



MACRO- AND MICROELEMENTS IN EEL (*ANGUILLA ANGUILLA*) FROM THE NORTHERN REGIONS OF POLAND

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Abstract

The abundance of the eel (*Anguilla anguilla*) in Europe has been on the decline in recent years, to the extent that this fish species is now considered to be threatened with extinction. Hence, the current implementation of the Eel Management Plan in Poland, whose aim is to restore w stocks of this fish. The main natural habitats of eel are the transitional waters of the Vistula and Szczecin lagoons and lakes in northern Poland. The eel is highly valued by many consumers for the taste and texture of its meat.

The aim of the study was to determine differences in concentrations of macro- and microelements and toxic metals in muscles of the eel as a function of each specimen's length, mass and the origin. The results of the study also served an evaluation of the health benefits and risks to consumers of eel meat with regard to the content of macro- and microelements and toxic metals.

Specimens of the eel (*Anguilla anguilla*) were caught in 2011-2013, in five regions of Poland: the southern Baltic, inland lakes in northeast Poland, the Vistula and Szczecin lagoons, and in the Vistula River.

The concentrations of Ca, P, Mg, Na, K, Fe, Sr, Zn and As were determined with optical emission spectrometry. The flameless atomic absorption spectrometry method was used to measure concentrations of Cu, Cd and Pb. The content of Hg and Se were determined with atomic absorption, those of mercury with cold vapour, and of selenium with hydride generation.

The concentrations of macro- and microelements in most instances, with the exception of P and Zn, were negatively correlated with the eel's length and mass, which indicated that small fish contained more minerals than large specimens. The eel is a rich source of phosphorus, zinc, selenium and iron, and it can supply significant quantities of the daily requirements of human consumers for these minerals. The other minerals occurred in eel muscle at levels that ranged from 2 to 6% of human daily requirements. Among the toxic metals, mercury was the cause for concern, while cadmium and lead occurred at low levels in all of the specimens examined regardless of their size. The mean concentration of mercury ranged from 0.147 to 0.273 mg kg⁻¹

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and was positively correlated with specimen length and mass. The content of mercury in large eel exceeded 0.500 mg kg^{-1} , while small eel (up to 70 cm) contained lower levels of mercury. Large eel exceeding 70 cm can pose a threat to the consumer's health because of mercury, and especially its organic form of methylmercury. This is why consumers should limit long-term consumption of larger eel, while it is safe to consume smaller specimens since they contain less mercury and more minerals than do large eel.

Key words: eel, macroelements, microelements, toxic metals.

INTRODUCTION

The European eel (*Anguilla anguilla*) is a catadromous species that migrates to the Sargasso Sea to spawn after attaining sexual maturity. After spawning, larvae hatch at the spawning grounds, and then drift on the Gulf Stream eventually reaching river mouths in western Europe. Only a small number of eels ever reach Polish waters naturally, and this species is currently threatened with extinction. This is why the Polish Eel Management Plan, based on Council Regulation (EC) no. 1100/2007 of 11 September 2007 that sets forth methods for restoring European eel resources, is being implemented. Natural eel habitats in Poland include nearly all water types. The least consequential are rivers, which provide corridors for feeding and spawning migrations, while the most important are the transitional waters of the Vistula and Szczecin lagoons, as well as lakes in the lake regions of northern Poland. In these areas, male eels grow to reach average lengths of 50 cm, while females can reach lengths of up to 1.5-2 m and masses of 4-6 kg. The European eel (*Anguilla anguilla*) is a predatory species at the top of the aquatic environment food chain, and it consumes various elements, including metals, along with its prey that occur at various levels of the chain. The eel's feeding habits combined with its stationary life strategy before migration and the fact that it inhabits various types of continental waters means that it is often used in environmental evaluations. As a bioindicator of pollution, the eel has been used in studies of aquatic environments in many European countries (FARKAS et al. 2000, USERO et al. 2003, LINDE et al. 2004, DURRIEU et al. 2005, HAS-SCHON ET AL. 2006, STORELLI et al. 2007, ICES 2010). However, there are few data regarding concentrations of metals, including macro- and microelements, in eels from Polish regions. Marine fish are an essential component of a proper human diet, because they are food rich in essential fatty acids, protein, vitamins and minerals. Minerals such as calcium, magnesium, and phosphorus are nutrients that are essential for the proper functioning of the human body. The physiological function of macroelements is well documented, and the requirements of these for human health are known (JAROSZ 2001). Microelements, however, present a different problem because the quantities necessary for the proper functioning of the human body often border on toxic levels. Iron, zinc, manganese and copper are included in the group of essential trace elements required for maintaining cellular function and being

integral components of numerous metal-containing enzymes. Fish also contain toxic elements including toxic metals (cadmium, lead, mercury), which have harmful impacts on human health. People appreciate the organoleptic properties of eel meat primarily in countries where these fish are distributed. Estimating the content of essential minerals and toxic compounds could help to determine the benefits and risks to consumers of eating this fish species.

