals and heavy metals, and write-up of results. This support to Francis contributed to building capacity in Ghana.

How the scientific results were disseminated

The scientific results are being disseminated through publication in a peer-reviewed journal, which has been drafted and is currently being revised by the team before submission. In addition, we are drafting a policy brief that will be shared with relevant government agencies (e.g., Ghana Health Service, Department of Fisheries) and local organizations (e.g., Densu Oyster Pickers Association) that will provide the results, key conclusions, and recommendations. After sharing the brief, we will hold a Zoom meeting to invite all parties that received the briefing to discuss the results presented.

References

Ackah, M. (2017). Informal E-waste recycling in developing countries: review of metal (loid) s pollution, environmental impacts and transport pathways. *Environmental Science and Pollution Research, 24*(31), 24092-24101.

Agbekpornu, H., Ennin, E. J., Issah, F., Pappoe, A., & Yeboah, R. (2021). Women in West African Mangrove Oyster (Crassostrea Tulipa) Harvesting, Contribution to Food Security and Nutrition in Ghana. *Oceanogr Fish Open Access J, 14 (1)*. doi:10.19080/OFOAJ.2021.14.555878

Agemian, H., Sturtevant, D., & Austen, K. (1980). Simultaneous acid extraction of six trace metals from fish tissue by hot-block digestion and determination by atomic-absorption spectrometry. *J Analyst, 105*(1247), 125-130.

Antoine, J. M. R., Fung, L. A. H., & Grant, C. N. (2017). Assessment of the potential health risks associated with the aluminium, arsenic, cadmium and lead content in selected fruits and vegetables grown in Jamaica. *Toxicol Rep, 4*, 181-187. doi:10.1016/j.toxrep.2017.03.006

Atindana, S. A., Fagbola, O., Ajani, E., Alhassan, E. H., & Ampofo-Yeboah, A. (2020). Coping with climate variability and non-climate stressors in the West African Oyster (Crassostrea tulipa) fishery in coastal Ghana. *Maritime Studies, 19*(1), 81-92. doi:10.1007/s40152-019-00132-7

B. Asare, B., Obodai, E. A., & Acheampong, E. (2019). Mangrove oyster farming: Prospects as supplementary livelihood for a Ghanaian fishing community. *J. Fish Coast. Mgt, 1*, 7-14.

Baggett, L. (2014). *Oyster habitat restoration monitoring and assessment handbook [Internet]*: Nature Conservancy [cited 2021 July 21] Available from: http://chnep.wateratlas.usf.edu/upload/documents/Oyster-Habitat-Restoration-Monitoring-And-Assessment-Handbook.pdf.

Bennett, E. (2005). Gender, fisheries and development. *Marine Policy, 29*(5), 451-459.

Bjorklund, G., Tippairote, T., Rahaman, M. S., & Aaseth, J. (2020). Developmental toxicity of arsenic: a drift from the classical dose-response relationship. *Arch Toxicol, 94*(1), 67-75. doi:10.1007/s00204-019-02628-x

Bristy, M. S., Sarker, K. K., Baki, M. A., Quraishi, S. B., Hossain, M. M., Islam, A., & Khan, M. F. (2021). Health risk estimation of metals bioaccumulated in commercial fish from coastal areas and rivers in Bangladesh. *Environ Toxicol Pharmacol, 86*, 103666. doi:10.1016/j.etap.2021.103666

Bukari, C., Aning-Agyei, M. A., Kyeremeh, C., Essilfie, G., Amuquandoh, K. F., Owusu, A. A., . . . Bukari, K. I. (2022). Effect of COVID-19 on Household Food Insecurity and Poverty: Evidence from Ghana. *Soc Indic Res, 159*(3), 991-1015. doi:10.1007/s11205-021-02766-9

Chew, K. W., Chia, S. R., Show, P. L., Ling, T. C., Arya, S. S., & Chang, J.-S. (2018). Food waste compost as an organic nutrient source for the cultivation of Chlorella vulgaris. *J Bioresource technology, 267*, 356-362.

