



HAL
open science

Seasonal Survey of Contaminants (Cd and Hg) and Micronutrients (Cu and Zn) in Edible Tissues of Cephalopods from Tunisia: Assessment of Risk and Nutritional Benefits

Moncef Rjeibi, Marc Metian, Tarek Hajji, Thierry Guyot, Ben Chaouacha-Chekir Rafika, Paco Bustamante

► To cite this version:

Moncef Rjeibi, Marc Metian, Tarek Hajji, Thierry Guyot, Ben Chaouacha-Chekir Rafika, et al.. Seasonal Survey of Contaminants (Cd and Hg) and Micronutrients (Cu and Zn) in Edible Tissues of Cephalopods from Tunisia: Assessment of Risk and Nutritional Benefits. *Journal of Food Science Education*, 2015, 80 (1), pp.199-206. 10.1111/1750-3841.12711 . hal-01119161

HAL Id: hal-01119161

<https://hal.science/hal-01119161>

Submitted on 21 Feb 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Seasonal survey of contaminants (Cd and Hg) and micronutrients (Cu and Zn) in edible tissues of cephalopods from Tunisia: assessment of risk and nutritional benefits

Moncef Rjeibi^{a,c}, Marc Metian^b, Tarek Hajji^c, Thierry Guyot^b, Rafika Ben Chaouacha-Chekir^c, Paco Bustamante^{b*}

^a *Faculty of Sciences of Tunis, University Campus, 2092 Tunis, Tunisia.*

^b *Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS-Université La Rochelle, 2 rue Olympe de Gouges, F-17042 La Rochelle Cedex 01, France.*

^c *Unité de recherche d'Ecophysiologie et de Procédés Agroalimentaires UR11-ES44, Institut Supérieur de Biotechnologie de Sidi Thabet (ISBST), Université La Manouba, BiotechPole Sidi Thabet, Sidi Thabet CP 2020, Tunisia.*

* Corresponding author: Prof. Paco Bustamante
Littoral Environnement et Sociétés
UMR 7266
Université de La Rochelle
2, rue Olympe de Gouges
F-17042 La Rochelle (France)
Tel.: (+33) 5 46 50 76 25
Fax: (+33) 5 46 45 62 84
E-mail: pbustama@univ-lr.fr

Abstract: Concentrations of cadmium (Cd), copper (Cu), mercury (Hg) and zinc (Zn) were determined by atomic absorption spectrophotometry in the muscle tissues (arms and mantle) of three commercial cephalopods (*Loligo vulgaris*, *Octopus vulgaris* and *Sepia officinalis*) caught in three different Tunisian coastal regions. The highest concentrations found correspond to the essential elements Cu and Zn. Octopuses and cuttlefish showed the highest levels of those elements while squid presented with significantly higher values of Hg in both muscular tissues. This may be related to different feeding behavior and detoxification processes among benthic and pelagic cephalopods. Variation of element concentrations between seasons was different between species and seemed to be mostly dependent on the sampling site. From a public health standpoint, average concentrations of Cd, Cu, Hg and Zn measured in edible tissues of cephalopods from this study did not reveal, in general, any risk for consumers. The estimated Target Hazard Quotients (THQs) for Cd and Hg for consumers of the selected species were below 1 and within the safety range for human health. Moreover, their consumption could provide in an important contribution to the daily dietary intake of Cu for the Tunisian population, especially regarding the consumption of octopus and cuttlefish muscles.

Keywords: Cephalopods; Tunisian coast; metals; PTWI; target hazard quotient; DRI.

Running head: Cephalopods are recommended for human consumption

Introduction

Marine resources constitute a mandatory part of the diet of many populations around the world. Seafood consumption is recommended for its nutritional qualities and benefits. The benefits of seafood derive mainly from its content of high quality proteins and its high content of omega3 polyunsaturated fatty acids - i.e. eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA] - and other nutrients (Castro-González 2002; Domingo and others 2007). Omega3 fatty acids have demonstrated protective effects in preventing coronary heart disease, reducing arrhythmias and thrombosis (Oomen and others 2000; Kris-Etherton and others 2002) lowering plasma triglyceride levels (Harris 1997; Ismail 2005) and reducing blood clotting tendency (Din and others 2004; Ismail 2005). Among the commercial marine resources, cephalopods represent one of the most important groups of species in the world to fisheries, comprising about 4% of total catches according to the FAO (2013). During the last decades, cephalopods have been recognized as an appropriate alternative to traditional marine resources and their consumption is likely to continue to increase in the future (Caddy and Rodhouse 1998; Piatkowski and others 2001). They are generally very rich in n-3 fatty acids (Ozogul and others 2008), as well as being excellent sources of proteins and of a large number of essential elements (Oehlenschläger 1997; Storelli 2009). In parallel to the nutritional benefits of consuming seafood, there is a non-negligible risk of contamination given the bioaccumulation/biomagnification capacities occurring in marine organisms. This is true certainly of cephalopods; they can be considered as “potentially” hazardous for consumers, given their ability to accumulate high levels of Cd, even in environments of low metal contamination (Bustamante and others 1998a; Dorneles and others 2007; Kojadinovic and others 2011).

In order to settle this specific controversy, several studies have been published examining various aspects of the risks and benefits of cephalopod consumption (Storelli and

Marcotrigiano 1999; Lourenço and others 2009; Storelli 2009; Storelli and others 2010). However, there is scarce or no data available on the nutritional and health risks associated with the consumption of cephalopods from Tunisian waters. Health organizations worldwide have compiled the following procedures. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has released guideline values and advisories to help consumers to manage risks and maximize benefits. JECFA has also established a Provisional Tolerable Weekly Intake (PTWI) of 7 and 4 $\mu\text{g kg}^{-1}$ body weight per week for Cd and Hg, respectively (JECFA 2006, 2010) and the Institute of Medicine has set the Dietary Reference Intakes (IOM 2001) and recommendations for the intake of micronutrients to prevent nutritional inadequacy, which could lead to nutrient deficiencies and consequent impairment of health.

In this context, the present study was undertaken to: (1) quantify the levels of non essential (Cd and Hg) and essential (Cu and Zn) metals in the edible parts (arms and mantle) of the European squid *Loligo vulgaris*, the common octopus *Octopus vulgaris*, and the common cuttlefish *Sepia officinalis* caught from three different Tunisian coastal regions during different seasons; (2) to estimate the sanitary and nutritional qualities of these fishing products. The selected species are commercially relevant because of their abundance and high consumption in Tunisia which. The country is ranked among the top countries with high per capita supply of cephalopods ($0.3 \text{ kg capita}^{-1} \text{ year}^{-1}$ in 2009; FAOSTAT 2013).

