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12. Heavy metal bio-accumulation in tissues of sturgeon species of the Lower Danube River, Romania

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ABSTRACT. This study investigates bio-accumulation of heavy metals in tissues of sturgeons of the North-Western Black Sea and Lower Danube River (LDR). Samples (10 – 30 gr) of liver, muscle, fat, gonads and skin tissues collected in October 2003 from 21 adult specimens of three sturgeon species: *Acipenser stellatus* (10), *A. gueldenstaedtii* (2), and *Huso huso* (9) were analysed for content in Cd, Cu, Zn, Pb, Mn, Fe and Ni, using VARIAN Spectra A100. The highest concentrations of Zn, Cu and Cd were found in liver and the smallest in muscles of sturgeons. The highest heavy metal content was detected in tissues of stellate sturgeons, followed by Russian sturgeons. In all three species Cd and Cu content of the liver as well as of the stellate sturgeon muscle surpassed the admitted limits for human consumption (Cd – 0.05; Zn - 50; Cu – 5.0; Pb – 0.3 [mg / kg wet weight]). In view of a future re-opening of the commercial fishing of wild sturgeons it is strongly recommended testing the heavy metal level prior delivering sturgeon products to the market. Avoiding human consumption of liver of sturgeons captured in the LDR is strongly recommended as well. In the case of Cd a bio-accumulation with age of sturgeons was visible. In all species males seem to accumulate more heavy metals in their tissues. We explain this as effect of more frequent spawning migration of males in the LDR, the major contamination source. Beluga sturgeons show less heavy metal bio-accumulation of tissues.

Key words: bio-accumulation, heavy metal, sturgeons, Danube River, Black Sea

INTRODUCTION

Stocks of Eurasian sturgeon species dramatically decreased during the last decades due to the poorly regulated fishery, illegal catch, overharvesting, loss of spawning habitat, water quality, and serious chemical pollution. The chemical contamination seems to be one of the most significant factors influencing sturgeon populations and the impact of pollutants on sturgeons is highly topical worldwide ([***1***]; [***5***]; [***10***]; [***13***]; [***15***]; [***17***]; [***18***]; [***23***], [***27***]).

Fish, as part of the aquatic food chain, are the most likely route of human exposure to contaminants. Due to urbanization, industry, agriculture or mining, human activities dictate the frequency and intensity of water contamination by heavy metals [***27***].

Heavy metals may enter the fish either through direct consumption of water or organisms (zooplankton, phytoplankton and benthic fauna) or by uptake through epithelia like the gills, skin and digestive tract. Fish assimilate the metals by digestion, ion exchange of dissolved metals across lipophilic membranes. Bioaccumulation of heavy metals causes irregularities in physiological processes in fish, depending on their uptake concentration in tissues ([***11***]; [***27***]).

Due to the high lipid content of sturgeon body, long lives, long juvenile stage and benthivorous diet, sturgeons are at a high potential risk for accumulating metals in their tissues ([***3***]; [***7***]). Fish feeding on benthic organisms, such as sturgeons, are directly exposed to contaminated sediments while others are exposed when toxicants from sediments are re-suspended into the water column [***27***]. Moreover, heavy metals were reported to affect various developmental processes during the embryonic period of fish, resulting in a decrease of offspring quantity and quality [***9***].

Although iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) are essential elements in organism nutrition and fulfil many biochemical functions in organisms, they can also produce toxic effects at excessive concentrations. Other metals as mercury (Hg), lead (Pb) and cadmium (Cd) are toxic, even in trace amounts ([***10***]; [***20***]).

The levels of contamination by cadmium in fish became of wide interest because an important source for humans cadmium intake is actually the fish consumption. Most of the cadmium content in fish or other seafood is highly absorbable and in humans cadmium is particularly accumulated in kidney [***20***].

Lead is a non-essential metal and is a common contaminant throughout the world, detectable in practically all phases of the inert environment and in all biological systems. Once absorbed, lead accumulates in high concentrations in bone, teeth, liver, lung, kidney, brain, and spleen, and it can penetrate the blood–brain barrier and the placenta ([***14***]; [***20***]).

Zinc is an essential element for humans and animals and it is required for the optimum function of over 300 enzymes, including those required for nucleic acid, protein, and membrane metabolism, as well as cell growth and division ([***12***]; [***22***]).

Copper is another essential trace element for all biological organisms, its essentiality arising from its incorporation into a large number of proteins.

In the Upper, Middle and Lower Danube the heavy metal contamination in the water suspended matter, fine sediments and fish was studied. A general increase in the lower section, which is reflected most clearly in cadmium and mercury (Hg) was reported [***25***]. Using an evaluation scheme [***25***] “very heavy pollution” levels (III-IV) and excessive pollution levels (IV) and higher concentrations of Cd, Cr, Cu, Hg and Zn in the lower reaches of the LDR were found [***26***].

In the Lower Danube concentrations of metals such as Cd and Pb are nearly two orders of magnitude higher compared to the upstream Danube regions [***19***].

Our previous study [***4***], the first one conducted on sturgeons of the Lower Danube River, drew attention to the level of bioaccumulation of heavy metals in tissues of migratory sturgeons in the Danube, a latent problem that might affect the future of these species.

Aim of the current study was to assess the contamination level with heavy metals (Cd, Cu, Zn, Pb, Mn, Fe and Ni) of liver, muscle, fat, gonads and skin of the migratory sturgeon species: Stellate sturgeon (*Acipenser stellatus*), Russian sturgeon (*A. gueldenstaedtii*), and Beluga sturgeon (*Huso huso*) collected in October 2003 from legally captured specimens in the Black Sea and in the Saint George Branch of the Danube River, in view of future re-opening of commercial fishing for sturgeons in the NW Black Sea and the LDR.

MATERIALS AND METHODS

Determination of trace metals

The analytical procedure was described elsewhere [***21***], using VARIAN atomic absorption spectra photometer type Spectra A 100.

Sturgeon age

Age of sturgeons was determined on cross-sections in the pectoral bony fin rays, according to the methods described earlier [***2***].