Chien, L. C., Hung, T. C., Choang, K. Y., Yeh, C. Y., Meng, P. J., Shieh, M. J., & Ha, B. C. (2002). Daily intake of TBT, Cu, Zn, Cd and As for fishermen in Taiwan. *Sci Total Environ, 285*(1-3), 177-185. doi:10.1016/s0048-9697(01)00916-0

Cho, Y.-H., & Nielsen, S. S. (2017). Phosphorus determination by Murphy-Riley method. In *Food Analysis Laboratory Manual* (pp. 153-156): Springer.

Chuku, E. O., Duguma, L., Abrokwah, S., Bah, A., Adotey, J., Effah, E., . . . W., A. D. (2020). Selection of Locations for Site Based Research [Internet]. USAID Women Shellfishers and Food Security Project. Kingston, RI, USA: University of Rhode Island Coastal Resources Center at the Graduate School of Oceanography and Department of Nutrition and Food Science; University of Ghana; University of Cape Coast; World Agroforestry; and TRY Oyster Women's Association. WSFS2020_04_CRC. 47 pp [cited 2022 March 10]. Available from: https://www.crc.uri.edu/download/WSFS2020_04_CRC_FIN508.pdf.

Chuku, E. O., Okyere, I., Adotey, J., Abrokwah, S., Effah, E., Adade, R., & Aheto, D. W. (2022). Site-Based Assessment of Oyster Shellfisheries and Associated Bio-Physical Conditions in Ghana and The Gambia [Internet]. Centre for Coastal Management (Africa Centre of Excellence in Coastal Resilience), University of Cape Coast, Ghana and Coastal Resources Center, Graduate School of Oceanography, University of Rhode Island. Narragansett, RI, USA. 105 pp [cited 2023 Jun 6]. Available from: https://www.crc.uri.edu/download/WSFS2022_05_CRC_FIN508.pdf.

Coates, J., Swindale, A., & Bilinsky, P. (2007). Household Food Insecurity Access Scale (HFIAS) for measurement of food access: Indicator Guide (v. 3) [Internet] . FHI 360/FANTA. Washington, D.C. [cited 2021 Dec 16]. Available from: https://www.fantaproject.org/sites/default/files/resources/HFIAS_ENG_v3_Aug07.pdf. In.

Domellöf, M. (2021). Microminerals: Iron, Zinc, Copper, Selenium, Manganese, Iodine, Chromium and Molybdenum. *Nutritional Care of Preterm Infants, 122*, 140-148.

Edgell, K. (1989). USEPA Method Study 35, SW Method 3005, Acid Digestion of Waters for Total Recoverable or Dissolved Metals for Analyses by Flame Atomic Absorption Spectroscopy. *Available from the National Technical Information Service, Springfield, VA. 22161, as PB 89-190573. Price codes: A 13 in paper copy, A 01 in microfiche. Report*.

EU/FAO. (2006). Commission Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs [Internet]. Official Journal of the European Union L 364, 20 December 2006, pp. 5-24 [cited 2022 Mar21]. Available from: http://extwprlegs1.fao.org/docs/pdf/eur68134.pdf. In.

FAO. (2021). Minimum dietary diversity for women: An updated guide for measurement, from collection to action [Internet]. Food and Agriculture Oraganization [cited 2022 Feb 19]. Available from: https://doi.org/10.4060/cb3434en. In.

FAO. (June, 2012). West African Food Composition Table. *Available at: http.fao.org/docrep/015/i2698b/i269800.pdf*.

FDA. (2018). Lead in Food, Foodwares, and Dietary Supplements [Intenet]. US Food and Drugs Administration, Silver Spring, MD, USA (Accessed 2023 Apr 07]. Available from: https://www.fda.gov/food/metals/lead-food-foodwares-and-dietary-supplements. In.

FSANZ. (2020). Maximum levels for arsenic in food [Internet]. Food Standards Australia and New Zealand, Wellington 6140, New Zealand [cited 2022 Dec 28]. Available from: https://www.foodstandards.gov.au/consumer/chemicals/arsenic/Pages/default.aspx. In.

