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HEAVY METALS AND PROXIMATE COMPOSITION OF BLACK SEA SPRAT (*SPRATTUS SPRATTUS*) AND GOBY (*NEOGOBIUS MELANOSTOMUS*)

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Abstract

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The aim of the present study were to determine and compare the heavy metals content (Pb, Cd, Hg and As) and proximate composition in edible part of two commercially important fish species from Bulgarian Black Sea – sprat (*Sprattus sprattus*) and goby (*Neogobius melanostomus*). Determination of As, Cd, and Pb were carried out on a Perkin Elmer Zeeman 3030 spectrometer with an HGA-600 atomizer, whereas Hg was analyzed by Milestone Direct Mercury Analyzer. The levels of Cd and Pb were relatively low in both analyzed species while those for As concentration show higher value for sprat. The amounts of Hg for sprat and goby are also under permitted levels for fishes for human consumption. Proximate composition of the following nutrients was determined using standard procedures of AOAC (1991): moisture, crude protein and total lipids. Crude protein in fish samples was in the range 17.15–18.10%, while fat content was from 1.60 to 4.30 g.100 g^{-1} w.w. Energy values have been calculated using FAO/WHO specific factors and were in interval 373–437 kJ.100 g^{-1} w.w. Results showed that observed heavy metal contents have lower concentration of mean values than the permissible limits set by FAO/WHO in analyzed samples. It can be concluded that both species studied are safe to be consumed and have a good nutrition quality.

Key words: Heavy metals, proximate composition, Black Sea Sprat, Goby

Introduction

Heavy metals are natural trace components of the marine environment, but they constitute one of the most hazardous substances that could be accumulated in biota. According to Munoz-Olivas and Camara (2001) heavy metals are classified as: potentially toxic (e.g. aluminum, arsenic, cadmium, lead, mercury), probably essential (e.g. nickel, vanadium, cobalt) and essential (e.g. cooper, zinc, selenium). Fish populations with commercially important often live in coastal area environments that contain high levels of heavy metals, coming from industrial and agriculture wastes or human activities. The marine organisms accumulateits from water, food, sediment and some suspended particulate materials. Furthermore fish species accumulate heavy metals to concentrations many times higher than presented in water or sediments andtherefore they have been extensively used for marine pollution monitoring (Agusa et al., 2005; Bat et al., 2012).

The nutritional benefits of fish are mainly due to thecontent of high-quality protein (fish provide 17% of the total animal protein and 6% of all protein consumed byhumans), and other essential nutrients. Thequality of fish tissue is function of their body compositions and energy values, which that vary among different species. Determination of proximate composition as protein contents, carbohydrates, lipids, moisture contents

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and ash percentage is often necessary to ensure that fish tissues have a good nutrition quality and that they meet the requirements of food regulations and commercial specifications (WHO/FAO, 2011).

Pelagic fish species (e.g. Clupeidea, Osmeridae) are usually high in lipid content and energy levels (kJ/100g raw tissue), whereas demersal species (e.g. Gobiidae, Cottidae) generally have lower lipid and energy values (Ball et al., 2007). Many studies have been conducted on the effect of marine fish lipids on coronary heart disease, atherosclerosis, thrombosis and blood pressure (Ball et al., 2007).

In recent years a number of investigators have assessed the levels of various pollutants in several fish species and in different water basins (Tuzen et al., 2003; Uluozlu et al., 2007; Alina et al., 2012). Black Sea is considered as one of the most polluted seas, and the increasing concentration of nutrients in recent years has led to a higher degree of eutrophication. Few studies have considered heavy metal contents and proximate composition of fish species as shad, horse mackerel, garfish and goby from Southeast (Guner et al., 1998; Bat et al., 2012) and Northwest (Creteanu, 2009) parts of Black Sea.

During the last 20 years, the sprat has been most abundant and commercially important fish species in the western Black Sea and for Bulgarian local fish markets. This small pelagic forage fishis also of great importance for the ecosystem since it represents animportant link between the plankton community and its predators in Black Sea food web.

Gobiidae species are carnivorous fishes, feeding mainly on *Mytilus galloprovincialis,* but also on other invertebrates (*Mytilaster, Cerastoderma, Gammarus,* decapods) and fish (sprat) (Creteanu et al., 2009). The main commercially target species from catches for the Bulgarian market has occurred to be *Neogobius melanostomus* (Vassilev et al., 2009).