Sturgeon tissue samples

Five types of tissues (liver, muscle, fat, gonads and skin) ranging between 10 – 30 gr, were collected in October 2003 by a commercial fishing company from 21 adult specimens of migratory sturgeons: *A. stellatus* (10 specimens), *A. gueldenstaedtii* (2 specimens) and *H. huso* (9 specimens) (**Table 1**) legally captured within the Romanian CITES quota in October 2003 on Danube River / Saint George Branch (RKm 10 and RKm 18) and in the Black Sea in Câșla Vădanei - Perișor area by a sturgeon fishing company [***30***]. Samples were kept at -20° C until used.

All samples were taken from specimens of adult sturgeons carrying a commercial tag, as specified in the data base of catches from the Danube Delta Biosphere Reserve of year 2003 [***30***].

Tabel 1.

Species, gender and number of sturgeon specimens analysed in the current study

Species	Gender		Total
	males	females	
Beluga sturgeon (*Huso huso*)	2	7	**9**
Russian sturgeon (*Acipenser gueldenstadtii*)	0	2	**2**
Stellate sturgeon (*Acipenser stellatus*)	8	2	**10**
Total	**10**	**11**	**21**

Statistical analysis

The relationship between fish age, gender and each metal concentration in tissue was investigated by univariate and multivariate regression analysis. Statistical analyses were performed using Statistica 7 software (StatSoft, Inc. 1984 - 2004). T-test for independent samples was performed in MS Excel. P-values less than 0.05 were accepted as significant.

Regulations on contamination of foodstuffs

The mean concentrations of Cd and Pb in sturgeon samples were compared with the limits imposed by European Commission Regulation (EC) No 1881/2006 of 19 December 2006, setting maximum levels for certain contaminants in foodstuffs and Commission Regulation (EC) No 629/2008 of 2 July 2008 [***29***] amending Regulation (EC) No 1881/2006 [***28***].

The mean concentrations of Zn and Cu in sturgeon samples were compared with the acceptable limits proposed by the United Kingdom's Ministry of Agriculture, Fisheries and Food (according to Mashroofeh et al., 2012 [***10***]).

RESULTS

Level of heavy metals including Cd, Cu, Zn, Pb, Mn, Fe and Ni was determined in different tissues: liver, muscle, fat, gonads and skin, collected from 21 specimens of migratory sturgeons species (10 beluga, 2 Russian and 9 stellate sturgeons). Mean values of heavy metals concentrations (mg/kg wet weight) determined in samples of organs (liver, kidney, intestine, spleen, skin and muscle) of Beluga (*Huso huso*), Russian sturgeon (*Acipenser gueldenstadtii*) and Stellate sturgeon (*Acipenser stellatus*) are shown in **Fig. 1 - 3**.

Concentrations of examined heavy metals were varying from 0.002 mg/kg wet weight for Ni in muscle and fat of beluga sturgeon (**Fig. 1B**) to 211.930 mg/kg wet weight for Fe in a liver sample of stellate sturgeon (**Fig. 3A**).

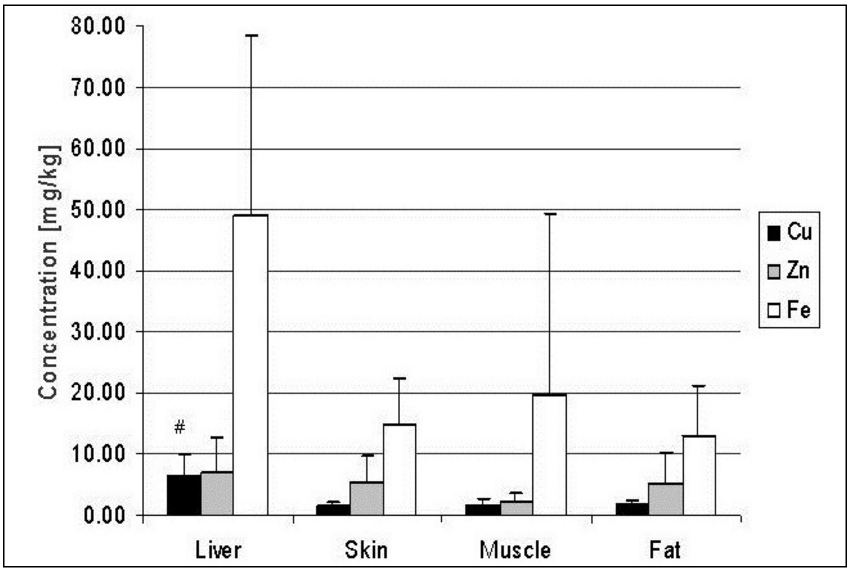

Fig. 1A. Comparison of Cu, Zn and Fe concentrations (mg/kg wet weight) in liver, skin, muscle and fat samples of Beluga sturgeon (*Huso huso*).

Note. Bars indicate the mean and standard deviation (SD) respectively. # indicates exceeding concentration limits.

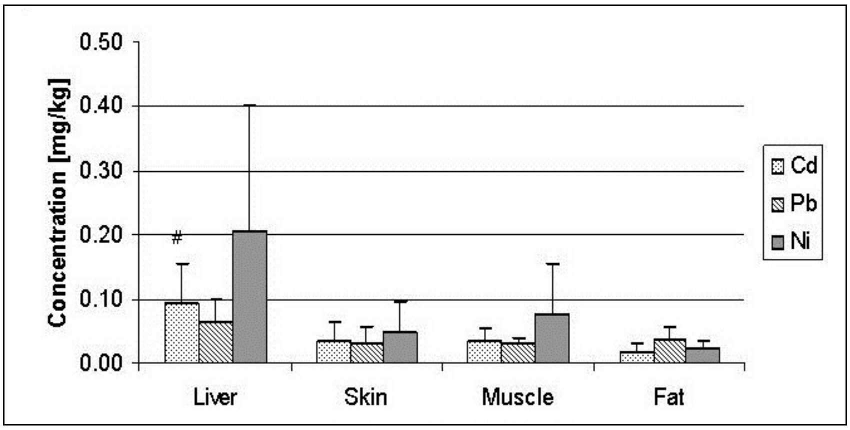

Fig. 1B. Comparison of Cd, Pb and Ni concentrations (mg/kg wet weight) in liver, skin, muscle and fat samples of Beluga sturgeon (*Huso huso*).

Note. Bars indicate the mean and standard deviation (SD) respectively. # indicates exceeding concentration limits.