Goyer, R. A. (1997). Toxic and essential metal interactions. *Annu Rev Nutr, 17*, 37-50. doi:10.1146/annurev.nutr.17.1.37

Greenfield, H., & Southgate, D. A. (2003). *Food composition data: production, management, and use*: Food & Agriculture Org.

GSS/GHS/ICF. (2015). Ghana Demographic and Health Survey 2014 [Internet]. Ghana Statistical Service, Ghana Health Service, and ICF International. Rockville, Maryland, USA [cited 2019 Oct 17]. Available from: https://dhsprogram.com/pubs/pdf/FR307/FR307.pdf.

Gyamfi, O., Sørensen, P. B., Darko, G., Ansah, E., Vorkamp, K., & Bak, J. L. (2021). Contamination, exposure and risk assessment of mercury in the soils of an artisanal gold mining community in Ghana. *Chemosphere, 267*, 128910.

Holloman, E. L., & Newman, M. C. (2012). Expanding perceptions of subsistence fish consumption: evidence of high commercial fish consumption and dietary mercury exposure in an urban coastal community. *Sci Total Environ, 416*, 111-120. doi:10.1016/j.scitotenv.2011.10.003

Iannotti, L. L., Blackmore, I., Cohn, R., Chen, F., Gyimah, E. A., Chapnick, M., & Humphries, A. (2022). Aquatic Animal Foods for Nutrition Security and Child Health. *Food Nutr Bull, 43*(2), 127-147. doi:10.1177/03795721211061924

IOM. (2011). Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc

[Internet]. Institute of Medicine (US) Panel on Micronutrients, Washington (DC) [cited 2022 Mar 01]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK222310/. In.
IPA. (2017). The Poverty Probability Index [Internet]. Innovations for Poverty Action (IPA), Washington, DC, USA [cited 2022 June 7]. Available from: https://www.povertyindex.org/. In.
Jamil, K. M., Rahman, A. S., Bardhan, P., Khan, A. I., Chowdhury, F., Sarker, S. A., . . . Ahmed, T. (2008). Micronutrients and anaemia. *Journal of health, population, and nutrition, 26*(3), 340.
Jastrzębska, A. (2009). Modifications of spectrophotometric methods for total phosphorus determination in meat samples. *J Chemical Papers, 63*(1), 47-54.
Joseph, A., Iwok, E., & Ekanem, S. (2021). Public health threats of heavy metals due to the consumption of Achachatina marginata (African Giant Land Snail) from a partially remediated site in Ikot Ada Udo, Akwa Ibom State, South-South Nigeria. *Environ Pollut, 271*, 116392. doi:10.1016/j.envpol.2020.116392
Lartey, A., Marquis, G. S., Mazur, R., Perez-Escamilla, R., Brakohiapa, L., Ampofo, W., . . . Adu-Afarwuah, S. (2014). Maternal HIV is associated with reduced growth in the first year of life among infants in the Eastern region of Ghana: the Research to Improve Infant Nutrition and Growth (RIING) Project. *Maternal & Child Nutrition, 10*(4), 604-616. doi:10.1111/j.1740-8709.2012.00441.x
Li, G., Taljaard, M., Van den Heuvel, E. R., Levine, M. A., Cook, D. J., Wells, G. A., . . . Thabane, L. (2017). An introduction to multiplicity issues in clinical trials: the what, why, when and how. *Int J Epidemiol, 46*(2), 746-755. doi:10.1093/ije/dyw320
Lim, W. Y., Aris, A. Z., & Zakaria, M. P. (2012). Spatial variability of metals in surface water and sediment in the langat river and geochemical factors that influence their water-sediment interactions. *ScientificWorldJournal, 2012*, 652150. doi:10.1100/2012/652150
Lorenzana, R. M., Yeow, A. Y., Colman, J. T., Chappell, L. L., & Choudhury, H. (2009). Arsenic in seafood: speciation issues for human health risk assessment. *Human and Ecological Risk Assessment, 15*(1), 185-200. doi:10.1080/10807030802615949
Densu Delta Community-Based Fisheries Management Plan, Greater Accra Region, Ghana [Internet]. Ministry of Fisheries and Aquaculture Development, and Fisheries Commission. 59 pp. Accra, Ghana [cited 2022 May 01]. Available from: https://www.crc.uri.edu/download/GH2014_ACT139_MOFAD_FC_FIN508.pdf (2020).
Montojo, U. M., Baldoza, B. J. S., Cambia, F. D., Benitez, K. C. D., Perelonia, K. B. S., & Rivera, A. T. F. (2021). Levels and health risk assessment of mercury, cadmium, and lead in green mussel (Perna viridis) and oyster (Crassostrea iredalei) harvested around Manila Bay, Philippines. *Food Control, 124*, 107890.
Moslen, M., & Miebaka, C. A. (2017). Heavy Metal Contamination in Fish (Callinectis amnicola) From an Estuarine Creek in the Niger Delta, Nigeria and Health Risk Evaluation. *Bull Environ Contam Toxicol, 99*(4), 506-510. doi:10.1007/s00128-017-2169-4
Mwangi, M. N., Phiri, K. S., Abkari, A., Gbané, M., Bourdet-Sicard, R., Braesco, V. A., . . . Prentice, A. M. (2017). Iron for Africa - Report of an expert workshop. *Nutrients, 9*, 576.
Nielsen, S. S. (2017). *Food analysis laboratory manual*: Springer.
Ogden, L. E. (2017). Fisherwomen—The Uncounted Dimension in Fisheries Management: Shedding light on the invisible gender. *BioScience, 67*(2), 111-117. doi:10.1093/biosci/biw165
Olafsdottir, A. S., Thorsdottir, I., Gunnarsdottir, I., Thorgeirsdottir, H., & Steingrimsdottir, L. (2006). Comparison of women's diet assessed by FFQs and 24-hour recalls with and without underreporters: associations with biomarkers. *Ann Nutr Metab, 50*(5), 450-460. doi:10.1159/000094781
Osei, I. K., Chuku, E. O., Effah, E., Kent, K., & Crawford, B. (2021). Participatory Assessment of Shellfisheries in the Estuarine and Mangrove Ecosystems of Ghana [Internet]. Centre for Coastal Management (Africa Centre of Excellence in Coastal Resilience). University of Cape