Sprat and goby are domestic (non-migrated) species. The catches in the Black Sea account for nearly 90% of total fish production in Bulgaria. Most of the fishing activities are carried out in territorial waters (up to 12 miles). Fiching catch activities are located along the entire coast,but two of main fishing ports are in Nessebar and Baltchik (NSP, 2007).

There are limited information about heavy metals pollution and proximate composition of sprat and goby from Bulgarian Black Sea coast.Therefore, considering the various health risk and the nutritional benefits associated with fish consumption; it has therefore become important that, fish's heavy metals content and proximate composition and their health status be assessed in order to establish the safety level of these commercially important species from Bulgarian Black Sea prior their consumption.

This work presents a comparative evaluation of accumulation of four heavy metals and proximate composition of edible tissue of Black Sea sprat (*Sprattus sprattus salinus*) and goby *(Neogobius melanostomus)* from Bulgarian Black Sea.

Materials and Methods

Sample preparation

Fresh fishes were purchased from Varna local fish markets in 2010, during fishing season. The species were caught from two fishing areas -Balchik (North part of Bulgarian Black Sea coast) and Nessebar (South part of Bulgarian Black Sea coast). Specimens of similar body weight and length were selected from all the captured species. Biological characteristics, as body weight (g), length (cm), habitats were determined (Table 1) and the fish were dissected immediately after catch.

A minimum nine specimen from each individual species were gutted, filleted and minced for analysis and stored at – 20ºC prior to analysis.

Table 1
Biometrical and biological characteristics of the analyzed fishes (mean ±SD)

Fish species	N	Sampling	Habitats	Weight, g	Length, cm
Sprat (*Sprattus sprattus*)	6	South (Nesebar)	Pelagic	8.0 ± 1.0	10.0 ± 1.5
Goby (*Neogobius melanostonus*)	6	North (Balchik)	Demersal	60.0 ± 5.0	16.0 ± 2.0

Heavy metals content analysis

All solutions were prepared with analytical reagent grade chemicals and ultra-pure water (18 MΩ cm) was used for all dilutions. HNO_3 was of superb quality purchased from Fluka. All the plastic and glassware were cleaned by soaking in 2 M HNO_3 for 48 h, and rinsed five times with distilled water, and then five times with deionized water prior to use. Stock standard solutions of As, Hg, Cd, and Pb (1000 μg mL^{-1} Titrisol, Merck in 2% v/v HNO_3) were used for preparation of calibration standards.

Fish tissues were dissected and thoroughly washed with MQ water. To assess the total metal contents, microwave assisted acid digestion procedure was carried out. Microwave digestion system „Multiwave", „Anton Paar" delivering a maximum power and temperature of 1000 W and 300°C, respectively, and internal temperature control was used. Reactors were subjected to microwave energy at 800 W in five stages program. Arsenic, cadmium and lead were determined by electrothermal atomic absorption spectrometry on a Perkin Elmer Zeeman 3030 spectrometer with an HGA-600 atomizer. Pyrolytic graphite-coated graphite tubes with integrated platforms were used as atomizers. Pd as $(NH_4)_2PdCl_4$ was used as modifier for ETAAS measurements of As and Cd. Total mercury was determined by Milestone Direct Mercury Analyzer DMA-80. Samples were analyzed in triplicate. Whole data were subjected to a statistical analysis.

Proximate composition analysis

Test portions of homogenized fish tissue (2.000 ± 0.005 g) were dried at 105 ± 2°C in air oven to constant weight for 16–18 hours (AOAC 950.46). The moisture [%] was calculated as weight loss. Crude protein content was calculated by converting the nitrogen content, determined by Kjeldahl's method (BDS 9374:1982). Total lipids (TL) were determined according to Bligh and Dyer procedure (1959) and the results were presented as g per 100g wet weight (g.100g^{-1} ww). Since carbohydrate content is generally low in fish and its contribution to the energy value is practically zero, this component was not measured (Anthony et al., 2000; Eder and Lewis, 2005). Carbohydrate content and energy value were calculated by subtracting the total of protein ,TL and water from the whole and by multiplying fat, protein and carbohydrate with appropriate coefficients (WHO/FAO, 2010).