The aim of the study was to determine differences in concentrations of macro- and microelements and toxic metals in eel muscle as functions of specimen size, mass and the region they inhabit. The results of the study also served to determine the health benefits and risks to eel consumers in terms of minerals and toxic metals.

MATERIAL AND METHODS

The eel (*Anguilla anguilla*) specimens used in the study were caught in 2011, 2012 and 2013, in five regions of Poland: the southern Baltic (Puck Bay, in the vicinity of Świnoujście and Mielno); lakes in northeastern Poland (Jamno, Bukowo, Święcajty, Nidzkie, Śniardwy, Mamry); the Vistula and Szczecin lagoons; the Vistula River. In all, 160 samples were collected. Eel individuals with masses in excess of 900 g comprised a single sample, whereas samples of smaller specimens comprised from 2 to 7 specimens of the same length.

The samples tested were handled in clean rooms (Class 10 000) up to the stage of measuring metal concentrations. Metal analyses were performed on eel muscles. The muscle was excised from the fish and homogenized. Approximately 2 g of wet weight sample, 6 ml of nitric acid (65%), and 2 ml of (30%) hydrogen peroxide (H_2O_2) were placed into PTF vessels. The sample in the vessel was then subjected to a Microwave Digestion System (MDS 2100 and MARS 5). A sub-sample of homogenized tissue was preserved intact for subsequent mercury analysis.

Concentrations of zinc, phosphorous, calcium, magnesium, sodium, potassium, iron, strontium and arsenic were determined by optical emission spectrometry with induction in plasma (Varian ICP-OES, Vista MPX). Copper, cadmium and lead concentrations were determined with atomic absorption spectrophotometry using a Perkin-Elmer 4100 atomic absorption spectrometer equipped with a graphite furnace. Mercury concentrations were determined using the cold vapour atomic absorption technique in a mercury analyzer (AMA 254). Concentrations of selenium were measured with the atomic absorption method using hydride generation and a Perkin-Elmer spectrometer combined with a Fias 200.

The average values with variation in two replicates of < 10% were used to interpret and analyze the results. Each measurement series was preceded

by analyzing freeze-dried reference materials throughout the validation process, and the chosen parameters of the method applied were designated as detection limits and recovery (Table 1).

Table 1

Limit of detection and recovery

Metal	Certified material	Certified value (mg kg ⁻¹)	Recovery (%)	Limit of detection (mg kg ⁻¹)
Ca	SRM 1566b	838 ± 20	104.3	0.05
K	SRM 1566b	6520 ± 90	98.8	2.0
Na	SRM 1577b	2420 ± 60	97.9	1.0
P	SRM 1577b	11000 ± 300	104.1	0.1
Mg	SRM 1577b	601 ± 28	104.8	0.01
Sr	SRM 1566b	6.8 ± 0.2	102.9	0.001
Fe	CRM 422	5.46 ± 0.3	92.0	0.03
Se	SRM 1566b	0.73 ± 0.06	98.4	0.001
Zn	CRM 422	19.6 ± 0.5	98.1	0.75
Cu	CRM 422	1.05 ± 0.07	97.4	0.10
Cd	CRM 422	0.017 ± 0.002	110.8	0.001
Pb	CRM 422	0.085 ± 0.015	89.2	0.01
Se	CRM 422	1.63 ± 0.07	95.7	0.01
Hg	SRM 1566b	0.037 ± 0.001	98.6	0.001
Hg	CRM 422	0.559 ± 0.016	98.8	0.001
As	IAEA 436	1.98 ± 0.17	105.5	0.08

CRM 422 – cod muscle; SRM 1566b – oyster tissue; SRM 1577b – bovine liver; IAEA 436 – tuna fish

Statistical analysis was performed with Statistica 8.0 (Stat Soft 2005, version 7). Fish biological parameters and metal concentrations in muscle were examined initially for normal distribution and homogeneity of variances with the Shapiro-Wilks test. Variables from almost all of the samples were not normally distributed. The non-parametric Kruskal-Wallis test was then performed to test differences in the metal concentrations in the muscles of eel from the different sampling regions. Correlations between metal concentration in eel muscles and length as well as mass were assessed using the non-parametric Spearman's test. The level of significance was $p < 0.05$.