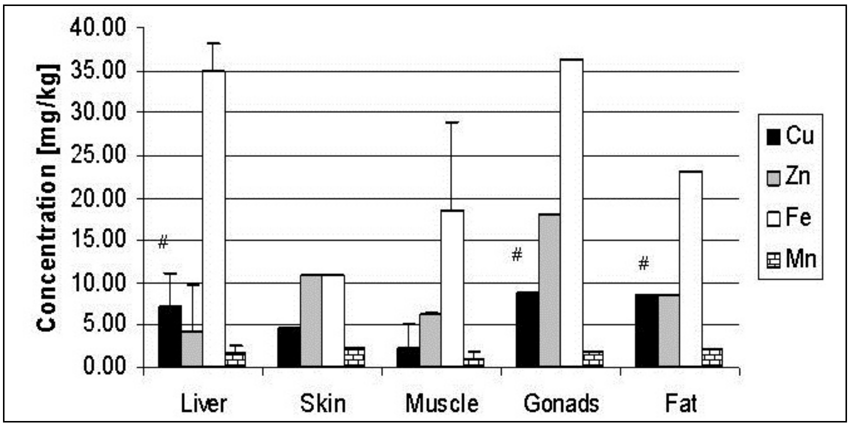

Fig. 2A. Comparison of Cu, Zn, Fe and Mn concentrations (mg/kg wet weight) in liver, skin, muscle, gonads and fat samples of Russian sturgeon (*Acipenser gueldenstadtii*).

Note. Bars indicate the mean and standard deviation (SD) respectively. # indicates exceeding concentration limits.

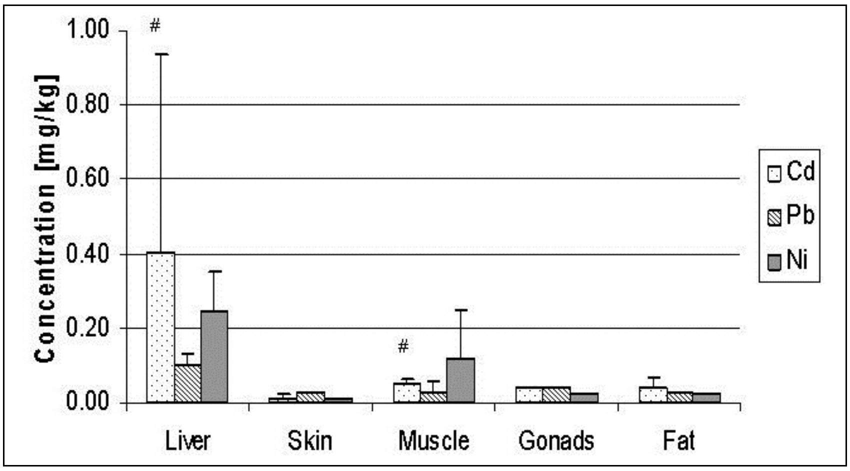

Fig. 2B. Comparison of Cd, Pb and Ni concentrations (mg/kg wet weight) in liver, skin, muscle, gonads and fat samples of Russian sturgeon (*Acipenser gueldenstadtii*).

Note. Bars indicate the mean and standard deviation (SD) respectively. # indicates exceeding concentration limits.

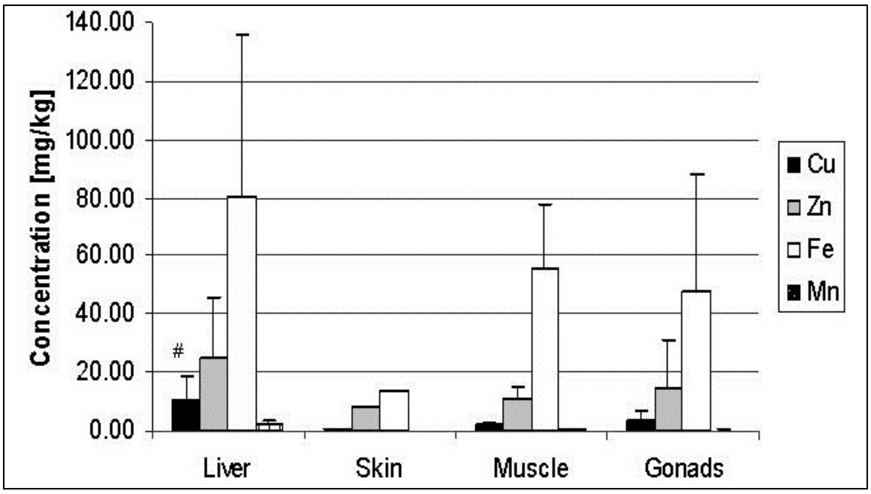

Fig. 3A. Comparison of Cu, Zn, Fe and Mn concentrations (mg/kg wet weight) in liver, skin, muscle and gonads samples of Stellate sturgeon (*Acipenser stellatus*).

Note. Bars indicate the mean and standard deviation (SD) respectively. # indicates exceeding concentration limits.

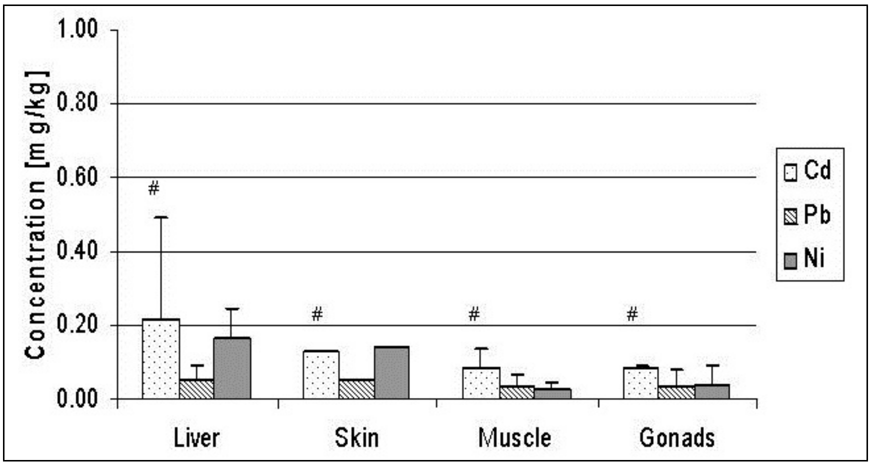

Fig. 3B. Comparison of Cd, Pb and Ni concentrations (mg/kg wet weight) in liver, skin, muscle and gonads samples of Stellate sturgeon (*Acipenser stellatus*).