Materials and methods

Sample collection and preparation

Common octopus (*Octopus vulgaris*), European squid (*Loligo vulgaris*) and common cuttlefish (*Sepia officinalis*), were collected seasonally (spring, summer, autumn and winter) over a one-year period (December 2009-November 2010) from three major commercial harbours (Bizerte, Monastir and Sfax) spread evenly across the Tunisian coast (north, east and

southeast, respectively; see Figure 1). The collected samples were immediately stored in hermetically-closed plastic bags, placed on ice, stored in thermo-insulated containers and transported to the laboratory at the University of Tunis. On arrival at the laboratory, dorsal mantle length and total weight of samples were determined (Table 1). Samples of arm muscle (A) and mantle muscle (M) were collected and vacuum-sealed in individual plastic bags, coded for easy identification, stored at -25°C and kept for elemental analysis.

Sample analysis

Separated tissue samples were either dried to a constant weight for several days at 60°C or freeze-dried and then homogenized in a mortar and porcelain pestle. An aliquot of ~ 300 mg of each dried material was digested with 5 mL of 65% ultrapure HNO₃ (Merck ®) at 80°C on a hotplate until the solution was clear. Next, the acid was evaporated and the residue was dissolved in 0.3 M ultrapure nitric acid. Blanks were carried through the procedure in the same way as the samples. Cu and Zn were determined by flame atomic absorption spectrophotometry (AAS) while Cd was analyzed by graphite furnace AAS using a Hitachi spectrophotometer Z5000 with Zeeman correction.

The total Hg concentrations were determined by analyzing Hg directly in the powder obtained from the tissues with an Advanced Mercury Analyzer (ALTEC AMA 254) on aliquots ranging from approx. 10 mg of dry sample weighed to the nearest 0.01 mg (Bustamante and others 2006a). Hg determination involved evaporation of the metal by progressive heating until 800°C was reached, the sample was then held under oxygen atmosphere for 3 min., and subsequently amalgamated in a gold-net. Afterwards, the net was heated to liberate the collected Hg, which was then measured by atomic absorption spectrophotometry.

Accuracy and reproducibility of the preparation were tested by preparing analytical blanks and replicates of Lobster Hepatopancreas (TORT-2), Dogfish liver (DOLT-3) and

Dogfish Muscle (DORM-2) reference standards (National Research Council, Canada) along with each set of samples. Recovery rates were respectively equal to $92 \pm 7\%$ for Cd, $94 \pm 8\%$ for Cu, $100 \pm 3\%$ for Hg and $101 \pm 12\%$ for Zn. Detection limits (DL, $\mu\text{g g}^{-1}$, dry weight (dw)) were 0.034 (Cd), 0.5 (Cu), 0.006 (Hg) and 3 (Zn).

All data were computed on a $\mu\text{g g}^{-1}$ wet weight (ww) basis. Concentrations of elements in $\mu\text{g g}^{-1}$ ww were obtained from data in dw using the dry/wet weight ratios measured in all samples. These ratios indicated a mean moisture content of 78% in cuttlefish and squid and 83% in octopuses, which matched well with those previously reported for the same species (e.g. Ozogul and others 2008; Lourenço and others 2009).

Statistical analyses

Levels of elements in the edible parts of the studied species were represented by median rather than average values because of their non-normal distribution. Differences in median levels between species, as well as between seasons, were performed by Kruskal-Wallis (KW) tests followed by Wilcoxon tests for independent samples using Bonferroni's p -value correction. Levels of significance of the null hypotheses associated with these tests will be divided into classes of p -values represented by the following codes: NS ≥ 0.05 ; * < 0.05 ; ** < 0.01 ; *** < 0.001 . All statistical analyses were performed using the GNU R statistical system (The R Development Core Team 2008).

Assessment of risk and nutritional benefits for Human consumers

The risk to health was assessed by the calculation of the Estimated Weekly Intake (EWI) and the Target Hazard Quotient (THQ) for muscle tissues considering the average Cd and Hg concentrations in both arms and mantle of selected cephalopod species caught along the Tunisian coast. In this calculation, metal sources supplied by other meals or by drinking water on the same day or week were not taken into account; i.e., only metal intake coming from the seafood has been considered.

The Estimated Weekly Intakes (EWI) of non-essential elements (Cd and Hg) were calculated by multiplying the respective concentrations in each cephalopod species by the average weight of that species consumed (33 g/person/day; FAO 2009), and reporting to the average body weight for Tunisian people (60 kg). The obtained values were compared to the Provisional Tolerable Weekly Intake (PTWI) defined by the JECFA (Joint FAO/WHO Expert Committee on Food Additives) for these elements (i.e., 7 $\mu\text{g Cd kg}^{-1}$ body weight (JECFA 2006) and 4 $\mu\text{g Hg kg}^{-1}$ body weight (JECFA 2010)).

Moreover, the non-carcinogenic effects through cephalopod consumption by the local inhabitants were assessed based on the target hazard quotient (THQ) (USEPA 1989). The THQ is an estimate of the risk level (non-carcinogenic) due to pollutant exposure. Thus, a THQ below 1 means that the exposed human population is unlikely to experience obvious adverse effects. The methodology for estimating THQ was available in the US EPA region III Risk-based Concentration Table (USEPA 2013) and is described by the following equation:

$$\text{THQ} = \left(\frac{\text{EF} \times \text{ED} \times \text{FIR} \times \text{C}}{\text{RFD} \times \text{WAB} \times \text{TA}} \right) \times 10^{-3}$$

where EF is the exposure frequency (365 days/year); ED is the exposure duration (70 years), equivalent to the average lifetime; FIR is the food ingestion rate (33g/person/day; FAO 2009); C is the metal concentration in cephalopods ($\mu\text{g g}^{-1}$ ww; RFD is the US EPA's reference dose (Cd= $1 \times 10^{-3} \mu\text{g g}^{-1} \text{d}^{-1}$, Hg= $5 \times 10^{-4} \mu\text{g g}^{-1} \text{d}^{-1}$) (USEPA 2013); WAB is the average body weight (60 kg), and TA is the average exposure time for non-carcinogens (365 days/year x ED).