RESULTS AND DISCUSSION

Muscle tissue from eel inhabiting the northern areas of Poland contained different concentrations of macro- and microelements (Table 2). Phosphorus and potassium dominated at concentrations several times higher than those of calcium, sodium and magnesium. Eel contained phosphorus and potassium at levels close to those of marine fish – herring, salmon, cod, sprat, mackerel (SZLINDER-RICHERT et al. 2011) and freshwater fish – carp, roach, perch,

Table 2

Average length, weight and concentration of minerals in eel from regions (mg kg⁻¹ wet weight)

Metal	Baltic Sea	Lakes	Vistula River	Vistula Lagoon	Szczecin Lagoon	KW ($p < 0.001$)
Length (cm)	67 - 101	56 - 97	35 - 64	50 - 88	50 - 86	12.4
Weight (g)	162 - 1920	270 - 2030	92 - 438	250 - 1610	185 - 1605	17.8
Ca	248 ± 61	240 ± 49	742 ± 122	205 ± 63	225 ± 74	38.1
K	2384 ± 215	2284 ± 147	2361 ± 242	2218 ± 207	2210 ± 164	31.5
Na	566 ± 103	602 ± 59	671 ± 68	632 ± 85	579 ± 69	15.7
P	2396 ± 248	2139 ± 175	3036 ± 956	1843 ± 113	1879 ± 130	120.2
Mg	201 ± 17	192 ± 14	220 ± 47	173 ± 15	175 ± 12	71.6
Sr	0.36 ± 0.22	0.38 ± 0.32	5.81 ± 5.0	0.46 ± 0.35	0.28 ± 0.22	25.2
Fe	7.85 ± 3.7	3.36 ± 0.99	11.21 ± 1.85	7.44 ± 4.75	6.10 ± 2.85	43.3
Cu	0.18 ± 0.06	0.24 ± 0.06	0.30 ± 0.04	0.37 ± 0.14	0.34 ± 0.09	83.8
Zn	27.9 ± 4.1	20.7 ± 5.1	21.5 ± 3.1	22.5 ± 2.5	19.4 ± 3.5	93.1
Se	0.152 ± 0.05	0.159 ± 0.05	0.209 ± 0.02	0.163 ± 0.04	0.256 ± 0.05	51.0
Cd	0.001±0.001	0.002 ± 0.001	0.024 ± 0.022	0.002 ± 0.002	0.002 ± 0.001	2.38(Ns)
Pb	0.018 ±0.007	< 0.01	0.012 ± 0.003	0.010 ± 0.006	0.014 ± 0.014	61.5
Hg	0.273 ± 0.22	0.149 ± 0.10	0.222 ± 0.165	0.147 ± 0.05	0.166 ± 0.09	26.1
As	0.607 ±0.17	0.465 ± 0.3	0.224 ± 0.19	0.273 ± 0.13	0.382 ± 0.22	51.9

KW – Kruskal-Wallis test

breem, pikeperch (BRUCKA-JASTRZEBSKA et al. 2009), but higher than cultured fish imported to Poland – oil fish, Nile perch, African catfish, pangasiid catfish (POLAK-JUSZCZAK 2007).

Eel muscles also contained large quantities of microelements (zinc, copper, selenium, iron, strontium), with the highest amounts of iron and strontium noted in eel from the Vistula River, similarly to those of the macroelements of calcium and phosphorus. These elements occurred in fish from the other regions of Poland at levels similar to those recorded in fish from Polish culture ponds (BRUCKA-JASTRZEBSKA et al. 2009). Copper and selenium dominated in specimens from the lagoons, while zinc did so in eel from the Baltic, and concentrations of it were several-fold higher than those in other species of marine or freshwater fish (BRUCKA-JASTRZEBSKA et al. 2009, SZLINDER-RICHERT et al. 2011, RAJKOWSKA, PROTASOWICKI 2013). These data indicate that eel is a good source of minerals, especially of phosphorus, selenium, zinc and iron, which are essential for the human body. A 100 g portion of eel provides the following percentage ranges of the adult daily requirements for these minerals: phosphorus – 26.8-43.4%; selenium – 27.6-46.5%; zinc – 17.6-20.4%; iron – 3.4-11.2% (Table 3). This portion also provides about 5% of the adult daily requirements for the remaining macro- and microelements. These wide percentage ranges arise from differences in mineral concentrations in eel depending on the region inhabited and fish size. The concentrations of macro- and microelements, with the exception of phosphorus and zinc, were negatively correlated with eel length and mass (Table 4), but the