Note. Bars indicate the mean and standard deviation (SD) respectively. # indicates exceeding concentration limits.

The estimated age of adult sturgeons was ranging between 17 and 24 years for beluga sturgeon, 18 years for Russian sturgeon and 6 and 17 for stellate sturgeon.

There were significant positive correlations between the age of stellate sturgeon and levels of Cd, Pb, Zn in tissues, and in beluga sturgeon between age and level of Cu, Zn, Cd, Pb, Mn and Ni in tissues. This phenomenon was obvious in increasing Cd level in liver of stellate sturgeon with age, indicated by significant correlation ($p < 0.01$) (**Fig. 4**). No statistic analysis could be performed for Russian sturgeon due to the low number of specimens (N=2). In all three sturgeon species males seem to accumulate higher heavy metal levels in their tissues ($p > 0.05$).

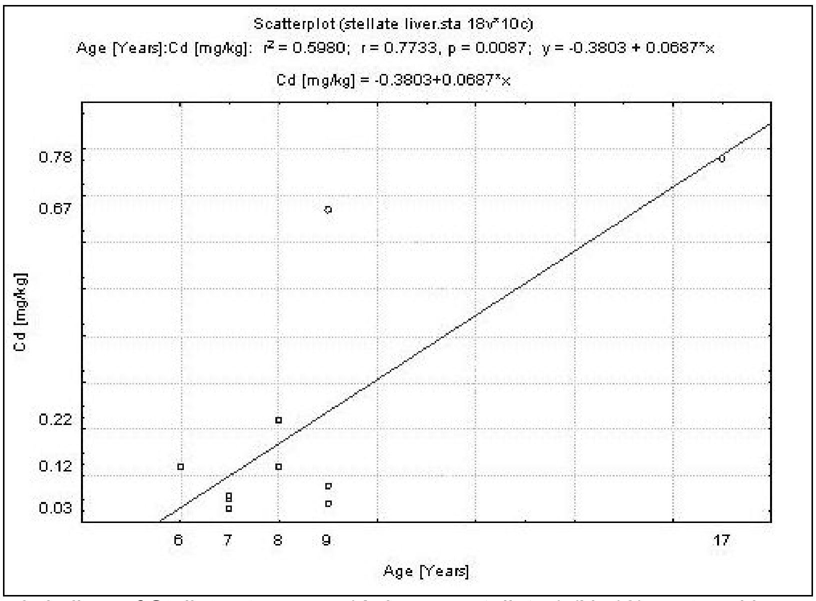

Fig. 4. Scatterplot of Cd levels in liver of Stellate sturgeons (*Acipenser stellatus*) (N=10) captured in year 2003 in Black Sea (Câșla Vădanei – Perișor) and Sfântu Gheorghe branch (output of Statistica 7.0 by StatSoft Inc.).

High level of Zn (74.59 mg / Kg) from the liver of a 9 years old stellate sturgeon male (commercial tag 5807) captured at Rkm18 on Sfântu Gheorghe branch of the Danube River, surpasses the admitted limits for human consumption.

Cadmium level was exceeding the permissible limits in liver of all three sturgeon species (**Fig. 5A** and **5B**). The highest values were determined in Russian sturgeon, 0.78 mg/kg wet weight, in 18 years old female (commercial tag 5987) captured in the Black Sea, Ciotica area, as well as in two stellate sturgeons, 0.78 and respectively 0.67 mg/kg wet weight, in a 17 years old female (commercial tag 5124) captured in the Black Sea, Southern Bay and a 9 years old male (commercial tag 5873) captured in Sahalin area, Black Sea. In stellate sturgeon the mean Cd values exceeded the permissible limits of 0.05 mg/kg wet weight [***29***] in all analysed organs. Consistent lower values of Cd concentration were determined in skin, muscle and fat of beluga sturgeon. These values were ranging between 0.018 ± 0.012 mg/kg wet weight in fat and 0.034 ± 0.030 mg/kg wet weight in beluga sturgeon skin.

Lead level did not exceed the permissible limits of 0.3 mg/kg wet weight [***29***] in any of the organs of the analysed sturgeon species. The highest value was determined in stellate sturgeon, 0.16 mg/kg wet weight, in an 8 years old male (commercial tag 5574) captured in Sahalin area, the Black Sea.

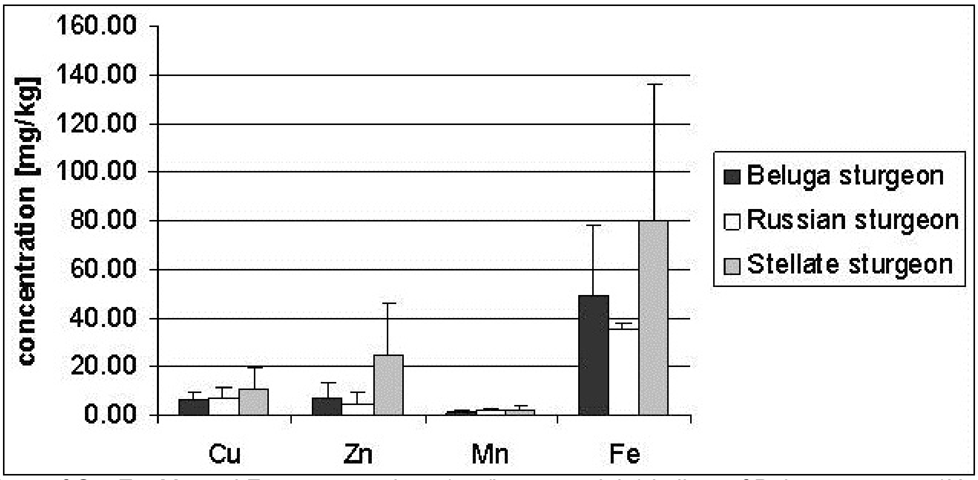

Fig. 5.A. Comparison of Cu, Zn, Mn and Fe concentrations (mg/kg wet weight) in liver of Beluga sturgeon (*Huso huso*), Russian sturgeon (*Acipenser gueldenstadtii*) and Stellate sturgeon (*Acipenser stellatus*).

Note. Bars indicate the mean and standard deviation (SD) respectively. # indicates values exceeding concentration limits.