Statistical Analysis

The data were analyzed usingGraph Pad Prism 5.0 software. T-test was used for calculation of means and standard deviations. One-way ANOVA (nonparametric test) statistical analysis was used to estimate differences between fish species. The significance level was $p < 0.05$.

Results and Discussion

Heavy metals content

The data obtained for heavy metals content in analyzed fishes are presented in Table 2.

For both species, the metal concentrations increase in the order: Cd < Pb < Hg < As. The non-essential elements as Hg, Pb and As in edible tissue presented species differences ($p < 0.001$).

Lead

Lead is toxic to humans, with the most deleterious effects on the hemopoietic, nervous, reproductive systems and the urinary tract. The Joint FAO/WHO (2004) Expert Committee on Food Additives establishes a provisional tolerable weekly intake (PTWI) for lead as 0.3 mg.kg^{-1} body weight. European Community (No 1881/2006) and Bulgarian Food Codex (No 31/2006) set maximum permitted level for Pb in fish of 0.4 mg.kg^{-1} w.w. In present study the higher concentration of lead was measured in sprat from Nessebar - 0.08 mg kg^{-1} w.w., whereas goby presented only 0.03 mg kg^{-1} w.w. Tuzen et al. (2003) re-

Table 2
Heavy metals content (mg.kg^{-1} wet weight) in sprat and goby (means ±SD)

Fish species	N	Pb	Cd	As	Hg
Sprat (*Sprattus sprattus*)	6	0.08 ± 0.02	0.005 ± 0.001	0.73 ± 0.05	0.12 ± 0.02
Goby (*Neogobius melanostonus*)	6	0.03 ± 0.01***	0.006 ± 0.001	0.66 ± 0.05***	0.05 ± 0.01***

SD – standard deviation, *** – p <0.001

ported for fish species from the middle Black Sea lead levels in the range of 0.22-0.85 mg kg^{-1}. Uluozlu et al. (2007) were investigated lead contents of fish species from Black and Aegean seas and were found values in range of 0.33-0.93 mg kg^{-1} for edible fish tissue. In current research Bat et al. (2012) presented three times higher concentrations for Pb (0.28 mg.kg^{-1}) for sprat from Sinop region (Turkey Black Sea coast) compared to our results. Creteanu et al. (2007) was investigated heavy metals content in three subspecies of Gobidae family from Mangalia, (Romania, Northwestern part of Black Sea) and was determined a lower Pb levels comparison to our results in the same goby species. In general, both analyzed species showed significantly lower Pb concentration in comparison to species from middle Black Sea and Aegean Sea.

Cadmium

Cadmium is a non-essential, highly toxic and ecotoxic metal. The occupational levels of Cd exposure prove to be a risk factor for chronic lung disease and testicular degeneration. Cadmium could originate from water, sediments and food and may accumulate in the human body as may induce kidney dysfunction, skeletal damage and reproductive deficiency (2). The approximately equal concentrations of Cd were obtained for both analyzed species (Table 2). The European Community (No 1881/2006) and Bulgarian Food Regulation recommended the maximum levels permitted for Cd in sea fish as 0.05 mg.kg^{-1} w.w. Moreover, the Joint FAO/WHO has recommended the provisional tolerable weekly intake (PTWI) as 0.007 mg.kg^{-1} body weight for this element (8). Bat et al. (2012) was determined tenfold higher levels for Cd (0.05 mg.kg^{-1}) for sprat from Sinop region (Turkey Black Sea coast) compared to our results, as well as Creteanu et al. (2007) presented significantly higher concentrations for goby from Mangalia region (Romanian Black Sea coast). We can summarize that both analyzed species showed significantly lower Cd concentration in comparison to same species monitored in middle and Northwest Black Sea.