Table 3

Estimated daily intake of metals on consumption of 100 g week⁻¹ ell *Anguilla anguilla*, and comparison with food standards

Metal	RDA (mg day ⁻¹) Male adult	% coverage of standard	RDA (mg day ⁻¹) Female adult	% coverage of standard
Ca	1000	2.1 - 7.4	1200	1.7 - 6.2
Mg	400	4.3 - 5.5	320	5.4 - 6.9
K	4700	4.7 - 5.0	4700	4.7 - 5.0
Na	1500	3.8 - 4.5	1500	3.8 - 4.5
P	700	26.8 - 43.4	700	26.8 - 43.4
Fe	10	3.4 - 11.2	10	3.4 - 11.2
Cu	0.9	2.0 - 4.1	0.9	2.0 - 4.1
Zn	8	24.3 - 28.1	11	17.6 - 20.4
Se	55*	27.6 - 46.5	55*	27.6 - 46.5
Toxic metal	Maximum levels** (µg kg ⁻¹)	PTWI (µg kg ⁻¹ body)	PTWI (µg 70 kg ⁻¹ body week)	% PTWI
Hg	1000	4.0	280	5.2 - 9.8
MeHg	-	1.3	91	14.2 - 26.4
Cd	100	2.5	175	0.06 - 1.4
Pb	300	1.5	105	0.9 - 1.7
As	4000	3	210	10.7 - 28.9

* µg day⁻¹; RDA (Recommended Dietary Allowance) by Jarosz 2001; ** Commission Regulation (EU) No 1881/2006 of 19 December 2006; PTWI (Provisional Tolerance Weekly Intake) by European Food Safety Authority (EFSA, 2010, 2011, 2012)

Table 4
Spearman correlation coefficients between minerals content and length, weight and region; Rs (p)

Metal	Length	Weight	Region
Ca	-0.50 (0.00)	-0.54 (0.00)	0.15 (0.03)
Zn	0.59 (0.00)	0.57 (0.00)	0.61 (0.00)
K	-0.19 (0.02)	-0.22 (0.006)	0.37 (0.00)
Mg	-0.31 (0.00)	-0.38 (0.00)	0.56 (0.00)
Na	-0.20 (0.01)	-0.22 (0.006)	-0.04 (Ns)
P	0.19 (0.03)	0.12 (0.02)	0.70 (0.00)
Sr	-0.46 (0.00)	-0.47 (0.00)	0.06 (Ns)
Fe	-0.21 (0.03)	-0.19 (0.04)	0.37 (0.00)
Cu	-0.21 (0.003)	-0.13 (Ns)	-0.68 (0.00)
Se	-0.27 (0.00)	-0.20 (0.04)	-0.49 (0.00)
Cd	-0.51 (0.00)	-0.57 (0.00)	-0.18 (0.01)
Pb	0.14 (Ns)	0.15 (Ns)	0.31 (0.00)
Hg	0.40 (0.00)	0.61 (0.00)	0.23 (0.001)
As	0.09 (Ns)	0.06 (Ns)	0.47 (0.00)

correlation coefficient was low (R_s from 0.2 to 0.5). The correlations were assessed with the non-parametric Spearman's test for all eel from the different regions; however, this correlation was also negative for eel from particular regions, but the correlation coefficient was substantially higher (R_s from 0.5 to 0.8). The negative correlation indicates that small eel contained more mineral than did large eel. This is confirmed by the higher concentrations of macro- and microelements in eel from the Vistula River, which were the smallest (35-64 cm). As noted earlier, and in contrast to the remaining minerals, phosphorus and zinc were positively correlated with the eel's length and mass. The correlation between phosphorus and length and mass was low ($R_s = 0.19$) and not highly significant. However, the positive correlation between the concentrations of zinc and length was high ($R_s = 0.59$), which was confirmed by the highest concentration of this element in large eel (67-101 cm).

In summation, the results of the study indicate that smaller eel contain more macro- and microelements, which means that smaller fish deliver to the consumer larger doses of minerals that are essential for good health.