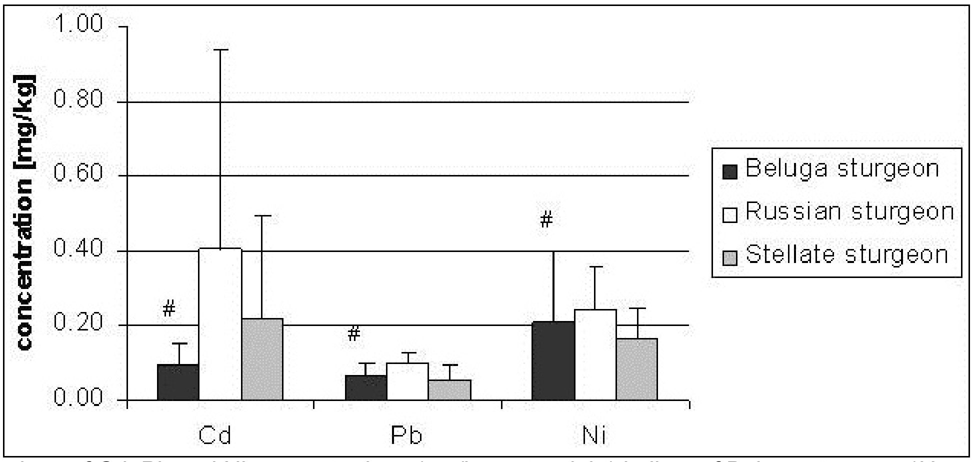

Fig. 5.B. Comparison of Cd, Pb and Ni concentrations (mg/kg wet weight) in liver of Beluga sturgeon (*Huso huso*), Russian sturgeon (*Acipenser gueldenstadtii*) and Stellate sturgeon (*Acipenser stellatus*).

Note. Bars indicate the mean and standard deviation (SD) respectively. # indicates values exceeding concentration limits.

In gonads Cd concentration was ranging between 0.040 and 0.090 mg/kg wet weight, the exceeding value being determined in gonads of a stellate sturgeon 6 years old male (commercial tag 5885) captured in area Zatoane, the Black Sea (**Table 2**).

Table 2.

Concentration (mean ± SD, mg/kg wet weight) of metals in gonad tissue samples of sturgeon from NW Black Sea and Danube River (N=4).

Species	N	Gender	Fe	Zn	Cu	Mn	Cd	Ni	Pb
Stellate sturgeon (*Acipenser stellatus*)	3	males	47.766 +/- 40.456	14.827 +/- 16.175	3.770 +/- 3.510	0.293 +/- 0.146	0.083 +/- 0.006	0.040 +/- 0.052	0.034 +/- 0.048
Russian sturgeon (*Acipenser gueldenstaedtii*)	1	female	36.400	18.050	8.720	1.940	0.040	0.021	0.040

DISCUSSIONS

Our results indicate the liver of sturgeon specimens as the prone organ to bioaccumulation of heavy metals (**Fig. 1-4**). This is consistent with previous observations of Wachs [**26**], showing that the highest concentrations of heavy metals vary in different tissues of fishes, although tissues as kidney and spleen were not included in our study:

Zn: Kidney > Liver > Spleen > Muscle
Cu: Liver > Spleen > Kidney > Muscle
Cd: Kidney > Liver > Spleen > Muscle

In literature the levels of Zn in fish were reported to be very high, up to over 300 µg/g dry weight. The Pb and Cu maximum levels are low and usually do not exceed 10 µg/g dry weights, while Cd and Hg are accumulating in fish organs in very low amounts, below 1 µg/g dry weight. It was shown that metal accumulation in fish depends on pollution, and may differ for various fish species despite of living in the same water body [***8***].

In live fish the metal levels are known to differ: Fe > Zn > Pb > Cu > Cd > Hg [***8***].

The level of heavy metals in beluga sturgeon liver in the current study was: Fe > Zn > Cu > Mn > Ni > Cd > Pb, in Russian sturgeon: Fe > Cu > Zn > Mn > Cd > Ni > Pb and in stellate sturgeon Fe > Zn > Cu > Mn > Cd > Ni > Pb.

Unlike the stellate sturgeon from Southern Caspian Sea, where the mean concentration level of trace metals was: muscle tissue Pb > Cd, liver Pb > Cd [***6***], in stellate sturgeon specimens captured in the NW Black Sea, the mean level of Pb was higher in liver than in muscle, skin or gonads (**Fig. 3**) and these mean values were significantly lower ($p < 0.05$) than corresponding values of Cd for the same organs.

In Persian sturgeon (*Acipenser persicus*) from the Caspian Sea the mean concentrations of the metals followed in the order of Zn > Cu > Mn in both caviar and muscle [***10***], similar to our results.

A study focused on twenty trace elements accumulation in muscles of five sturgeon species (*A. gueldenstaedtii, A. persicus, A. nudiventris, A. stellatus* and *H. huso*) from the southern part of the Caspian Sea reported amounts of toxic trace elements (Cd, Cu, Pb and Zn) markedly below the international guidelines for human consumption for all cases [***16***].

Little is known regarding the heavy metal contamination of sturgeons in the Danube River. Jarić et al. [***7***] measured slightly increased cadmium levels in the muscle of sterlet (*Acipenser ruthenus*) from Danube River in Serbia. Heavy metal analysis of sterlet sampled along the Danube River, at three different sites isolated from each other by dams, in Hungary and Serbia, indicated the liver as the main heavy metal storage tissue, while the muscles accumulate the lowest levels of heavy metals [***15***], in accordance with results reported in the current study. Excepting partly for Cd, heavy metal concentrations in the sterlet muscle were at acceptable levels for human consumption [***15***].

The heavy metals (Zn, Cu, Mn) detected in Persian sturgeon samples from the Caspian Sea indicated significantly higher concentrations in caviar than muscle tissue [***10***]. Our findings revealed comparable levels of these elements in muscle and gonads of analysed specimens. Moreover, comparing to data on Persian sturgeon muscle tissue, the mean concentrations [mg / kg wet weight] of Zn, Cu, Mn (7.49 ± 3.07, 1.00 ± 0.32 and respectively 0.34 ± 0.24), were similar to the mean concentrations of those metals in stellate sturgeon (11.24 ± 3.68, 1.81 ± 1.19 and 0.50 ± 0.46) analyzed in the current study. In beluga sturgeon muscle tissue Zn concentration was lower than in Persian sturgeon, whereas the determined values for Cu and Mn were comparable (Zn, Cu and Mn - 2.05 ± 1.71, 1.65 ± 1.16 and 0.62 ± 0.27). In Russian sturgeon muscle tissue, elevated values were determined for Cu and Mn, comparing to Persian sturgeon (Zn, Cu and Mn - 6.23 ± 0.15, 2.33 ± 2.77 and 0.93 ± 1.03).