Coast, Ghana and Coastal Resources Center, Graduate School of Oceanography, University of Rhode Island. Narragansett, RI, USA [cited 2023 Jan 02]. Available from: https://www.crc.uri.edu/download/WSFS2021-Ghana-Report-FIN508.pdf. In.

Otchere, F. A. (2019). A 50-year review on heavy metal pollution in the environment: Bivalves as bio-monitors. *African Journal of Environmental Science and Technology, 13*(6), 220-227.

Reilly, C. (2002). *Metal contamination of food: its significance for food quality and human health. Third ed. Blackwell Science, Inc, MA, USA*.

Romero-Estévez, D., Yanez-Jacome, G. S., Dazzini Langdon, M., Simbana-Farinango, K., Rebolledo Monsalve, E., Duran Cobo, G., & Navarrete, H. (2020). An overview of cadmium, chromium, and lead content in bivalves consumed by the community of Santa Rosa Island (Ecuador) and its health risk assessment. *Frontiers in Environmental Science, 8*, 134.

Stoltzfus, R. J. (2003). Iron deficiency: global prevalence and consequences. *Food and nutrition bulletin, 24*(4_suppl_1), S99-S103.

Taylor, S. F., Roberts, M. J., Milligan, B., & Ncwadi, R. (2019). Measurement and implications of marine food security in the Western Indian Ocean: an impending crisis? *Food Security, 11*(6), 1395-1415.

UG/GroundWork/UWisconsin-Madison/KEMRI/UNICEF. (2017). University of Ghana, GroundWork, University of Wisconsin-Madison, KEMRI-Wellcome Trust, UNICEF [Internet]. Ghana Micronutrient Survey. Accra, Ghana [cited 2022 May 25]. Available from: https://groundworkhealth.org/wp-content/uploads/2018/06/UoG-GroundWork_2017-GHANA-MICRONUTRIENT-SURVEY_Final_180607.pdf. In.