Arsenic

Arsenic is a widely distributed metalloid, occurring in rock, soil, waterand air. General population exposure to arsenic is mainly *via* intake of foodand drinking water. The most toxic forms of arsenicare the inorganic arsenic (III) and (V) compounds. Only a few percent of the total arsenic in fish is present in inorganic form, which is the only form about which a PTWI has been developed by JECFA (WHO/FAO, 2004). Chronic exposure to inorganic arsenic may cause serious impact on peripheraland central nervous system (Jarep, 2003).In this study the higher concentration of As was measured in sprat from Nessebar – 0.73 mg kg^{-1} w.w., whereas goby presented 0.66 mg kg^{-1} w.w.The WHO/FAO (2004) and Bulgarian Food Regulation recommended the maximum levels permitted for As in sea fish as 5.00 mg.kg^{-1} w.w. There are limited data about the arsenic content in fish species, especially from Black Sea region, in the literature. Falko et al. (2006) was estimate As concentration in 14 edible marine species from Mediterranean Sea and described the three times higher levels of As in red mullet (16.6mg.kg^{-1} w.w) comparison with WHO/FAO recommendations. Current investigations presented As levels in 12 fish species from Malaysia (Alisa et al., 2012) in range from 0.25 to 6.57 mg.kg^{-1} w.w. In general, all of the results obtained below the maximum As level permitted for fish according to Bulgarian Food Codex – 5.0 mg.kg^{-1}w.w. (1) and compared to other fish species from Mediterranean Sea and Malaysian waters.

Mercury

Hg is one of the most toxic elements among the studied heavy metals and exposure to high level of this element could permanently damage the brain, kidneys and developing foetus (WHO/FAO, 2004). The higher detected values of Hg was in sprat (0.12 mg.kg^{-1} w.w), whereas goby contains twice as lower value (0.05 mg.kg^{-1} w.w). According to Bulgarian Food Codex and European Community the maximum mercury level permitted for sea fish is 1.0 mg/kg w.w. (1.5). There is insufficient information about the Hg content in fish species from Black Sea region in the literature. The highest mercury concentrations measured in our study is lower then that reported by Yabanli et al. (2012) in frozen European sea bass and gilthead sea bream fillets produced and marketed in Turkey. Compared to our results Falko et al. (2006) presented similar concentration for Hg in 14 edible marine species from Mediterranean Sea, whereas Alina et al. (2012) were determined significant higher concentration for Hg in range from 1 to 6.5 mg/kg w.w in 12 fish species from Malaysia. Generally, the results obtained for both analyzed species are below the maximum Hg level permitted for fish according to Bulgarian Food Codex – 1.0 mg.kg^{-1} w.w. (1) and compared to other fish species from Mediterranean Sea, Malaysian waters and Turkish markets.

Differences in metal concentrations related to diet and feeding habits of benthic and pelagic fish species. Bat et al. (2012) supposed that demersal species generally accumulate higher concentrations of heavy metals than pelagic fish. Whereas, Topping (1973) suggested that mainly plankton feeding fish contain much higher concentrations of some heavy metals than bottom feeding fish. Sprat is zooplanctonivorous fish and has high metabolic rate. According to their food habits, sprat can be suitable as tools for descriptions of environmental conditions of coastal waters. It is also known that metal concentrations in fish tissues are related to the pollution status of the regions. Sprat is migrating between the open sea and inshore areas. The results in present study confirm assumption about pelagic species. Bat et al. (2012) presented similar results for higher heavy metal content in sprat compared with other demersal species from Sinop region of South Black Sea.

Based on the analyses of fish samples, heavy metal concentrations in fish species from Bulgarian Black Sea coastal waters are low. Both fish samples tested were well within the limits set by the recommendations of European Community (No 1881/2006) and Bulgarian Food Regulation.

Proximate composition and energy values

The proximate composition in most fish is primarily water, proteins, and lipids. In fish meat these constituents make up about 98% of the total mass, and the other minor constituents include carbohydrates, vitamins, and minerals (WHO/FAO, 2011). The average values of this constituents of two Black sea species are shown in Table 3.

Proximate data on different fish species are collected in different databases (such as www.fishbase.org), however, the chemical composition of fish generally varies due to geographical locations, stages of maturity, and sizes. It is known that edible fish tissue contains 60–84% water, 15–24% protein and 0.1–22% lipids. The proportions of the constituents are species-specific and the main variations in proximate composition between species occurs in moisture and lipids content . (WHO/FAO, 2010; Boran et al., 2011).

Moisture content

Water is required for the normal functioning of many biological molecules. It is present in two forms in the tissues, bound to the proteins and in the free form. These forms have well defined biological roles. There exists an inverse relationship between the water and lipid content of fish and the summation of both frequently spans a range of 78 to 88 %. As shown in Table 3, the moisture content in demersal species (goby) was higher than in the pelagic species (sprat). Similar results were obtained by other authors for Black Sea sprat (Guner et al., 1998). Not data available in literature for proximate composition of Black Sea goby.