The concentration of Cd was varying among sturgeon species in muscle tissue samples. Therefore, the highest mean values were determined in stellate sturgeon (0.08 ± 0.05 mg/kg wet weight) and the lowest values in beluga sturgeon (0.03 ± 0.02 mg/kg wet weight). These data indicate exceeding of limits permissible for human consumption in stellate sturgeon meat. Nevertheless, the stellate sturgeon meat was delivered to the market in year 2003.

The age of adult sturgeons was estimated by cross-sections in the pectoral bony fin rays as ranging between 17 and 24 years for beluga sturgeon, 18 years for Russian sturgeon and 6 and 17 for stellate sturgeon. Significant positive correlations were observed between the age of stellate sturgeon and levels of Cd, Pb, Zn in tissues, and in beluga sturgeon between age and levels of Cu, Zn, Cd, Pb, Mn and Ni in tissues.

Similarly to year 1996, as previously reported [***4***], our data indicate an increase of bio-accumulation of Cd in sturgeon organs, especially in liver, with age. This phenomenon is obvious in stellate sturgeon where the mean Cd values exceeded the permissible limits of 0.05 mg/kg wet weight [***29***] in all analysed organs even more than 10 times fold, with a maximum value of 0.78 in liver of a 17 years female (commercial tag 5124), captured in the Black Sea, Southern Bay.

Due to the species ecology, stellate sturgeon and Russian sturgeon prefer marine habitats located close to the Danube River mouths, feeding mainly on bottom fauna. After the downstream migration to the sea during the first year of their life, beluga sturgeons feed mainly on pelagic fish, such as anchovy, horse mackerel, pontic shad and sprat shoals, which feed on other pelagic organisms as well. As a consequence beluga sturgeons show less heavy metal bio-accumulation of tissues.

On the other hand, males seem to accumulate more heavy metals in their tissues than females in the three anadromous sturgeon species. This might be the effect of more frequent spawning migration of males in the LDR, the major contamination source. However confirmation of this hypothesis needs further investigations.

Moreover, it is expected that the autumn migrants, entering the river in late October – November and spend the winter in the wintering holes, to continue their migration to the spawning sites in the following spring, accumulate higher levels of heavy metals than the spring migrants. This hypothesis is supported by findings of Wachs ([***24***]; [***26***]) which indicate that heavy metal contamination in fish is facilitated by their contact with water suspended matter and fine sediments containing heavy metal ions.

According to the mean values of heavy metals in sturgeon muscle, the river or marine habitats were ecologically classified [***25***] (**Table 3**). Therefore, according to the mean values of Pb determined in the current study, habitats of migratory sturgeons in LDR are classified in the II category, while according to mean values of Cd, Zn and Cu they vary from the II category to III-IV.

Tabel 3.

Ecological classification of habitats of sturgeons in the LDR and the Black Sea according to Wachs [25] based on heavy metal content of muscle tissue (mean values in [mg / kg wet weight])

Species	N	Cd	Categ.	Zn	Categ.	Cu	Categ.	Pb	Categ.
Beluga sturgeon (*Huso huso*)	9	0.034	III	2.045	II	1.650	III- IV	0.031	II
Russian sturgeon (*Acipenser gueldenstaedtii*)	2	0.050	III - IV	6.225	III	0.050	II	0.030	II
Stellate sturgeon (*Acipenser stellatus*)	10	0.084	III - IV	11.240	III - IV	1.810	III - IV	0.034	II

The high content of lipids in sturgeon body makes them prone to bio-accumulation of heavy metals in their organs, including the gonads. Although not exceeding the maximum permissible limits for human consumption, heavy metals were detected in caviar samples collected from Persian sturgeon [***10***], as well as in gonad tissue analysed in this study, except the gonad of a 6 years old stellate sturgeon male (commercial tag 5885) captured in Zatoane area (in the Black Sea).

Besides the fact that accumulation of heavy metal in sturgeon tissues is known to induce irregularities in physiological processes in fish ([***11***]; [***27***]; [***20***]), it affects the number and quality of the larvae, causing high frequency of malformations and reduced viability. The egg shell cannot fully protect the embryo against metal penetration that occurs particularly during the swelling phase and therefore, metals may accumulate in the egg [***9***].

Early life stages of fish are sensitive to water pollution and depending on level / type of metal contamination, it could lead from developmental disturbances to death of the embryo. Early stages post-fertilization were reported to be particularly sensitive to metal intoxication, when most disturbances and the highest embryonic mortality occur. Delay in the hatching process, premature hatching, deformations and death of newly hatched larvae induced by heavy metals were described in fish [***9***]. For instance, a recent study reported that white sturgeon (*Acipenser transmontanus*) early life stages are sensitive to aqueous copper exposure under laboratory conditions. The greatest Cu sensitivity of white sturgeon early life stage, comparing to other fish species, coincides with the beginning of active feeding and close association with sediment [***23***]. According to our data, the ecological classification of sturgeon habitats in LDR based on Cu mean value is ranging from II to IV (**Table 3**). Thus, the natural spawning success and annual recruitment of the young-of-the-year (YOY) of sturgeon species spawning in the LDR could be affected by the aqueous Cu level in the spawning site. Further studies aiming to determine the level of contaminants and their dynamics during the spawning period and early developmental phase of YOY needs to be carried out.

Therefore, sitespecific water quality guidelines and criteria should be evaluated closely to ensure adequate protection [***23***]. Comparative analysis for the two periods, previous data on sturgeon captured in 1996 [***4***] and current data on those captured in 2003 show an evident reduction of heavy metal contamination of sturgeons, mostly in case of Cu and Zn, and less in Cd.