UNICEF-World Bank. (2022). COVID-19 and child poverty: Two out of three households lost income during the pandemic: new global report [Internet]. New York, USA [cited 2022 May 26]. Available from: https://www.unicef.org/ghana/press-releases/covid-19-and-child-poverty-two-out-three-households-lost-income-during-pandemic-new. In.

USDA. (2020). FoodData Central [Internet]. US Department of Agriculture, Washington DC [cited 2022-Mar-01]. Available from: https://fdc.nal.usda.gov/index.html. In.

USDA. (February, 2011). *National Nutrient Database for Standard Reference* National Agriculture Office.: Release 24. Available at: http//ndb.nal.usda.gov

USEPA. (2018). Integrated Risk Information System (IRIS)- Final IRIS assessments [Internet]. Environmental Protection Agency, Washington, D.C. [cited 2022 Mar 20]. Available from: https://iris.epa.gov/AdvancedSearch/. In.

Vieira, K. S., Delgado, J. F., Lima, L. S., Souza, P. F., Crapez, M. A. C., Correa, T. R., . . . Fonseca, E. M. (2021). Human health risk assessment associated with the consumption of mussels (Perna perna) and oysters (Crassostrea rhizophorae) contaminated with metals and arsenic in the estuarine channel of Vitoria Bay (ES), Southeast Brazil. *Mar Pollut Bull, 172*, 112877. doi:10.1016/j.marpolbul.2021.112877

Wang, W. X., & Lu, G. (2017). Heavy metals in bivalve mollusks. In: Schrenk, D. and Cartus A (Eds.), Chemical Contaminants and Residues in Food. 2nd Edition. Woodhead Publisher, Kidlington, United Kingdom. PP 553. ISBN: 978-0-08-100674-0. In.

WHO. (2014). *Global nutrition targets 2025: Policy brief series (WHO/NMH/NHD/14.2). World Health Organization, Geneva, Switzerland.* Geneva: WHO.

Wong, C., Roberts, S. M., & Saab, I. N. (2022). Review of regulatory reference values and background levels for heavy metals in the human diet. *Regul Toxicol Pharmacol, 130*, 105122. doi:10.1016/j.yrtph.2022.105122

World Bank Group. (2020). Ghana: Country Environmental Analysis [Internet]. The World Bank, Washington DC. [cited 2022 Aug 02]. Available from: https://openknowledge.worldbank.org/bitstream/handle/10986/33726/Ghana-Country-Environmental-Analysis.pdf?sequence=1#page=51. In.

World Economic Forum. (2021). Women are a mainstay of fishing in West Africa. But they get a raw deal [Internet]. Geneva Switzerland [cited 2022 May 26]. Available from: https://www.weforum.org/agenda/2021/05/why-women-in-the-west-african-fishing-industry-need-more-financial-safety-nets. In.

World Health Organization (WHO). (2020). Prevalence of anemia in women of reproductive age (%). Retrieved from https://www.who.int/data/gho/data/indicators/indicator-details/GHO/prevalence-of-anaemia-in-women-of-reproductive-age-(-)

Yap, C. K., Wong, K. W., Al-Shami, S. A., Nulit, R., Cheng, W. H., Aris, A. Z., . . . Al-Mutairi, K. A. (2020). Human Health Risk Assessments of Trace Metals on the Clam Corbicula javanica in a Tropical River in Peninsular Malaysia. *Int J Environ Res Public Health, 18*(1), 195-217. doi:10.3390/ijerph18010195

Young, M. F., Oaks, B. M., Tandon, S., Martorell, R., Dewey, K. G., & Wendt, A. S. (2019). Maternal hemoglobin concentrations across pregnancy and maternal and child health: a systematic review and meta-analysis. *Annals of the New York Academy of Sciences, 1450*(1), 47-68.

Zhou, L., Li, X., Li, S., Wen, X., Peng, Y., & Zhao, L. (2021). Relationship between dietary choline intake and diabetes mellitus in the National Health and Nutrition Examination Survey 2007-2010. *J Diabetes, 13*(7), 554-561. doi:10.1111/1753-0407.13143.

Appendices