In the case of essential trace metals (Cu and Zn), a nutritional evaluation was carried out by comparing the estimated dietary intakes from the consumption of edible tissues of the studied cephalopod species with the Dietary Reference Intake values, i.e., the Recommended

Dietary Allowance (RDA) recommended by the Institute of Medicine (IOM 2001) for these elements.

Results and discussion

Metal levels in edible tissues

Considering the results of all combined sites and for all four seasons, the median concentrations of Cd, Cu, Hg and Zn in the edible tissues (arms and mantle) of European squid, common octopuses and common cuttlefish, expressed in wet weight basis, are presented in Table 2. The following sequence of metal concentration was observed in all muscular samples for all studied species: $Zn > Cu > Hg \approx Cd$. Metals such as Cu and Zn are essential for cephalopod metabolism since they play an important role in biological functions, while non-essential metals such as Cd and Hg have no known biological functions and can be toxic, especially in the most sensitive stages (Bryan 1976). In the present study, Zn levels vary over an order of magnitude ranging from 2.6 to 45.3 and 4.0 to 33.6 $\mu\text{g g}^{-1}$ ww in the arms and the mantle, respectively. The highest median concentration (KW, $p < 0.001$) was observed in octopuses (arms: 14.2 $\mu\text{g g}^{-1}$; mantle: 11.7 $\mu\text{g g}^{-1}$) and cuttlefish (arms: 15.0 $\mu\text{g g}^{-1}$; mantle: 11.1 $\mu\text{g g}^{-1}$) followed by squid (arms: 12.7 $\mu\text{g g}^{-1}$; mantle: 9.8 $\mu\text{g g}^{-1}$) (Table 2). These results fall within the same range of data obtained for the same species from the European coasts (Miramand and Bentley 1992; Raimundo and others 2004; Napoleão and others 2005; Seixas and others 2005; Lourenço and others 2009). For Cu, the levels found ranged from 0.50 to 10.30 $\mu\text{g g}^{-1}$ in the arms and from 0.59 to 21.13 $\mu\text{g g}^{-1}$ in the mantle in cephalopods from Tunisia. The lowest concentrations were detected in squid for both muscle tissues with median concentrations of 1.94 $\mu\text{g g}^{-1}$ and 1.22 $\mu\text{g g}^{-1}$ in the arms and mantle, respectively. Cuttlefish revealed significantly higher concentrations than octopuses in the arms but were lower in the mantle (Table 2). This situation is consistent with the results of Lourenço and others (2009) on cephalopods from Portugal (i.e., 2.6-8.1 $\mu\text{g g}^{-1}$ ww and 1.7-

10.3 $\mu\text{g g}^{-1}$ ww for octopus and cuttlefish, respectively). Furthermore, the concentrations of Cu in cuttlefish and octopus tissues are in agreement with results for mantle muscle of *Sepia officinalis* from Turkish waters (i.e., 7.31-8.02 $\mu\text{g g}^{-1}$ ww; Ayas and Ozogul 2011) and for *Octopus vulgaris* from Portuguese coasts (i.e., 7-16 $\mu\text{g g}^{-1}$ dw Carvalho and others 2005; 5.5–14 $\mu\text{g g}^{-1}$ dw Raimundo and others 2005). The high levels of such essential metals found in benthic species (octopus and cuttlefish) compared to those found in the pelagic species (squid) confirms the results found for the same species by Lourenço and others (2009).

Cephalopods are carnivorous, active predators and have very high feeding rates (Rodhouse and Nigmatullin 1996). Thus, their diet represents a major pathway of exposure for many elements including Cu and Zn (Bustamante and others 2002, 2004, 2006b). This should explain the high values of the essential elements reported in this study. The octopus, with the highest concentration of these elements in its tissues, consumes a wider range of prey than the two other species studied: in addition to polychaetes and mollusks, octopuses mainly consume crustaceans (Smith 2003) such as crabs that contain high levels of Cu and Zn, which may lead subsequently to a higher intake of these elements (Reijbi and others 2014). However, an uptake of waterborne elements also occurs from exposure to seawater (Bustamante and others 2002). To the best of our knowledge, no data have been published on the respective proportions in cephalopods of these elements incorporated from food and seawater. Among the three cephalopod species, squid show the lowest concentrations of essential elements. According to some authors (Pierce and others 1994; Coelho and others 1997), fish represents the main component of the squid diet, comprising about 90% of the prey found in their stomachs. This may explain the lower values of essential elements in this species, since fish contain fewer minerals than crustaceans, the preferred prey of octopuses.

Non essential Cd and Hg do not have a known biological function in cephalopods' metabolism and are therefore not regulated by homeostatic processes. The amount of Cd and

Hg in cephalopod organisms would therefore reflect the environmental levels of these metals in the ocean. The Cd content for all studied species was in the range of 0.001-0.20 $\mu\text{g g}^{-1}$ and 0.006-0.27 $\mu\text{g g}^{-1}$ in the arms and mantle, respectively. Regardless of the geographical origin, squid revealed significantly higher values in the arms with a median concentration of 0.041 $\mu\text{g g}^{-1}$, followed by octopuses and cuttlefish (median concentration: 0.026 $\mu\text{g g}^{-1}$). Although there were no significant differences between species detected in the mantle, squid showed the highest levels (0.045 $\mu\text{g g}^{-1}$) (Table 2). In cephalopods, Cd preferentially accumulates in internal organs like the digestive gland, but is poorly concentrated in the muscle tissues, where the concentrations are usually very low (Bustamante and others 1998b; Raimundo and others 2004, 2005; Miramand and others 2006). Whereas the biological half-life of Cd is relatively long in whole cephalopods (i.e., > than 257d; Bustamante and others 2002), this parameter remains to be quantified for muscle tissues specifically. The median Cd content for the octopuses and cuttlefish in this study was lower than that found in studies conducted in other locations, like the Portuguese waters (0.33 and 0.23 $\mu\text{g g}^{-1}$; Lourenço and others 2009) and the Adriatic sea (0.87 and 0.27 $\mu\text{g g}^{-1}$; Storelli and others 2006), but concerning squid, it was in the same range of that from Spain and the British Isles (Villanueva and Bustamante 2006; Pierce and others 2008).