CONCLUSION

Current study indicates exceeding limits permissible for human consumption in stellate sturgeon meat, which was delivered to the market in year 2003. In view of a future re-opening of the commercial fishing of sturgeons that is currently banned for a 10 years period (2006 – 2015) in Romania and until 2015 in Bulgaria, it is strongly recommended certifying of the heavy metal levels prior delivering sturgeon products on market and avoiding human consumption of liver of sturgeons captured in the LDR.

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REFERENCES

[*1*]. AGUSA (T.), KUNITO (T.), TANABE (S.), POURKAZEMI (M.), ABUREY (D. G.), 2004 – Concentrations of trace elements in muscle of sturgeons in the Caspian Sea. IN: *Marine Pollution Bulletin*, vol. 49, No. 9 - 10, pp. 789 - 800. DOI: 10.1016/j.marpolbul.2004.06.008; ISSN 0025-326X

[*2*]. BACALBAŞA-DOBROVICI (N.), 1997 – Endangered migratory sturgeons of the lower Danube River and its delta. IN: *Environmental Biology of Fishes*, vol. 48, pp. 201 - 207. DOI: 10.1023/A:1007343611333; ISSN 0378-1909

[*3*]. BILLARD (R.), LECOINTRE (G.), 2000 – Biology and conservation of sturgeon and paddlefish. IN: *Reviews in Fish Biology and Fisheries*, vol. 10, No. 4, pp. 355 - 392. DOI: 10.1023/A:1012231526151; ISSN 0960-3166

[*4*]. ENE (F.), SUCIU (R.), BOGDAN (L.), 1998 – Cercetări asupra sturionilor marini migratori în Dunăre. IV. Incidența parazitozelor și contaminarea cu metale grele a diferitelor specii de sturioni. IN: *Analele Științifice ale Institului Delta Dunării*, vol. VI, No. 1, pp. 305 – 312. Tulcea, Romania. [in Romanian]

[*5*]. GESSNER (J.), WIRTH (M.), KIRSCHBAUM (F.), KRÜGER (A.), PATRICHE (N.), 2002 – Caviar composition in wild and cultured sturgeons – impact of food sources on fatty acid composition and contaminant load. IN: *Journal of Applied Ichthyology*, vol. 18, No. 4 - 6, pp. 665 - 672. DOI: 10.1046/j.1439-0426.2002.00366.x ; ISSN 0175-8659

[*6*]. HEYDARI (S.), NAMIN (J. I.), MOHAMMADI (M.), RAD (F. M.), 2011 – Cadmium and lead concentrations in muscles and livers of stellate sturgeon (*Acipenser stellatus*) from several sampling stations in the southern Caspian Sea. IN: *Journal of Applied Ichthyology*, vol. 27, No. 2 (Special Issue: Proceedings of the 6th International Symposium on Sturgeon - Wuhan, China; October 25 - 31, 2009), pp. 520 - 523. DOI: 10.1111/j.1439-0426.2011.01672.x ; ISSN 0175-8659

[*7*]. JARIĆ (I.), VIŠNJIĆ-JEFTIĆ (Ž.), CVIJANOVIĆ (G.), GAČIĆ (Z.), JOVANOVIĆ (L.), SKORIĆ (S.), LENHARDT (M.), 2011 – Determination of differential heavy metal and trace element accumulation in liver, gills, intestine and muscle of sterlet (*Acipenser ruthenus*) from the Danube River in Serbia by ICP-OES. IN: *Microchemical Journal*, vol. 98, No. 1, pp. 77 - 81. DOI: 10.1016/j.microc.2010.11.008 ; ISSN 0026-265X

[*8*]. JEZIERSKA (B.), WITESKA (M.), 2006 – The metal uptake and accumulation in fish living in polluted waters. IN: NATO Science Series, IV (Earth and Environmental Sciences) vol. 69 (Proceedings of the NATO Advanced Research Workshop on Viable Methods for Soil and Water Pollution Monitoring, Protection and Remediation – Krakow, Poland, 27 June – 1 July 2005; eds.: TWARDOWSKA I, ALLEN H. E., HAGGBLOM M. H.), pp. 107 - 114. Publisher: Springer. The Netherlands. DOI: 10.1007/978-1-4020-4728-2_6; ISSN 1568-1238; ISBN 978-1-4020-4726-8 (available at: http://www2.bren.ucsb.edu/~dturney/port/Soil_Water_Pollution_Monitoring_Protection_Remediation/107.pdf ; accessed on 10 February 2013)

[*9*]. JEZIERSKA (B.), ŁUGOWSKA (K.), WITESKA (M.), 2009 – The effects of heavy metals on embryonic development of fish (a review). IN: *Fish Physiology and Biochemistry*, vol. 35, No. 4, pp. 625 – 640. DOI: 10.1007/s10695-008-9284-4 ; ISSN 0920-1742

[*10*]. MASHROOFEH (A.), BAKHTIARI (A.), POURKAZEMI, (M.), 2012 – Bioaccumulation of Zn, Cu and Mn in the Caviar and Muscle of Persian Sturgeon (*Acipenser persicus*) from the Caspian Sea, Iran. IN: *Bulletin of Environmental Contamination and Toxicology*, vol. 89, No. 6, pp. 1201 - 1204. DOI: 10.1007/s00128-012-0863-9 ; ISSN 0007-4681

[*11*]. MATASIN (Z.), IVANUSIC (M.), ORESCANIN (V.), NEJEDLI (S.), GAIGER (I. T.), 2011 – Heavy Metal Concentrations in Predator Fish. IN: *Journal of Animal and Veterinary Advances*, vol. 10, No. 9, pp. 1214 - 1218. DOI: 10.3923/javaa.2011.1214.1218 ; ISSN 1680-5593

[*12*]. MCCALL (K. A.), HUANG (C. C.), FIERKE (C. A.), 2000 – Function and mechanism of zinc metalloenzymes. IN: *Journal of Nutrition*, vol. 130, 1437S - 1446S (available at: http://www.idpas.org/pdf/2828FunctionandMechanismZinc.pdf ; accessed on 3 February 2013)

[*13*]. MIKRYAKOV (V. R.), LAPIROVA (T. B.), 1997 – Influence of salt of some heavy metals on the differential blood count of juvenile *Acipenser baeri*. IN: *Journal of Ichthyology*, vol. 37, pp. 458 - 462. ISSN 0032-9452

[*14*]. PLUMLEE (K. H.), 2004 – Chapter 22 - Metals and Minerals. Clinical Veterinary Toxicology. pp. 193 - 230. Edit. Mosby, St. Louis, MO