Protein content

Quantitatively, protein is the second major component in muscle tissues of fish. The protein content tends to vary much less widely from one species to another (WHO/FAO, 2010). Protein content of fish is considered low if it is below 15%. In this study, edible tissue from both Black sea fish showed good protein levels (16.30–18.73 g.100^{-1} g w.w.). Sorting by the protein content was sprat < goby ($p < 0.05$). Guner et

Table 3
Proximate chemical composition (% wet weight; means ± SD) and energy values (kJ.100 g^{-1} wet weight, means ± SD) of sprat and goby

Fish species	N	Protein	Lipid	Moisture	Carbohydrate	kJ
Sprat (*Sprattus sprattus*)	6	16.20 ± 0.55	4.30 ± 0.25	79.00 ± 1.80	0.00	437.0 ± 20.0
Goby (*Neogobius melanostonus*)	6	18.73 ± 0.73***	1.60 ± 0.08***	80.10 ± 2.05	0.00	373.0 ± 16.0***

SD – standard deviation, *** – $p < 0.001$

al, (1998) presented lower values for protein content of Black Sea sprat (15.40 g.100^{-1} g w.w.) from East Black Sea region.

Total lipid content

According to Ackman (1989), fish species can be classifeied into four categories: high fat (up to 8.0 g.100 g^{-1} w.w.); medium fat (4–8 g.100 g^{-1} w.w.); low fat (2–4) and lean (under 2). In this study, the lipid content ranged from 1.60 g.100 g^{-1} w.w. for goby to 4.30 g.100 g^{-1} w.w. for sprat. Based on this results goby could be classified as a lean, whereas sprat was medium-fat species. There are several factors that may resulting in significant differences observed in the lipid content of fish such as season, diet, geographical area (Guner et al., 1998; Tanakol et al., 1999). Guner et al. (1998) had found significantly higher values for sprat, whereas Tanakol et al. (1999) found similar total lipids for goby compared with our results.

Energy values

The energy released by oxidation of protein, fat, and carbohydrate is the basis for sets of conversion factors. The Atwater general factor system is the foundation for the most frequently used systems for energy conversion (WHO/FAO, 2010) and used energy conversion factors are 4.0 kcal/g (17 kJ/g) for proteins, 9.0 kcal/g (39 kJ/g) for lipids, and 4.0 kcal/g (17 kJ/g) for carbohydrates (calculated by difference, i.e., subtracting water, proteins, and lipids). These factors are based on process on combustion with adjustments for losses in digestion, absorption, and excretion of urea. Seafood show variable composition of proteins and fat, and energy content is dependent on this distribution. The lipid level in particular has high significance for the calorie content of fish, with implications for calculations in dietary studies and databases. In this study estimated energy values ranged from 343 kJ.100 g^{-1} w.w. (goby) to 437 kJ.100 g^{-1} w.w. for sprat ($p < 0.05$). An earlier study Guner et al. (1998) showed significantly higher energy content for Black Sea sprat (889 kJ.100 g^{-1} w.w.) due to higher lipids content determined for this species. Current investigations presented energy values in four Black Sea fish species from Sinop Region (Boran et al., 2012) in wide range 360 (garfish) to 1250 (shad) kJ.100 g^{-1} w.w. According to Danish Food Database Baltic sprat energy values (640 kJ) are higher compared to our results. Not data available in literature for energy value of Black Sea goby.

Conclusions

Fish is an important food resource for human consumption and a major component of the marine ecosystem, thus assessment of the heavy metal content in fish species is particularly important. Therefore, studies on the presence of heavy metals in marine fish will contribute to the accumulation of new data on their levels in commercially important species from Bulgarian Black Sea waters, including for estimating the risk for Bulgarian consumer. Based on the analyses of fish samples, heavy metal concentrations in sprat and goby from Bulgarian waters were well within the limits set by the recommendations of European Community (No 1881/2006) and Bulgarian Food Regulation. Both studied species were characterized by a good nutritional quality compared to other Black Sea fish species, mainly characterized by higher protein contents and low lipid levels. According to presented results of this study we can give a positive evaluation of the nutritional quality and safety of sprat and goby living in Bulgarian Black Sea waters for consumers.

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