Concerning Hg, levels varied from 0.010 to 0.68 $\mu\text{g g}^{-1}$ in the arms and from 0.010 to 0.95 $\mu\text{g g}^{-1}$ in the mantle. The same trend was detected for both muscle tissues in the three analyzed species: significantly higher concentrations (KW, $p < 0.001$) were found in the arms and mantle of squid (0.062 and 0.072 $\mu\text{g g}^{-1}$), followed by cuttlefish (0.049 and 0.057 $\mu\text{g g}^{-1}$) and octopuses (0.027 and 0.031 $\mu\text{g g}^{-1}$) (Table 2). This different Hg distribution among the studied species is in accordance with the process of bioaccumulation of this metal in cephalopods, which is dominated by the diet (Lacoue-Labarthe and others 2009). The highest concentration of Hg found in squid reflects their behavior of feeding mainly on fish and other cephalopod

species (Pierce and others 1994; Coelho and others 1997). Such a pelagic diet would lead to higher Hg exposure and thence to higher Hg bioaccumulation in pelagic cephalopods, since their prey contains higher organic Hg loads than those of benthic species (Cossa and others 1990; Chauvelon and others 2012). The concentration of Hg in the edible parts of the cephalopod species under investigation were generally slightly lower than the levels reported by authors in other areas of the world (Bustamante and others 2006a; Storelli and others 2006; Villanueva and Bustamante 2006; Lourenço and others 2009). In a previous study, we showed that these differences were not due to important differences either in size or in weight distribution, and thus they reflect actual geographic variations (Rjeibi and others 2014). Within the Tunisian zone, the eastern coast may be subjected to potential natural inputs from nearby Hg ferrous belts and previous mining areas (Joiris and others 1999), while the southern coast is especially contaminated by Cd from the phosphate extraction waste (Hamza-Chaffai and others; Smaoui-Damak and others 2003; Banni and others 2005, 2007).

Seasonal variations of heavy metal concentrations in the edible tissues of the three cephalopod species are shown in Table 3. In general, a similar seasonal profile was observed for the different metals in both muscular tissues. In most cases, the variation among seasons was different between species and seemed to be dependent on the sampling site. Considering the essential elements, the maximum concentration of Cu was obtained in spring for squid and cuttlefish from Bizerte and Monastir, and in autumn for octopuses from Monastir and cuttlefish from Sfax. In the case of Zn, higher levels were found in summer for the arms tissue of cuttlefish in all sampling sites, while maximum values were obtained in winter for the mantle. Squid caught from the eastern and southeastern coast and octopuses from the northern coast showed higher Zn levels in autumn (Table 3).

Considering the non-essential metals, Cd levels have been reported higher in winter for cuttlefish samples from Bizerte (KW, $p < 0.01$) and Monastir, and in autumn for octopuses

from Monastir (Table 3). For Hg, higher concentrations were detected in squid and octopus samples from the northern coast and in cuttlefish from the eastern coast in spring, whereas they tended to increase in summer for all selected species from the southern coast (Table 3).

For safety purposes, authorities and environmental agencies around the world have set standards for metal concentrations in food. The Ministry of Agriculture, Fisheries and Food in the United Kingdom (MAFF 1995) limits the levels of Cu and Zn in the muscle at $20 \mu\text{g g}^{-1}$ and $50 \mu\text{g g}^{-1}$ ww respectively, while the Western Australian Food and Drug Regulations List has established a Zn maximum limit in fish of $40 \mu\text{g g}^{-1}$ wwt. In Tunisia, there is no legislation regarding Cu and Zn levels in food or in products of animal or plant origin. Using the above mentioned values as a guideline, average concentrations of Cu and Zn found in this study were below the proposed limits, with the exception of one cuttlefish caught in winter on the northern coast for Cu in the mantle, and two octopuses from the southeastern coast for Zn in the arms.

For non-essential metals, the Tunisian authority (Directorate General for Veterinary Services) (DGSV 2006) as well as the European legislation (Official Journal of the European Communities 2001) stipulates that the maximum concentrations for Cd and Hg in cephalopods should be $1 \mu\text{g g}^{-1}$ and $0.5 \mu\text{g g}^{-1}$ ww, respectively. None of the muscle samples reached this limit for Cd. For Hg, only three squid caught in spring from the northern coast (Bizerte) exceeded the limit proposed by the European Community. Despite this fact, the average levels did not exceed the indicative value.

Assessment of contamination risk and nutritional benefits for human consumers

In the present study, the Cd and Hg weekly intakes for cephalopods from Tunisia, based on average concentrations, were calculated by considering a weekly average consumption of 231

g (7 days X 33 g) for the Tunisian population (FAO 2009). As shown in Table 4, the average estimated weekly intakes (EWIs) represented very low percentages of the reference values (ranging from 1.37 to 15.76% for a person weighing 60 kg). The highest dietary intake of Cd and Hg assessed in the present study was associated with the consumption of octopuses from Sfax (maximum of 26.18 $\mu\text{g week}^{-1}$) and of squid from Bizerte (maximum of 37.81 $\mu\text{g week}^{-1}$), respectively (Table 4), which are still far under the limits (420 $\mu\text{g week}^{-1}$ for Cd and 240 $\mu\text{g week}^{-1}$ for Hg). It is therefore unlikely that Cd and Hg intakes through cephalopod consumption would cause any risk for average consumers. Indeed, it would be necessary to eat more than seven times the daily average consumption in the specific case of the squid and octopus that presented the highest concentration in this study (381 g and 962 g, respectively). Assuming that most of the Hg found in cephalopod muscles is in methyl-Hg form (ranging from 67-83%; Bustamante and others 2006a) and that the standard PTWI value of 1.6 $\mu\text{g kg}^{-1}$ body weight (JECFA 2010), equals 96 $\mu\text{g MeHg}$ per week for a 60 kg person, we have also estimated the related risk. With the worst hypothesis (83% of MeHg), the average EWI accounted for MeHg was in the range of 15.11-32.7%, 6.31-7.43% and 7.94-13.43% of the PTWI due to the consumption of squid, cuttlefish and octopuses respectively. However, when calculating the maximum safe consumption using the highest MeHg concentrations, the PTWI was reached with only 183.5 g of squid from the northern coast (Bizerte). Taking this result into consideration, it seems that the Hg content in squid from Bizerte may present a health hazard if consumed excessively.

According to the report of USEPA (2013), the dose calculations are made using the standard assumptions for an integrated USEPA risk analysis, including the exposure over an entire 70 year lifetime and for a 60 kg body weight for an average Tunisian adult. In addition, it was assumed in accordance with the USEPA (1989) that the ingested dose is equal to the absorbed contaminant dose and that cooking has no effect on the contaminants (Cooper and

others 1991). Table 4 shows the results of estimated target hazard quotients (THQs) for Cd and Hg, caused by consuming muscle tissues of the selected species from different sampling locations. The obtained values were all below the acceptable safe value of 1. Therefore, our results infer that consumption of these cephalopod species at the present rate may not be hazardous to the human population.