[*15*]. POLEKSIC (V.), LENHARDT (M.), JARIC (I.), DJORDJEVIC (D.), GACIC (Z.), CVIJANOVIC (G.), RASKOVIC (B.), 2010 - Liver, gills, and skin histopathology and heavy metal content of the Danube sterlet (*Acipenser ruthenus* Linnaeus, 1758). IN: *Environmental Toxicology and Chemistry*, vol. 29, No. 3, pp. 515 - 521. DOI: 10.1002/etc.82 ; ISSN 0730-7268

[*16*]. POURANG (N.), TANABE (S.), REZVANI (S.), DENNIS (J. H.). 2005 - Trace elements accumulation in edible tissues of five sturgeon species from the Caspian Sea. IN: *Environmental Monitoring and Assessment*, vol. 100, No. 1 - 3, pp. 89 - 108. DOI: 10.1007/s10661-005-7054-7 ; ISSN 0167-6369

[*17*]. POURKAZEMI (M.), 2006 – Caspian Sea sturgeon conservation and fisheries: past, present and future. IN: *Journal of Applied Ichthyology*, vol. 22, Supplement 1, pp. 12 - 16. DOI: 10.1111/j.1439-0426.2007.00923.x ; ISSN 0175-8659

[*18*]. ROMANOV (A. A.), SHEVELEVA (N. N.), 1993 – Disruption of gonadogenesis in Caspian sturgeons (Acipenseridae). IN: *Journal of Ichthyology*, vol. 33, pp. 127 - 133. ISSN 0032-9452

[*19*]. SCHIEMER (F.), GUTI (G.), KECKEIS (H.), STARAȘ (M.), 2004 – Ecological status and problems of the Danube river and its fish fauna: a review. IN: RAP Publication 2004/16 - Proceedings of the Second International Symposium on the Management of Large Rivers for Fisheries, vol. 1 (Sustaining Livelihood and Biodiversity in the New Millenium: 11 – 14 February 2003, Phnom Penh, Kingdom of Cambodia; eds.: WELCOMME R. L., PETR T.), pp. 273 - 299. Published by FAO Regional Office for Asia and the Pacific. Bangkok, Thailand.

[*20*]. SQUADRONE (S.), PREARO (M.), BRIZIO (P.), GAVINELLI (S.), PELLEGRINO (M.), SCANZIO (T.), GUARISE (S.), BENEDETTO (A.), ABETE (M. C.), 2013 – Heavy metals distribution in muscle, liver, kidney and gill of European catfish (*Silurus glanis*) from Italian Rivers. IN: *Chemosphere*, vol. 90, No. 2, pp. 358 - 365. DOI: 10.1016/j.chemosphere.2012.07.028 ; ISSN 0045-6535

[*21*]. TEODOROF (L.), NĂSTASE (C.), ANUTI (I.), 2009 - Bioaccumulation of Heavy Metals in Fish from Dobrudja Aquaculture Farm. IN: *Revista de Chimie*, vol. 60, No. 11. pp. 1235 - 1241. București, Romania. ISSN 0034 - 7752 (copy of article available at: http://www.revistadechimie.ro/pdf/TEODOROF%2011.pdf ; accessed on 3 February 2013)

[*22*]. VALLEE (B. L.), AULD (D. S.), 1990 – Zinc coordination, function, and structure of zinc enzymes and other proteins. IN: *Biochemistry*, vol. 29, No. 24, pp. 5647 - 5659. DOI: 10.1021/bi00476a001 ; ISSN 0006-2960

[*23*]. VARDY (D.), OELLERS (J.), DOERING (J.), HOLLERT (H.), GIESY (J.), HECKER (M.), 2013 – Sensitivity of early life stages of white sturgeon, rainbow trout, and fathead minnow to copper. IN: *Ecotoxicology*, vol. 22, No. 1, pp. 139 - 147. DOI: 10.1007/s10646-012-1010-4 ; ISSN 0963-9292

[*24*]. WACHS (B.), 1998 – Heavy metal contamination of the Danubian Bioresource Fish. IN: *Danubius*, vol. 1 - 2, pp. 43 - 65. Belgrade.

[*25*]. WACHS (B.), 1998 – A qualitative classification for the evaluation of the heavy metal contamination in river ecosystems. IN: *Verhandlungen der Internationalen Vereinigung für Limnologie*, vol. 26, part 3 (Verhandlungen – Proceedings – Travaux: 26th Congress, Sao Paulo, 1995), pp. 1289 - 1294. Publisher: E. Schweizerbart'sche Verlagsbuchhandlung. Stuttgart, Germany. ISSN 0368-0770; ISBN 978-3-520-54047-1

[*26*]. WACHS (B.), 2000 – Heavy metal content in Danubian fish. IN: *Archiv für Hydrobiologie* - Supplementband. Large rivers, vol. 11, pp. 533 - 556. ISSN 0003-9136

[*27*]. ZRNČIĆ (S.), ORAIĆ (D.), ĆALETA (M.), MIHALJEVIĆ (Ž.), ZANELLA (D.), BILANDŽIĆ (N.), 2013 – Biomonitoring of heavy metals in fish from the Danube River. IN: *Environmental Monitoring and Assessment*, vol. 185, No. 2, pp.1189 - 1198. DOI: 10.1007/s10661-012-2625-x ; ISSN 0167-6369

[*28*]. ***, 2006 – Commission Regulation (EC) No. 1881 / 2006 of 19 December 2006 setting maximum levels for certain contaminants in foodsuffs (Text with EEA relevance). IN: *Official Journal of the European Union*, L 364 (published in 20 December 2006), pp. 5. Luxembourg. (available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R1881:20100701:EN:PDF ; accessed on 3 February 2013)

[*29*]. ***, 2008 – Commission Regulation (EC) No. 629 / 2008 of 28 July 2008 amending the Regulation (EC) No. 1881 / 2006 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance). IN: *Official Journal of the European Union*, L 173 (published on 3 July 2008), pp. 6 – 9. Luxembourg. (available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:173:0006:0009:EN:PDF ; accessed on 3 February 2013)

[*30*]. ***, 2008 – http://sturgeons.ddni.ro/sturgeons/index.php?option=com_content&view=article&id=29&Itemid=43 ; accessed on 3 February 2013

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