The concentrations of the toxic metals cadmium and lead were low in all the specimens analyzed (Table 2). The dose of these elements consumed with 100 g of eel corresponded to a maximum of 1.4% and 1.7%, respectively, of the TWI (Tolerable Weekly Intake) reference dose. The TWI values for cadmium and lead was determined based on studies and is recognized as safe for consumer health (EFSA 2009, 2010). These data indicate that the cadmium and lead in eel muscles does not pose a health risk, in contrast to mercury. This element occurred in eel muscles at high levels that exceeded significantly those in other fish species (SZLINDER-RICHERT et al. 2011). For this reason, and also in the light of its strong toxicity, special attention should be focused on the contents of mercury in eel muscles. Many authors report that fish accumulate mercury with age (BURGER et al. 2001, PINHO et al. 2002, GREEN, KNUTZEN 2003, POLAK-JUSZCZAK 2012). Length is used widely as a surrogate for age, and in eel this rule is particularly applicable, because specimens of this species attain large sizes. The eel analyzed in the present study had body lengths ranging from 35 to 101 cm. The largest specimen (101 cm) from the southern Baltic (Puck Bay) contained as much as 1010 mg Hg kg⁻¹, which exceeded the permissible mercury limit of 1000 mg kg⁻¹ for this species (according to Commission Regulation (EC) No 1881/2006 of 19 December 2006). A consumer who ingests 100 g of eel, in which the mercury content is 1000 mg kg⁻¹, also ingests 101 mg of mercury, which is 36% TWI (TWI for mercury according to the EFSA 2012 is 4 mg week⁻¹ per kg of body weight for an adult weighing 70 kg or 280 mg Hg week⁻¹). However, a 100 g portion of eel with a mercury content of 500 µg kg⁻¹ contains 50 µg of mercury, which is 18% of the reference dose. These data indicate that it is safer to consume small eel (up to 70 cm), in which mercury levels are below 500 µg kg⁻¹. The fact that mercury levels are lower in small specimens was also confirmed by the positive correlation between the concentrations of this

element and eel length and mass (Table 4). The organic form of mercury, methylmercury which comprised about 88% of the total mercury contained in the muscles of eel from the Baltic, is substantially more toxic to humans (BARSKA, SKRZYŃSKI 2003). The TWI reference dose of methylmercury determined by the EFSA (2012) is $1.6 \text{ mg week}^{-1} \text{ per kg of body weight}$, and for an adult 112 mg MeHg week . This limit can be exceeded by consuming 254 g of eel with a total mercury level of $500 \text{ } \mu\text{g kg}^{-1}$ or 127 g of eel with a level of $1000 \text{ } \mu\text{g kg}^{-1}$ in one week. It should be underscored that bodies assimilate MeHg to different degrees depending on the type of protein and fat contents in fish. According to KWAŚNIAK et al. (2012), the mean bioaccessibility of organic mercury in the digested Baltic fish muscles they examined was approximately 37%. In light of this study, the dose of methylmercury digested and assimilated by eel consumers is reduced nearly threefold; however, the long-term consumption of large portions of large eel could pose a risk to consumer health. This is why it is important to limit the consumption of eel meat from large specimens. The consumption of small specimens, which contain more minerals and lower levels of toxic metals – especially mercury, is significantly safer and more advantageous.

Arsenic occurred in eel muscles in the range of 0.224 to 0.607 mg kg^{-1} , and therefore posed no risk. The Polish regulations set the limit for this element at 4 mg kg^{-1} , while the UE regulations do not set a limit for it. The arsenic contained in fish poses no significant risk since it usually occurs in marine animals in the non-toxic form of an *arsenobetaine compound*. Although many compounds containing arsenic have been identified in marine organisms, arsenobetaine is almost invariably the major species present in marine animals with minor traces of inorganic arsenicals and other organoarsenicals (RODRIGUEZ et al. 2009). At present, there is no maximum EU threshold for either total As or inorganic As in fish muscles.

CONCLUSIONS

The European eel, *Aguilla anguilla*, is a good source of phosphorus, zinc, selenium and iron, and the daily requirements for these minerals can be largely met by consuming this fish. The concentrations of mineral components differ depending on the eel size and the region. Small eel (up to 70 cm) contain more macro- and microelements, hence their consumption is more beneficial to consumer health. Additionally, the consumption of small eel does not create health risk because of their lower levels of mercury. It should be borne in mind that methylmercury contained in large eel can be a hazard to consumer health, which is why it is necessary to limit long-term consumption of large eel (exceeding 70 cm), while it is safer to eat small specimens, which contain less mercury and more minerals than large eel do.

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