In parallel to the negligible risk related to the metal contamination of this seafood, results of essential elements (Cu and Zn) concentrations were analyzed from a nutritional point of view. Given the Dietary Reference Intakes (DRIs) published by the IOM (2001), the nutritional contribution that the edible part of the species offers in terms of essential elements was calculated (in DRI percent; see Table 4). The DRI relates to a set of the reference values (Recommended Dietary Allowance) given in concentration level per day (Table 4). The Estimated Daily Intake of each element was calculated using the average content obtained in each muscle tissues for the selected species and considering a meal of 33g of seafood. Results from Table 4 show that the contribution to daily intake through the consumption of muscle tissues of the selected species was lower than the required DRIs. The contribution of octopuses and cuttlefish from all sampling sites are more important than that of squid, especially for Cu.

Conclusions

In summary, levels of the metals analyzed (Cd, Cu, Hg and Zn) in the edible parts of three cephalopods species (*L. vulgaris*, *O. vulgaris*, *S. officinalis*) from various Tunisian coastal regions were broadly comparable to those reported in similar international studies for the same species. We showed that the average concentrations for these metals were below the limits provided by international authorities and environmental agencies. Additionally, the consumption of cephalopods from Tunisian waters did not exceed the provisional tolerable weekly intakes (PTWI) for the non-essential elements investigated, indicating that average

consumers should not be at risk. In fact, the studied cephalopods specie would provide a reasonable percentage of the Dietary Reference Intakes for the essential elements (Cu and Zn) in the average human diet.

Acknowledgments: MR benefits from a PhD fellowship provided by the Tunisian Ministry of Higher Education and Scientific Research. MM is Nippon Foundation Senior Nereus Fellow at the Stockholm Resilience Centre. We are grateful to C. Churlaud for her help in the Hg analyses. This study has been financially supported by the laboratory LIENSs and the CNRS.

Author Contributions

Conceived and designed the experiments: P. Bustamante, R. Ben Chaouacha-Chekir and T. Hajji

Performed the experiments: M. Rjeibi and T. Guyot

Analyzed the data: M. Rjeibi and M. Metian

Wrote the paper: M. Rjeibi, P. Bustamante, M. Metian

Conflict of interest

Authors declare they have no conflict of interest.

References

- Ayas D, Ozogul Y. 2011. The Effects of Season and Sex in the Metal Levels of Mature Common Cuttlefish (*Sepia officinalis*) in Mersin Bay, Northeastern Mediterranean. *J Food Sci* 76:121-124.
- Banni M, Dondero F, Jebali J, Guerbej H, Boussetta H, Viarengo A. 2007. Assessment of heavy metal contamination using real-time PCR analysis of mussel metallothionein mt10 and mt20 expression: a validation along the Tunisian coast. *Biomarkers* 12:369-83.
- Banni M, Jebali J, Daubeze M, Clerandau C, Guerbej H, Narbonne JF, Boussetta H. 2005. Monitoring pollution in Tunisian coasts: application of a classification scale based on biochemical markers. *Biomarkers* 10:105-16.

- Bryan, GW. 1976. Some aspects of heavy metal tolerance in aquatic organisms. In: A.P.M. Lockwood (ed.) Effects of pollutants on aquatic organisms. Cambridge University Press; p.7-34.
- Bustamante P, Caurant F, Fowler SW, Miramand P. 1998a. Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. *Sci Total Environ* 220:71-80.
- Bustamante P, Cherel Y, Caurant F, Miramand P. 1998b. Cadmium, copper and zinc in octopuses from Kerguelen Islands, Southern Indian Ocean. *Polar Biol* 19:264-271.
- Bustamante P, Teyssie J, Fowler S, Cotret O, Danis B, Miramand P, Warnau M. 2002. Biokinetics of zinc and cadmium accumulation and depuration at different stages in the life cycle of the cuttlefish *Sepia officinalis*. *Mar Ecol Prog Ser* 231:167-177.
- Bustamante P, Teyssie J, Danis B, Fowler S, Miramand P, Cotret O, Warnau M. 2004. Uptake, transfer and distribution of silver and cobalt in tissues of the common cuttlefish *Sepia officinalis* at different stages of its life cycle. *Mar Ecol Prog Ser* 269:185-195.
- Bustamante P, Lahaye V, Durnez C, Churlaud C, Caurant F. 2006a. Total and organic Hg concentrations in cephalopods from the North Eastern Atlantic waters: Influence of geographical origin and feeding ecology. *Sci Total Environ* 368:585-596.
- Bustamante P, Teyssie J, Fowler S, Warnau M. 2006b. Assessment of the exposure pathway in the uptake and distribution of americium and cesium in cuttlefish (*Sepia officinalis*) at different stages of its life cycle. *J Exp Mar Biol Ecol* 331:198-207.
- Caddy JF, Rodhouse PG. 1998. Cephalopod and groundfish landings: Evidence for ecological change in global fisheries? *Rev Fish Biol Fish* 8:431-444.
- Carvalho M, Santiago S, Nunes ML. 2005. Assessment of the essential element and heavy metal content of edible fish muscle. *Anal Bioanal Chem* 382:426-432.
- Castro-González MI. 2002. Ácidos grasos omega 3: Beneficios y Fuentes. *Interciencia* 27:128-136.
- Chouvelon T, Spitz J, Caurant F, Mèndez-Fernandez P, Autier J, Lassus-Débat A, Chappuis A, Bustamante P. 2012. Enhanced bioaccumulation of mercury in deep-sea fauna from the Bay of Biscay (north-east Atlantic) in relation to trophic positions identified by analysis of carbon and nitrogen stable isotopes. *Deep-Sea Res Part I Oceanogr Res Pap* 65:113-124.
- Coelho M, Domingues P, Balguerias E, Fernandez M, Andrade JP. 1997. A comparative study of the diet of *Loligo vulgaris* (Lamarck, 1799) (Mollusca: Cephalopoda) from

- the south coast of Portugal and the Saharan Bank (Central-East Atlantic). *Fish Res* 29:245-255.
- Cooper CB, Doyle ME, Kipp K. 1991. Risks of consumption of contaminated seafood: the Quincy Bay case study. *Environ Health Persp* 90:133.
- Cossa D, Thibaud Y, Roméo M, Gnassia-Barelli M. 1990. Le mercure en milieu marin. *Biogéochimie et écotoxicologie. Rapports scientifiques et techniques de l'IFREMER* 19 Brest.
- DGSV. 2006. Note de service n° 742/200 du 28 mars 2006 fixant les Limites Maximales de Résidus des médicaments vétérinaires et des contaminants dans les produits avicoles, les poissons d'élevage et le gibier sauvage à plumes.
- Din JN, Newby DE, Flapan AD. 2004. Omega 3 fatty acids and cardiovascular disease—fishing for a natural treatment. *Bmj* 328:30-35.
- Domingo JL, Bocio A, Martí-Cid R, Llobet JM. 2007. Benefits and risks of fish consumption: Part II. RIBEPEIX, a computer program to optimize the balance between the intake of omega-3 fatty acids and chemical contaminants. *Toxicology* 230:227-233.
- Dorneles PR, Lailson-Brito J, dos Santos RA, Silva da Costa PA, Malm O, Azevedo AF, Machado Torres JP. 2007. Cephalopods and cetaceans as indicators of offshore bioavailability of cadmium off Central South Brazil Bight. *Environ Pollut* 148:352-359.
- FAO. 2009. Food and Agriculture Organization. FAOSTAT. Statistical databases; [Accessed 2014 Feb 2]. Available from <http://faostat.fao.org> .
- FAO. 2013. Yearbook, Fishery and Aquaculture Statistics 2011. Food and Agriculture Organization of the United Nations: Rome.
- Hamza-Chaffai A., Pellerin J, Amiard JC. 2003. Health assessment of a marine bivalve *Ruditapes decussatus* from the Gulf of Gabès (Tunisia). *Environ Intern* 28:609-617.
- Harris WS. 1997. n-3 fatty acids and serum lipoproteins: human studies. *Am J Clin Nut* 65:1645-1654.
- IOM. 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc: The National Academies Press, Washington DC.
- Ismail HM. 2005. The role of omega-3 fatty acids in cardiac protection: an overview. *Front Biosci* 10:1079-1088.

- JECFA. 2006. Evaluation of certain food contaminants (Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 930.
- JECFA. 2010. Summary and conclusions of the seventy-second meeting of the joint FAO/WHO expert committee on food additives. Rome, 16–25 February 2010. Geneva: World Health Organisation.
- Joiris CR, Holsbeek L, Moatmeri NL. 1999. Total and Methylmercury in Sardines *Sardinella aurita* and *Sardina pilchardus* from Tunisia. *Mar Pollut Bull* 38:188-192.
- Kojadinovic J, Jackson CH, Cherel Y, Jackson GD, Bustamante P. 2011. Multi-elemental concentrations in the tissues of the oceanic squid *Todarodes filippovae* from Tasmania and the southern Indian Ocean. *Ecotoxicol Environ Saf* 74:1238-1249.
- Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am J Med* 113:71-88.
- Lacoue-Labarthe T, Warnau M, Oberhänsli F, Teyssié J-L, Bustamante P. 2009. Bioaccumulation of inorganic Hg by the juvenile cuttlefish *Sepia officinalis* exposed to ²⁰³Hg radiolabelled seawater and food. *Aquatic Biol* 6:91-98.
- Lourenço HM, Anacleto P, Afonso C, Ferraria V, Martins MF, Carvalho ML, Lino AR, Nunes ML. 2009. Elemental composition of cephalopods from Portuguese continental waters. *Food Chem* 113:1146-1153.
- MAFF. 1995. Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of waste at sea, 1993. Aquatic Environment Monitoring Report No. 44. Tech. rep., Directorate of Fisheries Research, Lowestoft, UK.
- Miramand P, Bentley D. 1992. Concentration and distribution of heavy metals in tissues of two cephalopods, *Eledone cirrhosa* and *Sepia officinalis*, from the French coast of the English Channel. *Mar Biol* 114:407-414.
- Miramand P, Bustamante P, Bentley D, Kouéta N. 2006. Variation of heavy metal concentrations (Ag, Cd, Co, Cu, Fe, Pb, V, and Zn) during the life cycle of the common cuttlefish *Sepia officinalis*. *Sci Total Environ* 361:132-143.
- Napoleão P, Pinheiro T, Sousa Reis C. 2005. Elemental characterization of tissues of *Octopus vulgaris* along the Portuguese coast. *Sci Total Environ* 345:41-49.

- Oehlenschläger J. 1997. Marine fish: A source for essential elements. In: Seafood from producer to consumer. Amsterdam, NL: Elsevier p.641-652.
- Official Journal of the European Communities. 2001. Official Journal of the European Communities L 77/1, (16 March 2001), Reg. No. 466/2001.
- Oomen CM, Feskens EJ, Räsänen L, Fidanza F, Nissinen AM, Menotti A, Kok FJ, Kromhout D. 2000. Fish consumption and coronary heart disease mortality in Finland, Italy, and The Netherlands. *Am J Epidemiol* 151:999-1006.
- Ozogul Y, Duysak O, Ozogul F, Özkütük AS, Türeli C. 2008. Seasonal effects in the nutritional quality of the body structural tissue of cephalopods. *Food Chem* 108:847-852.
- Piatkowski U, Pierce G, Morais da Cunha M. 2001. Impact of cephalopods in the food chain and their interaction with the environment and fisheries: an overview. *Fish Res* 52:5-10.
- Pierce GJ, Boyle PR, Hastie LC, Santos MB. 1994. Diets of squid *Loligo forbesi* and *Loligo vulgaris* in the northeast Atlantic. *Fish Res* 21:149-163.
- Pierce GJ, Stowasser G, Hastie LC, Bustamante P. 2008. Geographic, seasonal and ontogenetic variation in cadmium and mercury concentrations in squid (Cephalopoda: Teuthoidea) from UK waters. *Ecotoxicol Environ Saf* 70:422-432.
- Raimundo J, Caetano M, Vale C. 2004. Geographical variation and partition of metals in tissues of *Octopus vulgaris* along the Portuguese coast. *Sci Total Environ* 325:71-81.
- Raimundo J, Pereira P, Vale C, Caetano M. 2005. Fe, Zn, Cu and Cd concentrations in the digestive gland and muscle tissues of *Octopus vulgaris* and *Sepia officinalis* from two coastal areas in Portugal. *Cienc Mar* 31:243-251.
- Rjeibi M, Metian M, Hajji T, Guyot T, Ben Chaouacha-Chékir R, Bustamante P. 2014. Interspecific and geographical variations of trace metal concentrations in cephalopods from Tunisian waters. *Environ Monit Assess* 186(6): 3767-3783.
- Rodhouse P, Nigmatullin CM. 1996. Role as consumers. *PhilosTrans R Soc Lon B Biol Sci* 351:1003-1022.
- Seixas S, Bustamante P, Pierce GJ. 2005. Interannual patterns of variation in concentrations of trace elements in arms of *Octopus vulgaris*. *Chemosphere* 59:1113-1124.
- Smaoui-Damak W, Hamza-Chaffai A, Berthet B, Amiard JC. 2003. Preliminary study of the clam *Ruditapes decussatus* exposed in situ to metal contamination and originating from the Gulf of Gabès, Tunisia. *Bull Environ Contam Toxicol* 71: 961-970.

- Smith CD. 2003. Diet of *Octopus vulgaris* in False Bay, South Africa. *Mar Biol* 143:1127-1133.
- Storelli MM. 2009. Intake of Essential Minerals and Metals via Consumption of Seafood from the Mediterranean Sea. *J Food Prot* 72:1116-1120.
- Storelli MM, Garofalo R, Giungato D, Giacomini-Stuffler R. 2010. Intake of essential and non-essential elements from consumption of octopus, cuttlefish and squid. *Food Addit Contam Part B* 3:14-18.
- Storelli MM, Giacomini-Stuffler R, Storelli A, Marcotrigiano GO. 2006. Cadmium and mercury in cephalopod molluscs: Estimated weekly intake. *Food Addit Contam* 23:25-30.
- Storelli MM, Marcotrigiano GO. 1999. Cadmium and total mercury in some cephalopods from the South Adriatic Sea (Italy). *Food Addit Contam* 16:261-265.
- The R Development Core Team R. 2008. R: a language and environment for statistical computing. R foundation for statistical computing Vienna, Austria.
- USEPA. 1989. Risk assessment guidance for superfund volume I: Human health evaluation manual (Part A), interim final. EPA /540/1-89/002. United States Environmental Protection Agency.
- USEPA. 2013. EPA Region III Risk-Based Concentration (RBC) Table 2008 Region III. 1650 Arch Street, Philadelphia, Pennsylvania 19103.
- Villanueva R, Bustamante P. 2006. Composition in essential and non-essential elements of early stages of cephalopods and dietary effects on the elemental profiles of *Octopus vulgaris* paralarvae. *Aquaculture* 261:225-240.

Table 1: Mean \pm SD and ranges of Dorsal Mantle Length (DML, mm) and Weight (g) of cephalopod species from the sampling locations along the Tunisian coast. n: number of individuals.

| Species | Bizerte (Northern coast) | | | Monastir (Eastern coast) | | | Sfax (Southern coast) | | |
|--------------------------|--------------------------|--------------|---------------|--------------------------|--------------|---------------|-----------------------|--------------|---------------|
| | n | DML | Weight | n | DML | Weight | n | DML | Weight |
| European squid | 21 | 154 \pm 28 | 118 \pm 64 | 37 | 171 \pm 29 | 128 \pm 47 | 37 | 182 \pm 32 | 156 \pm 66 |
| <i>Loligo vulgaris</i> | | 122-227 | 58-318 | | 120-225 | 47-207 | | 120-256 | 59-297 |
| Common octopus | 20 | 112 \pm 18 | 506 \pm 228 | 27 | 104 \pm 21 | 476 \pm 208 | 25 | 117 \pm 22 | 655 \pm 256 |
| <i>Octopus vulgaris</i> | | 85-150 | 286-1267 | | 72-160 | 114-983 | | 72-155 | 194-1033 |
| Common cuttlefish | 35 | 110 \pm 22 | 171 \pm 90 | 38 | 108 \pm 16 | 155 \pm 55 | 38 | 108 \pm 18 | 163 \pm 99 |
| <i>Sepia officinalis</i> | | 70-145 | 40-396 | | 80-139 | 74-258 | | 65-170 | 41-682 |

Table 2: Concentrations of Cd, Cu, Hg and Zn ($\mu\text{g g}^{-1}$ wet weight) in cephalopods muscle tissues from the Tunisian coasts for four seasons (winter 2009 and, spring to autumn 2010). Inter-species comparison results are given in the last columns. The significances of the differences among species are indicated by letters. KW: Kruskal-Wallis; NS: not significant.

| Metal | Muscle tissue | European Squid | a | Common octopus | b | Common cuttlefish | a | Hypotheses Tests results |
|-------|---------------|------------------------|---|------------------------|---|-------------------------|---|--------------------------|
| | | n=95 Median (Range) | | n=72 Median (Range) | | n=111 Median (Range) | | |
| Cd | Arms | 0.041 (0.014-0.17) | a | 0.026 (0.001-0.20) | b | 0.026 (0.006-0.10) | b | KW: <0.001 |
| | Mantle | 0.045 (0.009-0.16) | a | 0.043 (0.006-0.27) | a | 0.041 (0.007-0.18) | a | KW: NS |
| Cu | Arms | 1.94 (0.50-7.22) | c | 3.29 (1.49-10.62) | b | 5.33 (2.31-10.30) | a | KW: <0.001 |
| | Mantle | 1.22 (0.59-6.13) | c | 4.90 (1.67-13.93) | a | 3.63 (0.80-21.13) | b | KW: <0.001 |
| Hg | Arms | 0.062 (0.030-0.68) | a | 0.027 (0.010-0.11) | c | 0.049 (0.020-0.15) | b | KW: <0.001 |
| | Mantle | 0.072 (0.030-0.95) | a | 0.031 (0.010-0.10) | c | 0.057 (0.019-0.22) | b | KW: <0.001 |
| Zn | Arms | 12.7 (2.8-19.7) | b | 14.2 (6.4-45.3) | a | 15.0 (2.6-39.4) | a | KW: <0.001 |
| | Mantle | 9.8 (4.0-18.7) | b | 11.7 (5.3-32.3) | a | 11.1 (6.2-33.6) | a | KW: <0.001 |

Table 4: Metal concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ ww), estimated Cd and Hg weekly intakes (EWIs; $\mu\text{g}\cdot\text{Kg}^{-1}$ body weight), estimated dietary intake for Cu and Zn ($\text{mg}\cdot\text{day}^{-1}$) and target hazard quotients (THQs) for Cd and Hg following the consumption^a of edible tissue (mantle and arms muscles) of cephalopods caught off three different Tunisian regions.

| Cephalopod species | Average Concentrations | | Estimated Weekly Intake ^a | | Percent of PTWI ^b | | Target Hazard Quotients | | Average Concentrations | | Estimated Daily Intake ^a | | Percent of DRIs ^c | | |
|--------------------|------------------------|----------------|--------------------------------------|-------|------------------------------|------|-------------------------|-------|------------------------|------|-------------------------------------|-------|------------------------------|-------|------------|
| | Cd | Hg | Cd | Hg | Cd | Hg | Cd | Hg | Cu | Zn | Cu | Zn | Cu | Zn | |
| | Bizerte | European squid | 0.039 | 0.164 | 0.15 | 0.63 | 2.17 | 15.76 | 0.02 | 0.18 | 2.26 | 12.34 | 0.07 | 0.41 | 8.31 |
| | Common octopus | 0.025 | 0.037 | 0.10 | 0.14 | 1.37 | 3.58 | 0.01 | 0.04 | 5.23 | 10.92 | 0.17 | 0.36 | 19.18 | 4.50- 3.28 |
| | Common cuttlefish | 0.040 | 0.040 | 0.15 | 0.15 | 2.18 | 3.83 | 0.02 | 0.04 | 5.55 | 14.74 | 0.18 | 0.49 | 20.36 | 6.08- 4.42 |
| Monastir | European squid | 0.053 | 0.079 | 0.20 | 0.31 | 2.92 | 7.66 | 0.03 | 0.09 | 1.67 | 11.58 | 0.06 | 0.38 | 6.13 | 4.78- 3.47 |
| | Common octopus | 0.026 | 0.031 | 0.10 | 0.12 | 1.44 | 3.04 | 0.01 | 0.03 | 4.08 | 13.28 | 0.13 | 0.44 | 14.98 | 5.48- 3.98 |
| | Common cuttlefish | 0.045 | 0.067 | 0.17 | 0.26 | 2.48 | 6.47 | 0.02 | 0.07 | 5.07 | 12.76 | 0.17 | 0.42 | 18.57 | 5.26- 3.83 |
| Sfax | European squid | 0.044 | 0.076 | 0.17 | 0.29 | 2.43 | 7.28 | 0.02 | 0.08 | 1.83 | 9.93 | 0.06 | 0.33 | 6.70 | 4.09- 2.98 |
| | Common octopus | 0.113 | 0.032 | 0.44 | 0.12 | 6.23 | 3.06 | 0.06 | 0.03 | 4.41 | 18.40 | 0.15 | 0.61 | 16.17 | 7.59- 5.52 |
| | Common cuttlefish | 0.038 | 0.066 | 0.14 | 0.25 | 2.06 | 6.37 | 0.02 | 0.07 | 4.44 | 13.95 | 0.15 | 0.46 | 16.26 | 5.75- 4.18 |

Notes: ^a Based on a daily consumption of 33 g of cephalopod (FAO, 2009) for a person weighing 60 kg.

^b PTWI: Provisional Tolerable Weekly Intake (Cd: $7 \mu\text{g kg}^{-1}$ b.w; Hg: $4 \mu\text{g kg}^{-1}$ bw; JECFA, 2006, 2010).

^c DRIs: Dietary Reference Intake (Cu: 0.9 mg day^{-1} ; Zn: 8 (women) -11 (men) mg day^{-1}) (IOM, 2001)

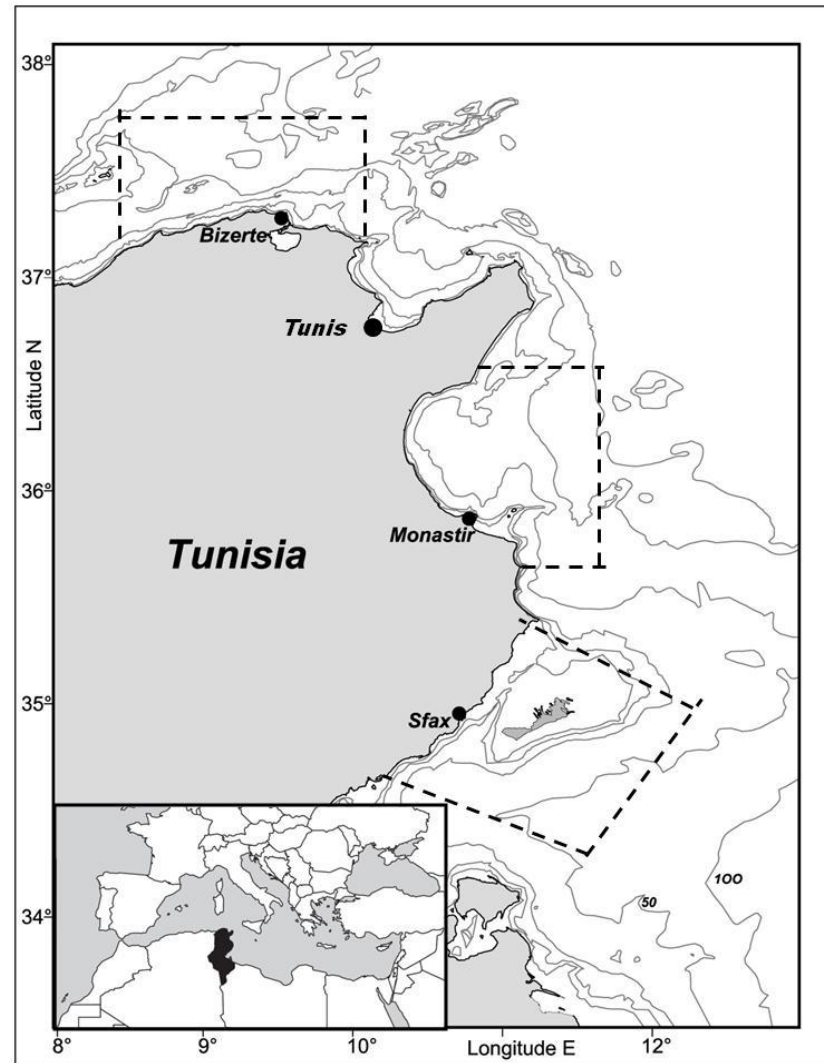


Figure 1: Sampling locations (Bizerte, Monastir and Sfax) along the Tunisian coast.